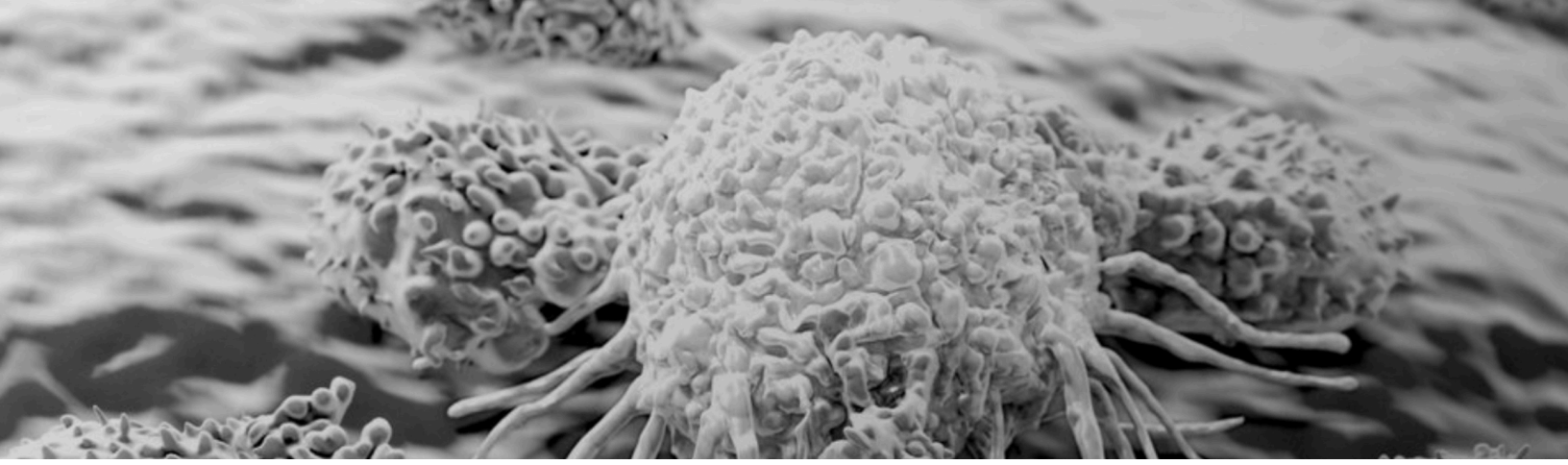


The Effects of EGFR Mutations in Tumours on the Outcome of an Abscopal Effect

Log Book

C. van der Raadt
Grade 12, Age 17

July 3rd

Vocabulary 3 Notes

- Armamentarium: The medicines, equipment, and techniques available to a medical practicioner.
- Metastasis: The development of secondary malignant growths at a distance from the primary sight of cancer.
- Abscopal effect: The ability of localized radiation to initiate an anti-tumour response that kills cancer cells at a distance from the primary target.
- Cryosurgery: surgery using the local application of intense cold to destroy unwanted tissue.

Apoptotic Signaling: Signaling the natural death of cells (usually as a part of growth or development).
Lesion: A region of an organ or tissue which has suffered damage through injury or disease.
Modality: A particular method or procedure.
Synergistic: in harmony.
Immune Checkpoint: Regulators of the immune system that are crucial for self-tolerance, preventing the immune system from attacking indiscriminantly.
Senescence: The process of deterioration with age.
Autophagy: the consumption of the body's own tissue as a metabolic process during starvation/certain diseases.
Dendritic Cells: Immune cells that act as messengers between innate and adaptive immune systems.
Cytokine: A type of substance that is secreted by certain cells of the immune system and have an effect on other cells
↳ Chemokine: A cytokine which attracts white blood cells to cites of infection
Adenocarcinoma: Cancer in mucus-secreting glands around the body
"Lung cancer is often insidious, producing no symptoms until the disease is well advanced."
Locoregional: Restricted to a localized region of the body
Immunohistochemistry: Using antibodies binding to antigens in biological tissues to detect the antigens and diagnose some types of cancer
Exon: A segment of DNA or RNA containing information for a protein or peptide sequence

The optimism surrounding stereotactic body Radiation therapy and immunemodulation

Kinase: An enzyme that transfers a phosphate group from ATP to a specified molecule

Pulmonary: Relating to the lungs

loss of IFNX pathway genes in liver cells as a mechanism of resistance to anti-CTLA-4 therapy

the abscopal effect of local radiotherapy: Using immune therapy to make a rare event clinically relevant

Abscopal effects in Radio-immunotherapy-Response analysis of Metastatic cancer patients with progressive disease under anti-PD1 immune checkpoint inhibition

overview of the biology of type 1 interferons

PD-1 – NCI Dictionary of Cancer Terms

Radiotherapy induces Responses of lung cancer to CTLA-4 blockade

Report of an abscopal effect induced by stereotactic body radiotherapy and Nivolumab in a patient with metastatic NSCLC

Interferon: Britannica

The optimal Radiation dose to induce Robust Systemic Anti-tumor immunity

Fractionated but not single-dose radiotherapy induces an immune-mediated abscopal effect when combined with anti-CTLA-4 antibody

Abscopal benefits of localized Radiotherapy Depend on Activated T-cell trafficking and distribution between Metastatic lesions

Notes

July 5th

4 Pillars of Cancer treatment: Radiation therapy, Chemotherapy, surgery, and immunotherapy
ABSCOPAL EFFECT: The Regression of lesions or tumour or Metastatic regions outside the radiation field
Immunogenic: Able to produce an immune response
Abscopal effect is most likely mediated by the immune system
Dependent on → RT-induced cell damage leading to the release of cell fragments, neoantigens, Cellular Danger-associated Molecular patterns (DAMPS), and Cytokines
one way to increase the likelihood of an abscopal effect occuring is by manipulating the tumour microenvironment

↓ Decluttering

- 4 Pillars of Cancer treatment: Radiation therapy, Chemotherapy, surgery, and immunotherapy
- ABSCOPAL EFFECT: The regression of lesions, or tumour or metastatic regions outside of the radiation field and caused by Radiotherapy (RT)
- Immunogenic: Able to produce an immune response
- Abscopal effect is most likely mediated by the activation of the immune system,
- Dependent on: RT-induced cell damage leading to the release of cell fragments, neoantigens, cellular danger-associated Molecular patterns (DAMPs), and cytokines
- One way to increase the likelihood of an abscopal effect occuring is by manipulating the tumour micro-environment
  - Radiation dose
    - Fractionation
  - Site of irradiation
    - Timing
  - Combined RT with other systemic therapies
- RT and immunotherapy (IT) can immunize the patient against the tumour (like a tumour vaccine)
- Antigen: A toxin/foreign substance which induces an immune response, especially the production of antibodies.
  - Neoantigen: An antigen to which the immune system has not been previously exposed, especially that arises from alteration of the host's antigens

- Danger-associated Molecular patterns (DAMPs): Molecules released passively or exerted actively by stressed/dying cells and further enhance inflammatory or cell-death signaling
- ☆ Immunotolerance: The inability to give rise to a specific immune response to a given antigen, as this antigen has previously been exposed to the immune system
- Immunotolerance hinders the abscopal effect at the tumor site
  - ↳ Tumors can cause/affect immune tolerance ~~repression~~

July 8th

CASE STUFF
- 47 y/o male, smoker (40 P/Y) (Stage IIIB lung adenocarcinoma)
- Complete Regression + Response to Abscopal effect

If I'm not good at effort post me → (I don't know if this is important)

"Radiation induced exposure of immunogenic mutations to the immune system"

"Patients who did not complete treatment [died or progressed despite treatment] had more advanced disease at study entry, with significantly more organs involved by metastasis, more frequently had bone metastases, and had received more courses of prior chemotherapy"
- PD-L1 expression in the tumor before treatment was not associated with a response
- CD8 T-cell infiltration was also not associated with a response
- Patients with EGFR Mutated cancers had Progressed Disease at a rate significantly higher Compared to patients with disease control
- In this case, Radiation regimen and location of the irradiated lesion did not affect treatment response significantly
- Interferon-β (IFNβ) correlated with the abscopal response, similar to mice trials

NECESSARY

- UPREGULATED: ~~Increase~~ in a cellular response to a Molecular Stimulus due to an increase in the number of receptors on the cell surface.

"[The] expansion of a large number of tumour-specific T cell clones in peripheral blood and their persistence over time correlate well with a successful abscopal response."

July 11th

"The different outcome might reflect RT's ability to elicit the activation of a response in the tumour that mimics a viral infection"

- Interferon-B is a CYTOKINE! $C_{908}H_{1408}N_{246}O_{252}S_7$ wow
  - ↳ Used to treat Multiple sclerosis
    - ↳ Produced by mammalian cells as a defense against pathogens (IFNB can cause or lower inflammation paradoxically) → Depending the context or immune response
- There are three forms of interferon - α (alpha) β (beta) and γ (gamma). Type 1 (α and β) can be produce by almost any cell upon stimulation by a virus whereas Type 2 interferon is secreted only by natural killer cells and T lymphocytes with the main purpose of signaling the immune system to respond to infectious agents or cancerous growths → ipilimumab (antibodies)
- ★ CTLA-4 or Cytotoxic T-lymphocyte-associated protein 4 is a protein receptor that functions as an immune checkpoint and DOWNREGULATES immune responses.
- ★ PD-1 or Programmed Cell Death Protein 1 is a protein on the surface of cells that promotes self-tolerance by downregulating the immune system and suppressing T cell inflammatory activity
  - ↳ Both prevent autoimmune disorders but also keep cancer hidden from the body
- "75% of ipilimumab non-responders harbour... genomic defects of the IFN-γ pathway genes."
  - ↳ Tumours with IFN-γ defects don't respond as well to immunotherapy as they won't respond to PD-1 or CTLA-4 anti-therapy drugs.
- "A total of 184 mutations were detected in the 12 non-responders including 142 copy number alterations (CNAs) and 42 single nucleotide variants of the IFN-γ pathway genes; whereas only 4 mutations were detected in the 4 responders, which were all SNV

↳ This suggests that CNAs are the dominant genomic traits associated with anti-CTLA-4 therapy.
↳ The CNAs included loss of key pathway genes and amplification of IFN-γ pathway inhibitors.
- "The higher dose... induced greater immune-mediated cell killing" in vitro and mouse models
  ↳ Must be balanced with healthy cell toxicity
- In terms of local tumour control, larger, single-dose regimes are shown to be superior or at least equivalent to fractionated (low-dose) regimes.

July 15th

- High-dose fractionated regimes have been shown to result in enhanced, systematic anti-tumour responses compared to single-dose therapy.
- "It's possible that the systemic immune effects [...] may be more effective in the elimination of subclinical [minor] metastasis and perhaps our initial focus should be on those with more limited metastasis."
- Stereotactic: involving or being used in a surgical technique for precisely directing the beam of radiation in three planes using coordinates provided by medical imaging in order to reach a specific locus in the body
- Cytotoxic: Toxic to living cells
- "the striking feature of abscopal effects occuring in immunologically eminent tumor types like RCC (Renal cell carcinoma) and HCC (hepatocellular carcinoma)."
- "a concurrent administration of RT and IT is superior to sequential treatments."
- (Not sure if this is important but here it is) "The clinical data suggests that... a time frame of several months is needed before a clinical response can be detected."
- "Since the patients in the ipilimumab alone group were more likely to have been treated with IL-2 beforehand, it is possible that this more heavily pre-treated group was less likely to respond to immunotherapy. Other studies don't show this, so it appears not."

- "Total dose of radiation, dose per fraction, and timing in relation to ipilimumab could have an effect but there seemed to be no pattern of note... It is worth exploring these variables, however" July 18th
- Tumour Burden: The number of cancer cells, the size of a tumour, and/or the amount of cancer cells in the body.
- NON-Small Cell lung Cancer or (NSCLC): A group of cancers named for the kinds of cells found and how the look under a microscope. The three main types are:
  - ↳ Adenocarcinoma: Cancer that begins in the glands or secretory cells (produce & release mucus & other fluids). This is the most common type of lung cancer.
  - ↳ Squamous Cell Carcinoma: Cancer in the thin, flat cells that line the lungs (inside).
  - ↳ Large cell/Undifferentiated Carcinoma: Cancer that is composed of large, abnormally shaped cells.
- Radiation therapy: The use of high-energy radiation (x-rays, gamma rays, neutrons, protons, etc.) to kill cancer, shrink tumors, and stop it from spreading.
- Radiotherapy works by destroying cancer cells and damaging DNA so that it stops dividing & growing. It's most effective on cells that grow & divide quickly (like cancer cells).
  - ↳ External: aka external beam therapy, this is the most common type of therapy where a machine directs a beam of radiation to the tumour on the body.
  - ↳ Internal: A radioactive substance is put in the body (like on the tumor) to kill the cancer cells.
- Chemotherapy: Treatment utilizing drugs to kill cancer cells. "targets fast growing/dividing cells (which is why people can lose their hair on there) and usually work by damaging DNA or preventing mitosis.
- Immunotherapy: treatment utilizing substances to stimulate or suppress parts of the immune system in order to help the body fight cancer.

There are many types of immunotherapy:

- Monoclonal antibodies: A laboratory-made protein that can bind to substances in the body, including cancer cells. This alone can cause the immune system to see cancer cells as an intruder OR they can also be used to carry toxins, drugs, or radioactive substances directly to cancer cells.
- Immune checkpoint inhibitors: A type of monoclonal antibodies that attach to immune checkpoint proteins on cancer cells so that the body no longer sees the cancer as part of itself and attacks.
- Immune checkpoints are proteins that prevent the body from attacking itself. Some cancer cells have a lot of them (PD-1 or CTLA-4) and so confuse the immune system.
  - ↳ PD-1 stops T cells from attacking other cells in the body by attaching to PD-L1 (which is on the surface of cells) Nivolumab!

July 22nd

  - ↳ CTLA-4 is another one of these. ~~Pembrolizumab~~! Ipilimumab!
- Interferon: A cytokine that is typically produced by white blood cells (and others) to fight infection and disease. It can also be lab-made to fight cancer for a strong immune response.
- Granulocyte colony stimulating factor (G-CSF) and Granulocyte-macrophage colony stimulating factor (GM-CSF) Blood growth factors that stimulate the bone marrow to make more granulocytes and macrophages, but can also be given to boost the immune system, Sargramostim!
  - ↳ Granulocyte: A type of immune cell that has granules with enzymes that release during infections, allergic reactions, and asthma. A type of white blood cell.
  - ↳ Macrophage: A type of white blood cell that surrounds and kills micro-organisms, removes dead cells, and stimulates other immune cells.
- Cancer staging can use TNM and/or numerical staging.
- TNM or tumour, nodes, metastasis,
- T → The size and extent of the main tumour or primary tumour.

July 24th

- Primary Tumour (T)
  - TX: Main tumour cannot be measured
  - T0: Main tumour cannot be found
  - T1, T2, T3, T4: Refers to the size/extent of the main tumour. The higher the number, the larger/more grown it is into nearby tissues. letters (ie T3a or T3b) can be added to provide more information.
- N → The number of nearby lymph nodes that have cancer
- Regional lymph nodes (N)
  - NX: Cancer in nearby lymph nodes cannot be measured
  - N0: There is no cancer in nearby lymphnodes
  - N1, N2, N3: Refers to the number and location of lymph nodes that contain cancer. The higher the number, the more lymph nodes that contain cancer.
- M → Whether the cancer has metastasized.
- Distant Metastasis (M)
  - MX: Metastasis cannot be measured
  - M0: Cancer has not spread to other parts of the body
  - M1: Distant metastasis or cancer has spread to other parts of the body
- Other terms
  - In situ: Abnormal cells are present but have not spread to nearby tissue
  - Localized: Cancer is limited to the place where it started, with no sign that it has spread
  - Regional: Cancer has spread to nearby lymph nodes, tissues, or organs
  - Distant: Cancer has spread to distant parts of the body
- Numerical Staging
  - Stage 0: Abnormal cells are present but have not spread to nearby tissue. Not cancer but can become cancer
  - Stage I, II, and III: Cancer is present. The higher the number, the larger the tumor and the more it has spread to nearby tissues
  - Stage IV: The cancer has metastasized

- Cancer occurs when a genetic mutation / DNA damage causes the cell to refuse to die when it should and divide uncontrollably.

Types of Cancer

- Carcinoma: Cancer that begins in the skin or epithelium which lines or covers organs. This is the most common type of cancer.
- Sarcoma: Cancer that starts in the connective tissue such as bone, muscle, fat, etc.
- Melanoma: Cancer that starts in melanocytes (ie skin or eyes)
- Blood Cancers: Cancer in the blood that doesn't necessarily produce a tumour
  - ↳ Leukemia: Starts in the bone marrow where blood cells are made. There will not be a tumour, but this cancer produces abnormal blood & bone marrow.
  - ↳ Lymphoma: Starts in the lymphocytes (white blood cells) and causes abnormal lymphocytes that build up in the lymph nodes, vessels, bone marrow, etc.
  - ↳ Multiple Myeloma: starts in the plasma cells (white blood cell that produces antibodies). These abnormal plasma cells (myeloma cells) can cause tumours of the bones or other tissues.
- Antibodies are specialized proteins that travel through bodily fluids and in the blood stream to identify and defend against antigens. They recognize specific antigens by identifying specific areas on their surface called antigenic determinants. Once recognized, it will bind to the antigen to tag it as an intruder labeled for destruction.
- Lymphocytes are a type of white blood cell that determine the specificity of the immune response.
- The two main types are B and T lymphocytes. Both originate from the bone marrow and are initially similar. Some migrate to the Thymus (T cells) and others stay in the bone marrow (B cells). Most are short lived, but some can live for years as immunologic "memory"

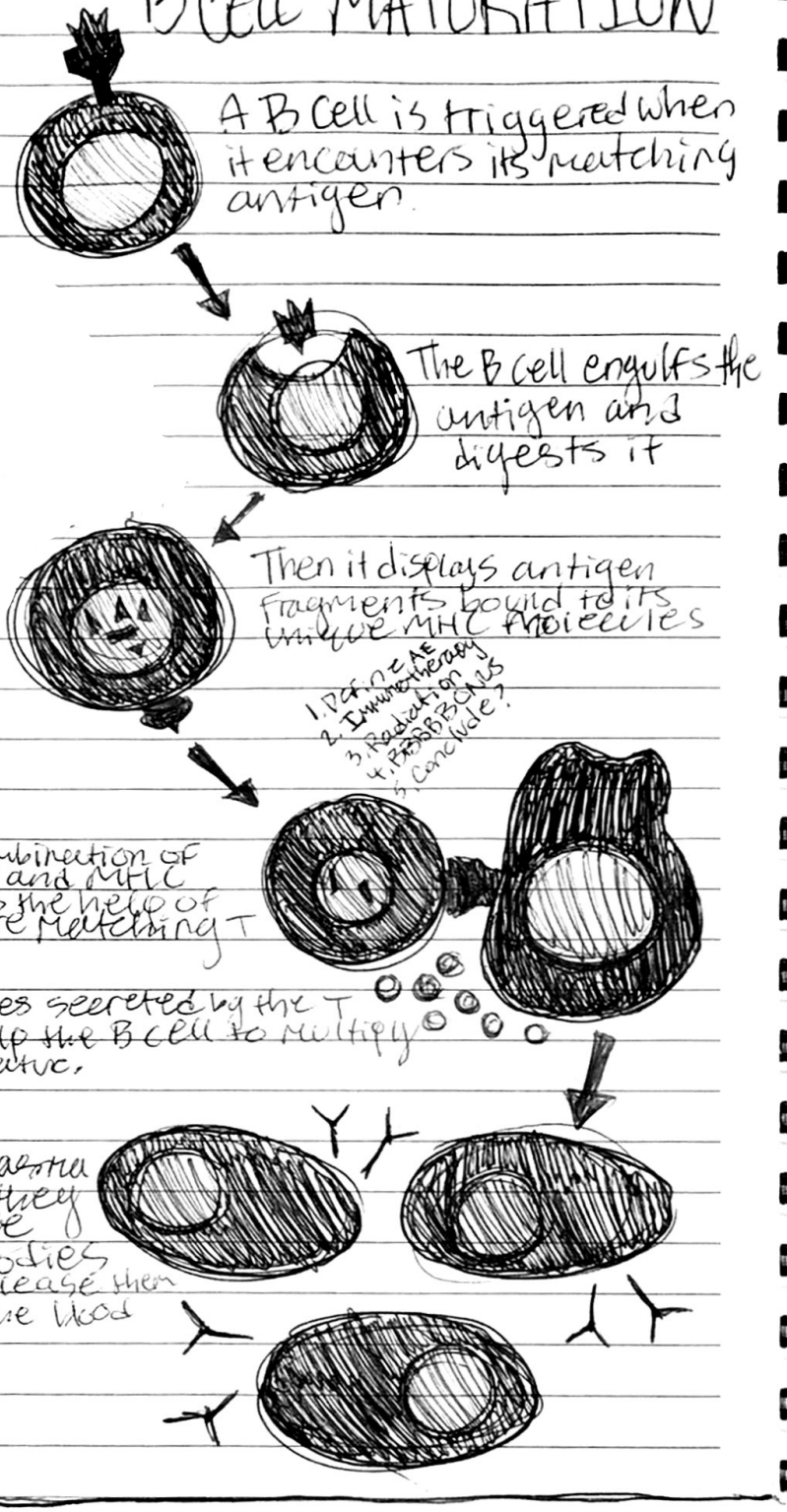
B

- Each lymphocyte has specific receptors for specific antigens. Once bound, the lymphocyte multiplies into identical clones. Some of the B cells differenciate into plasma cells and start producing antibodies. Some can become memory B cells
- In the thymus, T cells differeneiate into helper, regulatory, cytotoxic, or memory T cells
  - ↳ Helper T cells secrete cytokines once stimulated by the appropriate antigen (B cells → plasma cells)
  - ↳ Regulatory T cells control immune reactions
  - ↳ cytotoxic T cells bind to and kill infected/cancer cells
- Antigen: A substance capable of stimulating an adaptive immune response. This can include pollen, viruses, bacteria, etc.
- Neoantigen: An antigen to which the immune system has not previously been exposed to
- Immune Tolerance or Immunotolerance: The inability to give rise to a specific immune response to a specific antigen due to previous exposure
  - ↳ This tolerance is important so that disorders like autoimmune disease or food allergies don't occur.
- Innate Immune system: The first line defense against an antigen that depends on a group of molecules commonly found in pathogens but not in the host.
- It can take a while for the adaptive immune system to develop antigen-specific defenses, so the innate immune system is like the initial responders.
- Immunity occurs when, after the infection has ended, some activated T & B cells stay as memory cells, ready to activate if they encounter that antigen again.
- Immune tolerance occurs due to either the previous exposure of a substance or the recognition of self.
  - ↳ The latter is what cancer uses to trick the body
- The body recognizes itself by recognizing proteins on cell surfaces (like CTLA-4 or PD-1)

July 26th

- "The overall survival of patients who received radiotherapy with the mCH (Chemo) therapy regimine did not correlate with RT modality, dosage, or site of irradiation"
- "mCH alone vs mCH and RT did not show a significant difference in progression free survival."
- "The distribution of T-cells depends on the site of activation" (activated)
- "Activated T cell distribution is dependent on the anatomic distribution of metastatic sites, tumor volume of each metastasis, and site of activation." (from a math model not clinical trial)
- "[A smaller tumor burden] was associated with improved response and os. one rationale for [this] lies in T cell extinction."

B CELL MATURATION



- Lung cancer is the leading primary tumor that tends to metastasize to the brain.
- Thoracic: Relating to the Chest ¯\(ツ)/¯

Abscopal Effect

July 29th

- Localized Radiation Therapy (RT) induces cell death and the release of immunogenic factors through **Immunogenic Cell Death** (ICD)
  - ICD differs from the typical "silent Death" of cells that occurs everday through the release of DAMPs (Damage associated Molecular patterns) and in some cases PAMPs (Pathogen associated Molecular patterns).
- These DAMPs, like Calreticulin (used in maintaining Calcium levels and helping other proteins fold correctly), High-Mobility group box 1 Protein (HMGB1) (used in copying DNA into RNA or mRNA, inflammation, etc), and Adenosine Triphosphate (ATP) (used in cellular respiration) contribute to an immune response by triggering dendritic cells
  - Dendritic cells (DCs) are bone marrow-derived leukocytes responsible for the initiation of adaptive immune responses as they are the most potent antigen presenting cell.
  - Calreticulin is translocated to the surface of dying cells, stimulating DCs and production of Cytotoxic T lymphocytes.
  - HMGB1 acts as a pro-inflammatory Mediator, stimulating the production of many types of Cytokine
  - It also binds to DCs to stop the rapid degredation of antigens within.
  - Released ATP binds to Purine receptors on DCs, leading to inflammasome activation and the release of a cytokine.
- This immune reaction is often counterbalanced with the immunosuppressive effects of RT, which is why immunotherapy (with checkpoint inhibitors) is often necessary for the abscopal effect to occur

Sources

- www.ncbi.nlm.nih.gov/pmc/articles/PMC5346418/
  The Abscopal Effect of Radiation Therapy: What is it and How Can We Use it in Breast Cancer?

- www.ncbi.nlm.nih.gov/pmc/articles/PMC2818721/
  IMMUNOGENIC AND TOLEROGENIC CELL DEATH

- www.nature.com/scitable/topicpage/calreticulin-a-multifaceted-protein-14237270
  Calreticulin: A Multifaceted Protein

- www.ncbi.nlm.nih.gov/gene/3146
  HMGB1 high mobility group box 1 [Homo sapiens (Human)]

- www.immunology.org/public-information/bitesized-immunology/celulas/dendritic-cells
  Dendritic Cells

WWW.imgt.org/IMGTeducation/Tutorials/ImmuneSystem/UK/the_immune_system.pdf
Understanding The Immune System - How it Works
(U.S. Department of health and human services)

July 30th

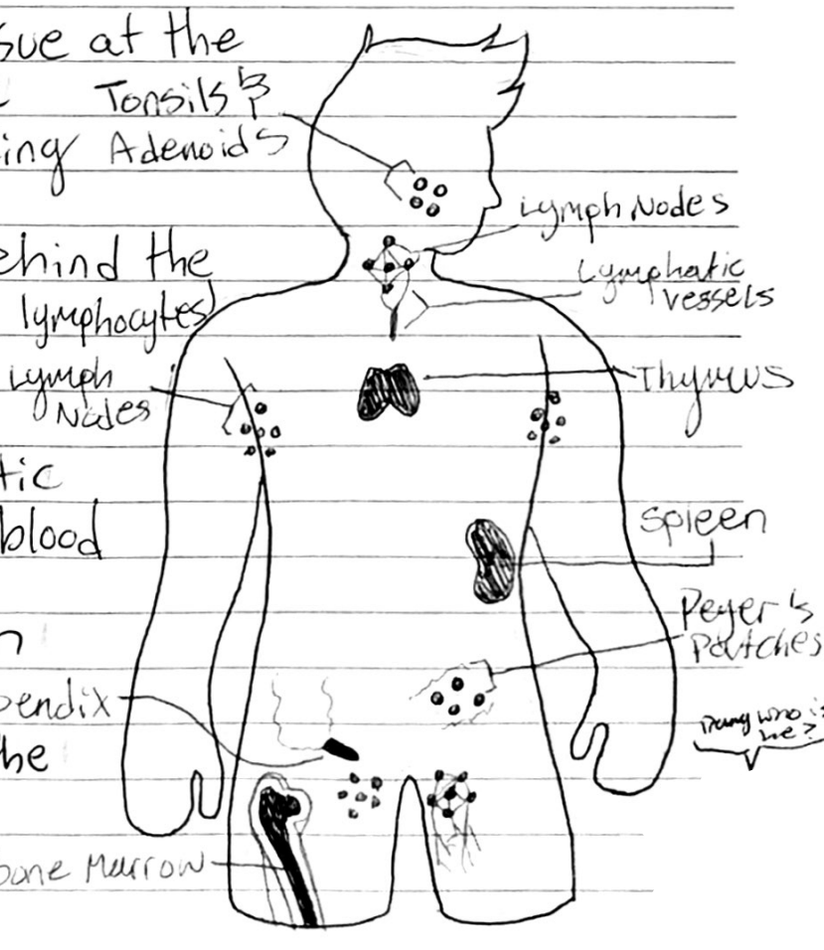
Immune System

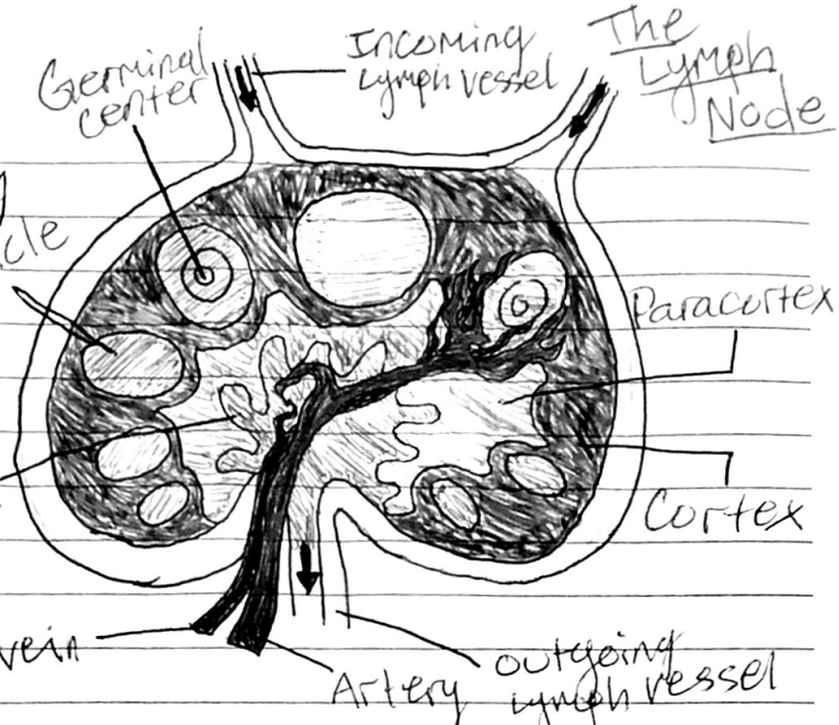
Self vs Nonself

- The key to a healthy immune system is the ability to distinguish between the body's own cells and foreign ones - AKA Self vs Nonself
- Cells carry distinctive markers on their surface (Ex: PD-1 and PD-L1, CTLA-4) that are recognized as "self"
- When immune cells see a foreign organism - denoted by its foreign markers - it launches an attack.
- Antigen - Anything that can produce an immune response
  - This can be a microbe, PARTS of a microbe, or even cells from another person (ie - blood types)
- Autoimmune disease stems from the mistaking of self for nonself. Allergy is when a harmless substance is mistaken for something dangerous, causing an immune response.

Structure of the Immune System

- The organs of the immune system are positioned throughout the body, called the lymphoid organs, home to lymphocytes, small white blood cells that are key in the immune system.
- Bone Marrow is the soft tissue at the centre of the bones, and is the source of all blood cells, including white blood cells.
- The Thymus is an organ behind the breastbone. Lymphocytes (T lymphocytes) mature here.
- Lymphocytes can travel through blood vessels or lymphatic vessels which closely parallel blood vessels.
- Cells and fluids pass between these vessels, allowing the lymphatic system to monitor the body for antigens.





- Lymphatic vessels carry Lymph - a clear fluid that bathes the body's tissues
- The lymph nodes are strung along the lymphatic vessels, within the neck, armpits, abdomen, and groin.
- Immune cells and antigens enter the lymph nodes via lymph vessels, or blood vessels.
- Lymphocytes exit ONLY through lymph vessels. They patrol the body for antigens, and then gradually drift back to the lymph nodes,

T-cells concentrate in the paracortex, B-cells in and around germinal centers, and Plasma in the medulla

- The spleen contains special structures where immune cells meet and work, and serves as a meeting ground for antigens and immune cells.
- Lymphoid tissue is also present in the lining of organs, like the lungs and digestive track, as this is where many substances enter the body. These include the tonsils, adenoids, and appendix.

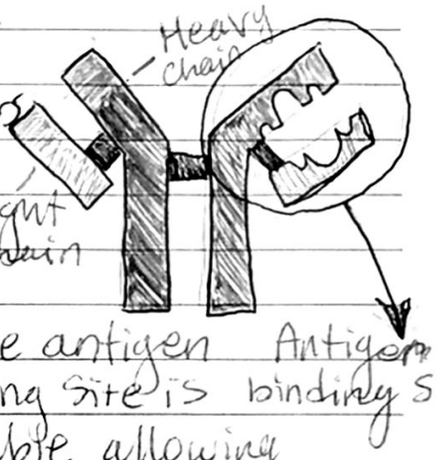
Immune Cells and Their Products

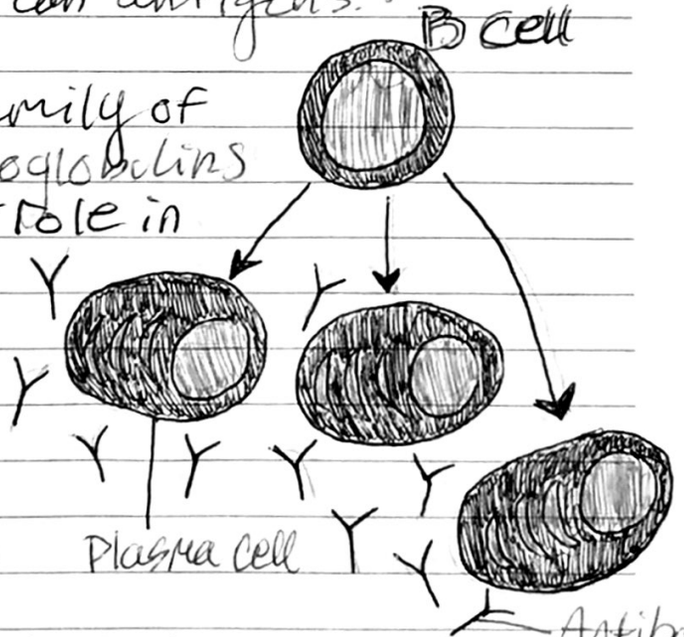
- Some immune cells take on all comers, while others are specialized.
- Immune cells work most efficiently together, communicating through direct physical contact or the release of chemical messengers.
- The immune system stores a few of each type of immune cell. When an antigen appears, the matching cells divide into an army. After the antigen is dealt with, their numbers fall and few are left behind to watch for future invasions.

- All immune cells start as stem cells in the bone marrow.
- In response to different cytokines and other signals, these cells grow into different immune cells, like T cells, B cells, and Phagocytes

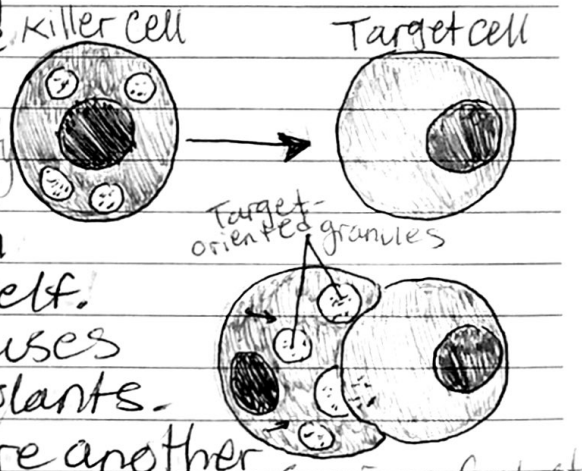
B Lymphocytes

- B & T cells are the main types of lymphocytes
- B cells are responsible mostly for secreating antibodies, which ambush antigens in the bloodstream and mark them for destruction by other immune cells.
- Each B cell is programmed to make one specific antibody. When it encounters its antigen, it gives rise to many large cells called Plasma cells.
- These cells produce antibodies en mass and secreate them into the bloodstream.
- Antigens match with antibodies like keys to a lock — they don't always match exactly, but can still interlock.
- Antibodies are part of a family of large molecules called immunoglobulins which each play a different role in immune defense.
- Immunoglobulin G (IgG) efficiently coats microbes, speeding up their uptake by other immune cells.
- IgM is very effective at killing bacteria
- IgA concentrates in bodily fluids (tears, saliva, etc.) to protect & guard entrances to the body.
- IgE, which is naturally supposed to protect against parasites, is responsible for allergies & allergic reactions.
- IgD remains attached to B cells and is key in initiating an early B cell response.





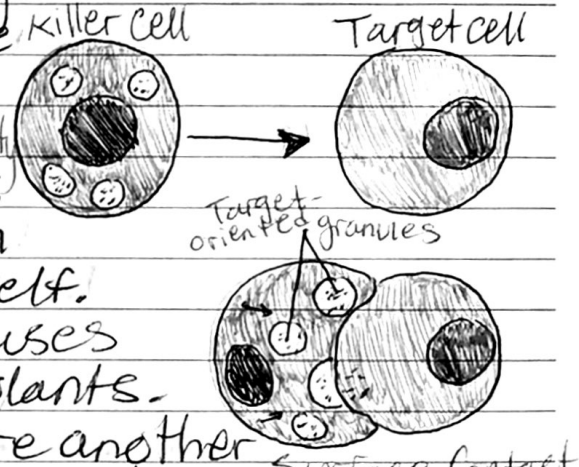
- **T cells**
  - T cells do not recognize free floating antigens, but rather contain antibody-like receptors that recognize antigen fragments on the surface of infected or cancerous cells.
  - T cells either direct to regulate immune responses or directly attack target cells.
  - Helper T cells coordinate immune responses by communicating with other cells. They stimulate nearby B cells to produce antibodies, call in phagocytes, ~~stim~~ activate other T cells, and more.
  - Killer T cells - or Cytotoxic T Lymphocytes (CTLs) - directly attack cells carrying foreign or abnormal molecules on their surfaces.
    - ↳ They are especially good at killing viruses, which hide from other immune cells in infected cells. CTLs recognize their fragments in the cell membrane.
  - In most cases, T cells only recognize antigens if they are carried on the surface by the body's own Major histocompatibility Complex (MHC). These proteins are recognized by T cells when distinguishing self from non-self.
    - ↳ MHC Molecules are what causes difficulty in organ/blood transplants.
  - Natural killer (NK) cells are another kind of lymphocyte/white blood cell. They are similar to killer T cells in that they contain granules filled with potent chemicals to kill.
  - The main difference is that NK cells recognize cells lacking MHC molecules rather than with foreign molecules, making them very versatile.
  - Both killer cells kill on contact, delivering a burst of chemicals.



- **Phagocytes and more**

July 31st

- **T Cells**
  - T cells do not recognize free floating antigens, but rather contain antibody-like receptors that recognize antigen fragments on the surface of infected or cancerous cells.
  - T cells either direct & regulate immune responses or directly attack target cells.
  - Helper T cells coordinate immune responses by communicating with other cells. They stimulate nearby B cells to produce antibodies, call in phagocytes, ~~stimulate~~ activate other T cells, and more.
  - Killer T cells - or Cytotoxic T Lymphocytes (CTLs) - directly attack cells carrying foreign or abnormal molecules on their surfaces.
    - ↳ They are especially good at killing viruses, which hide from other immune cells in infected cells. CTLs recognize their fragments in the cell membrane.
  - In most cases, T cells only recognize antigens if they are carried on the surface by the body's own Major histocompatibility Complex (MHC). These proteins are recognized by T cells when distinguishing self from non-self.
  - ↳ MHC Molecules are what causes difficulty in organ/blood transplants.
  - Natural Killer (NK) cells are another kind of lymphocyte/white blood cell. They are similar to Killer T cells in that they contain granules filled with potent chemicals to kill.
  - The main difference is that NK cells recognize cells lacking MHC molecules rather than with foreign molecules, making them very versatile.
  - Both Killer cells kill on contact, delivering a burst of chemicals.



- **Phagocytes and more**

July 31st

- Phagocytes are large white blood cells that can swallow and digest microbes and other large foreign particles.
- Monocytes are phagocytes which circulate the blood. When they migrate into tissues, they become macrophages, which can be specialized in many organs (ie brain, liver, lungs, etc).
- Macrophages play many roles. They rid the body of worn-out cells and other debris. They display bits of antigens to attract the attention of T lymphocytes, and then churn out a variety of chemical signals (monokines) which are necessary for immune responses.
- Granulocytes contain granules filled with potent chemicals to destroy microorganisms. Some of these chemicals (like histamine) contribute to inflammation and allergy.
- One type of granulocyte, neutrophils, is also a phagocyte. it ingests microbes and uses its packaged chemicals to break it down.
- Eosinophils and basophils "degranulate" and spray their chemicals on the target organism.
- Mast Cells are similar to basophils. They are present in the lining of organs and responsible for allergic reaction symptoms.
- Blood Platelets are cell fragments that contain granules and are responsible for clotting, wound repair, and activating some immune responses.

Cytokines

- Components of the immune system communicate by exchanging chemical messengers called Cytokines. These proteins are secreated by one cell and acted on another to coordinate an appropiate immune response.
- Cytokines include interleukins, interferons, growth factors and chemical switches to turn certain immune cells on & off.

- One cytokine, interleukin 2 (IL-2), causes the immune system to produce T cells.
  - ↳ This property is promising in the use for treating diseases like hepatitis C, cancer, and HIV/AIDs.
- Other cytokines chemically attract specific cell types. These chemokines are released by cells at sites of injury/infection and call immune cells to the region.
  - ↳ Chemokines are key in inflammation and are a promising target for new drugs.

Complement

- The Complement System is made up of about 25 proteins that assist antibodies in destroying bacteria and in ridding the body of antibody-coated antigens.
  - ↳ Complement proteins cause the symptoms that characterize an inflammatory response.
- Complement proteins circulate the blood in an inactive form until the first in the complement series is activated (usually by an antibody locked on an antigen) which sets off a domino effect called the Complement cascade.
- In the end, these compliment proteins come together and form a cylinder, which, when inserted into a cell's membrane, causes it to swell and burst.
- Other components of the complement system make bacteria more susceptible to phagocytosis or beckon other cells to the area.

Immunity: Natural and Acquired

- Some activated T and B cells become memory cells, allowing the body to be better prepared to fight a reoccuring illness.

- How long immunity lasts for depends on the type of antigen, the amount of antigen, and the route in which it enters the body
- Immunity is also affected by inherited genes- dictating how forceful a response may be

Immune Tolerance

- Immune Tolerance is the tendency of the immune system to ignore the body's own tissues.
- Central Tolerance occurs during lymphocyte development.
  - Lymphocytes with receptors for self-antigens are destroyed at an early stage of development through apoptosis. This process is called clonal deletion.
- Peripheral Tolerance occurs after the self-reactive lymphocytes have entered the bloodstream.
  - These lymphocytes can be "turned off" through the lack of certain signals. This leaves them unreactive through the induction of anergy
  - Regulatory T cells can also stop them from being activated by self-antigens.
    - Regulatory T cells (TREG) regulate & suppress activation, proliferation, and cytokine production.

SOURCES

- www.imgt.org/IMGTeducation/Tutorials/ImmuneSystem/UK/the_immune_system.pd
- www.astro.org/Patient-Care-and-Research/Research/Professional-Development/Research-Primers/Central-vs-Peripheral-Tolerance
- www.immunology.org/public-information/bitesized-immunology/cells/regulatory-t-ce-tregs

August 1st

Adaptive vs Innate Immune system

Innate Immunity: The nonspecific defense mechanisms that come into play immediately or very quickly when an antigen appears in the body. It is activated by the chemical properties of the antigen.

↳ These mechanisms include physical barriers like the skin, Chemicals in the blood, and immune cells that attack foreign cells.

Adaptive Immunity: The antigen-specific response that is much more complex than the innate. The antigen must be processed and recognized. Afterwards, an army of specifically designed cells is created to efficiently attack the antigen. Lastly, memory cells are created in preparation for the future.

↳ Lymphocytes, T cells, B cells, phagocytes, etc.

www.biology.arizona.edu/immunology/tutorials/immunology/page3.html

[Emailed paper (PDF) titled Immunomodularity Effects of Stereotaletic Body Radiation Therapy: Preclinical insights and Clinical Opportunities]

Page 13 → Second last Paragraph
Page 15 → All

- Metastasis: The Spread of cancer throughout the body.
- Metastasis can occur three different ways
  - ↳ The primary can grow directly into the surrounding tissue
  - ↳ Travel through the blood stream
  - ↳ Through the lymph vessels

cleveland clinic

Concepts

~~-Interferon~~
~~-PD-1, PD-L1~~
~~-CTLA-4~~
-EGFR and ~~ALK~~ → Q cases

Question

If a Patient with NSCLC, treated with Immunotherapy, has any EGFR Mutations in the tumour, will the outcome of an abscopal effect be impacted?

Variables

Independent → EGFR Mutations (exon 19, 21)
dependent → Abscopal effect (How do you quantify this?)
↳ Overall Survival
↳ Progression Free Survival
↳ Metastasis
Controlled → Where Patients are treated (Calgary + Edmonton)
→ Lung Cancer type (NSCLC) ← Adenocarcinoma
→ Lung Cancer Primaries (Only one)
Confounding → Lots! (Human body is a system, complex)
→ Amount & type of RT
→ Age (18+)
→ Stage of Cancer (I, II, III, IV)

Hypothesis

If a patient with NSCLC with EGFR Mutations, ← treated with Immunotherapy is the abscopal effect is less likely to occur. EGFR patients have reduced survival, increased lymph node metastasis, and insensitivity to chemo. EGFR-specific treatments also quickly lose efficacy due to gained resistance.

Data Collection

- Through ARIA-MO
  - ↳ Oncology Information System
- Biopsy Results, CT Scan Results, Referals
  - → Most info on mets

339 Patients → 239 (100 did not have adenocarcinoma)

226 EGFR−, 13 EGFR+

Demographics

variables	Freewency	percent
Female	133	55.6
male	106	44.4
smoked	213	89.1
Neversmoked	24	10
Unknown	2	0.8
STAGE IA	7	2.9
IB	9	3.8
IIA	1	0.4
IIB	7	2.9
IIIA	21	8.8
IIIB	36	15.1
IV	157	65.7
EGFR−	226	94.6
EGFR+	13	5.4
PDL1 −	125	52.3
PDL1 +	114	47.1
Recurrence No	217	90.8
Recurrence yes	22	9.2
No Radiation	60	25.1
Reediation	179	74.9
systemic TX	239	100

A B C

Mets: Brain, adrenal gland, Renal, Bone, liver, intrathoracic

D

Other

Statistics (Example Calculations)

mean: Average, middle value of a dataset
- Male: 172, Female: 160, All: 165.8

Heights (cm)	
Male	Female
180	160
170	156
165	166
175	161
170	157

Standard Error: How reliable a sample mean is as an approximation of the population mean (How reflective is it)

$$SE = \sqrt{\frac{\sum_{i=1}^{n}(x_i - \bar{x}_{mean})^2}{n-1}} \div \sqrt{n} \qquad SE = \sqrt{\frac{(8)^2 + (-2)^2 + (-7)^2 + (3)^2 + (-2)^2}{4}} \div \sqrt{5}$$

$$\boxed{SE = 2.55\,cm}$$ → shows how much calculated mean can vary within actual population's mean

Standard Deviation: Average distance from the mean

$$SD = \sqrt{\frac{\sum_{i=1}^{n}(x_i - \bar{x})}{n-1}} \qquad SD = \sqrt{\frac{64 + 4 + 49 + 9 + 4}{4}}$$

$$\boxed{SD = 5.70\,cm}$$

95% Confidence Interval → True mean (95%) is between these values

$$\bar{x} \pm Z\frac{SD}{\sqrt{n}} = Ci \qquad Ci = 172 \pm (1.960\ \frac{5.70}{\sqrt{5}})$$

(Z-value)

$$\boxed{Ci = 172 \pm 4.997}$$

Lower Bound	Upper Bound
167.00	176.997

Confidence Interval	Z
90%	1.645
95%	1.960
99%	2.576

Students T-test (P-value)

Compares means of 2 samples to determine if the difference between groups is significant enough to be found in separate Populations

Null Hypothesis (Hypothesis to disprove to support actual hypothesis): There is no difference between male and female heights

Test Statistic (Raw difference between means with Standard Errors taken into account)

Alternative Hypothesis (actual hypothesis): There is a difference between male and female heights.

$$t = \frac{\bar{X}_1 - \bar{X}_2}{\sqrt{\frac{SD_1^2}{n_1} + \frac{SD_2^2}{n_1}}} \qquad t = \frac{172 - 160}{\sqrt{\frac{5.10^2}{5} + \frac{3.94^2}{5}}}$$

$$\boxed{t = 3.87}$$

Degree of Freedom: Number of values that need to be known in order to know all of the values

$$n_1 + n_2 - 2 = df \qquad 5 + 5 - 2 = df$$

$$\boxed{df = 8}$$

P-levels: (0.05) The probability of mistakenly rejecting the null hypothesis when it is true

Critical T-Value

Degrees of Freedom	level of Probability (0.05)
8	±2.31

As the observed T value is before than the critical T-value, the probability that the variation is due to chance is less than 5%.

P Value from Chart → $0.0249 < 0.05$ ✓ Significant!

Chi-Square: For frequency (categorical) data. How much difference exists between the observed counts and the counts one would expect if the null hypothesis was true

Do the amount of Brown vs spotted cows fit the expected frequency? (50/50 chance of birthing a brown cow)

Null hypothesis: The Brown and spotted cows are equally represented in this sample

Brown	spotted
13	7

Expected value (based on known frequency): 10 brown 10 spotted

Observed chi-square (X^2) value: How much the observed counts deviates from the expected:

$$X^2 = \sum_{i=1}^{n} \frac{(O_i - E_i)^2}{E_i}$$

(O_i: observed frequency; E_i: expected frequency)

$$\frac{(13-10)^2}{10} + \frac{(7-10)^2}{7} = X^2$$

$$\boxed{X^2 = 1.8}$$

Degrees of Freedom: With 1 variable, it is the number of categories (1)

dF	Probability level (0.05)
1	3.84

When the observed X^2 value (1.80) is less than the critical value (3.84), the difference is due to chance alone and so the null hypothesis is accepted.

P value from Chart → $0.179 > 0.05$ X Not Significant