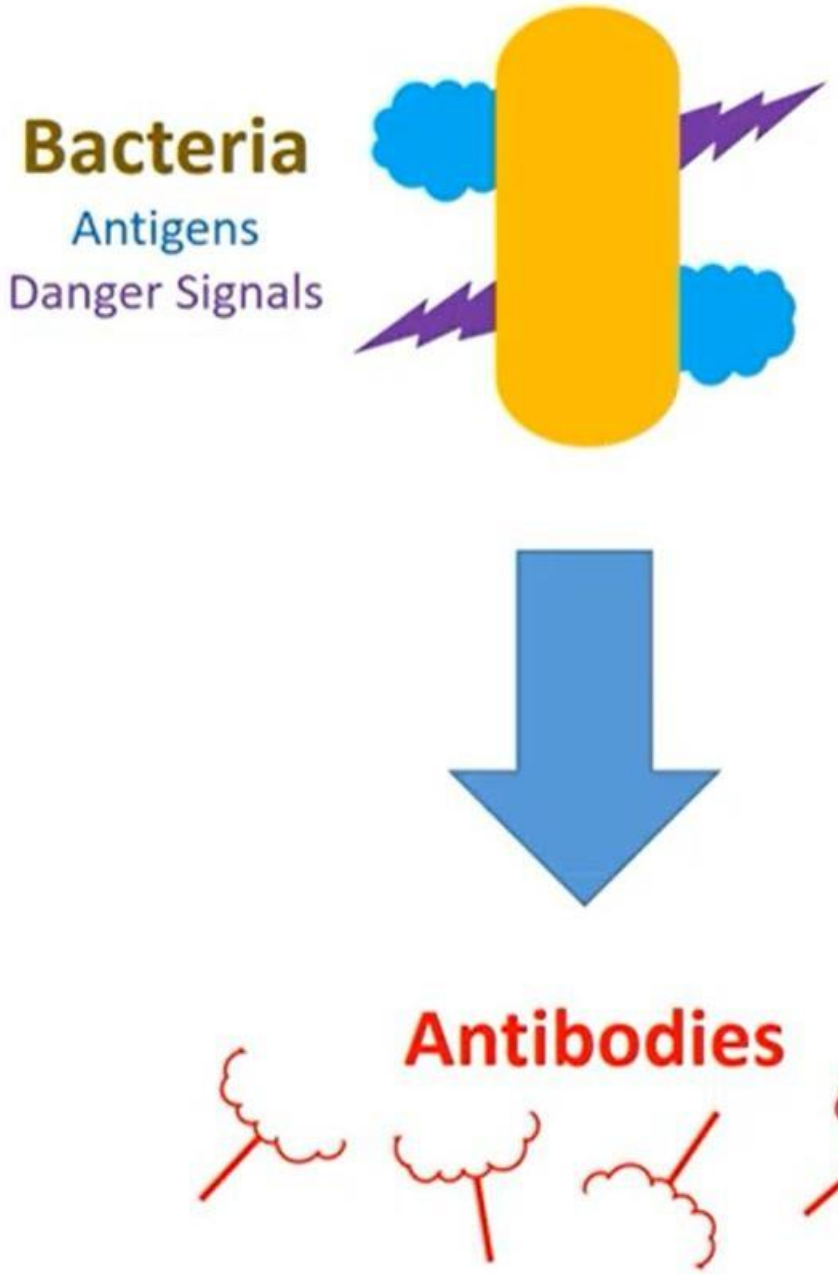
Engineered immune cells for cancer therapy

Immunity



Body makes antibodies

Antigen combined with Danger Signals

Our immune system has the ability to react with enormous and diverse foreign antigens and neutralizes them

WHILE

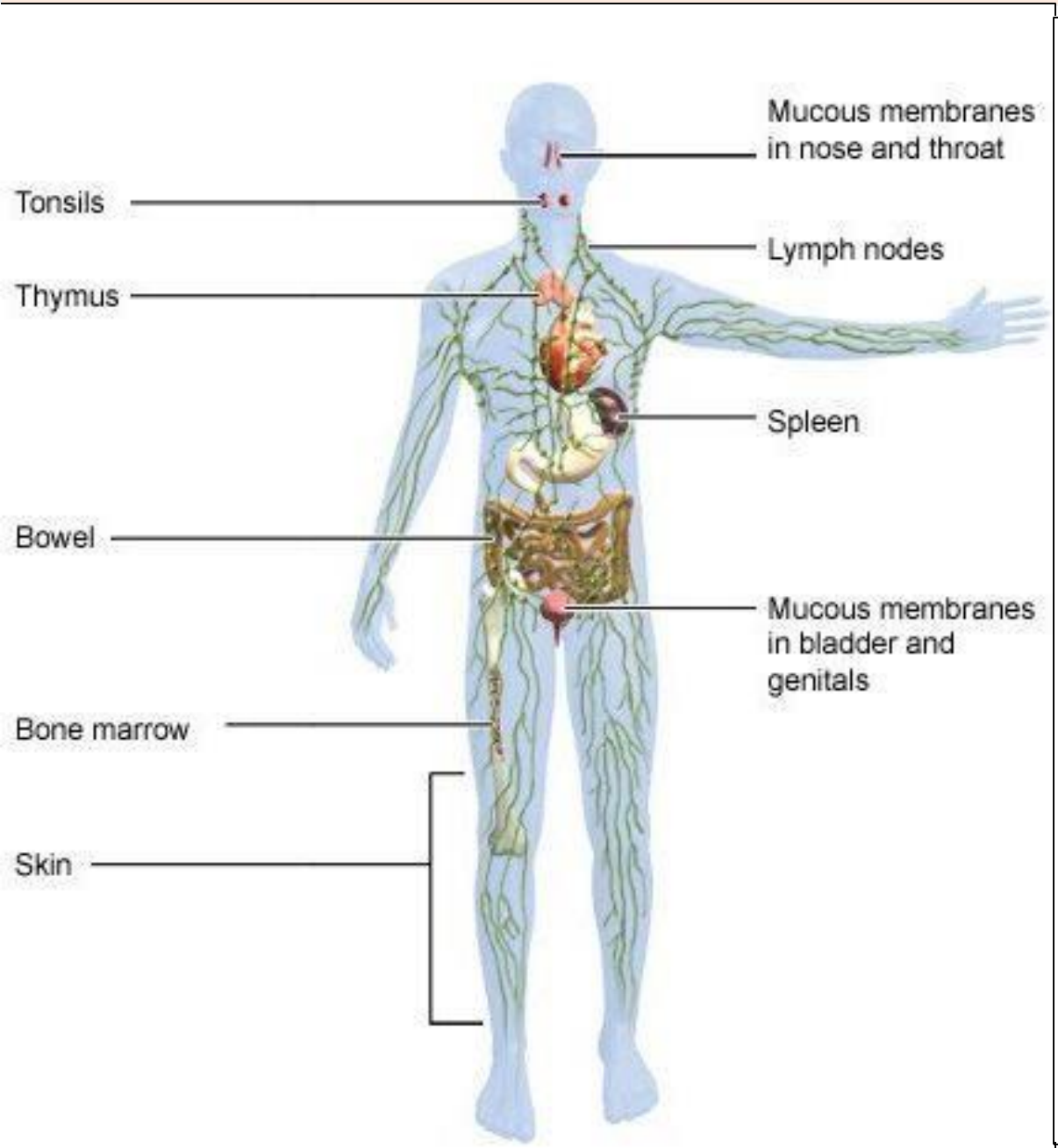
it is tolerant to self-antigens

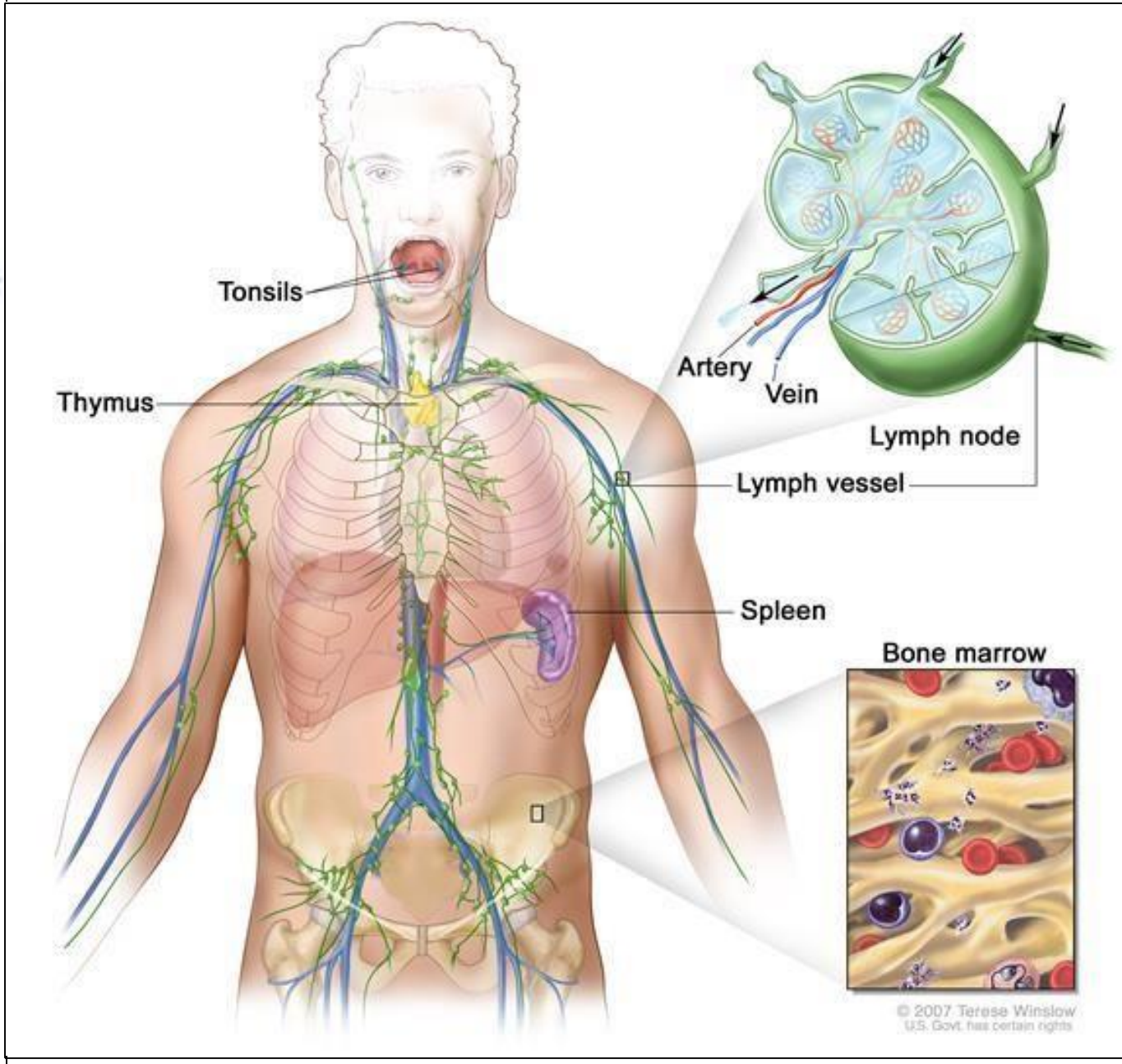
This means our immune system decides a "friend (self) from foe (foreign)"

Our body recognizes and accepts self-antigens

Does not attack them

Components of the Immune System – Organs and Tissues



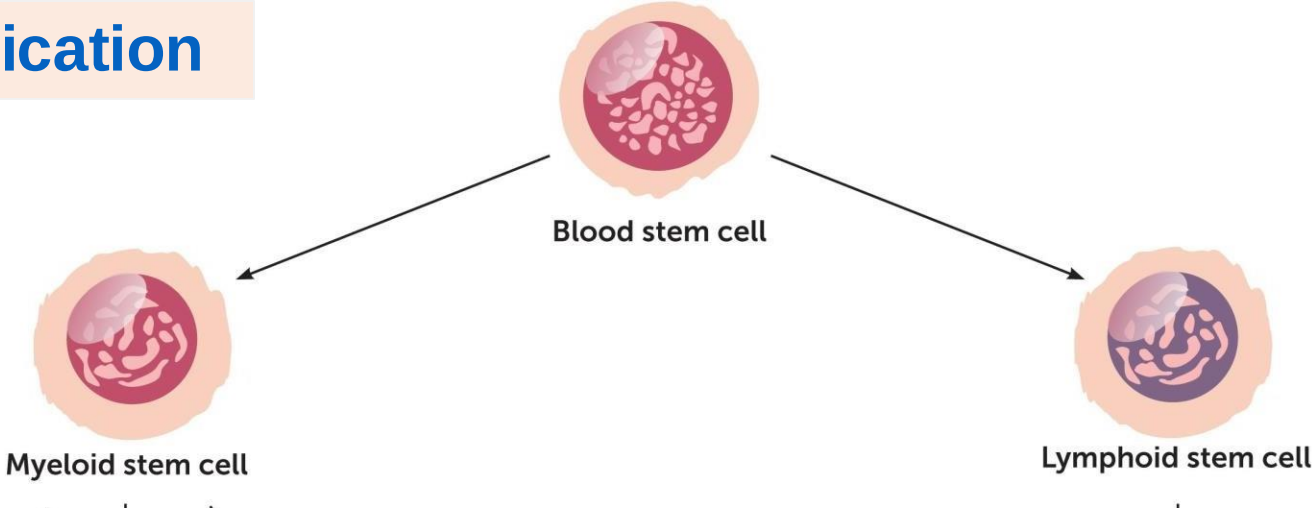


Components of the Immune System – Organs and Tissues

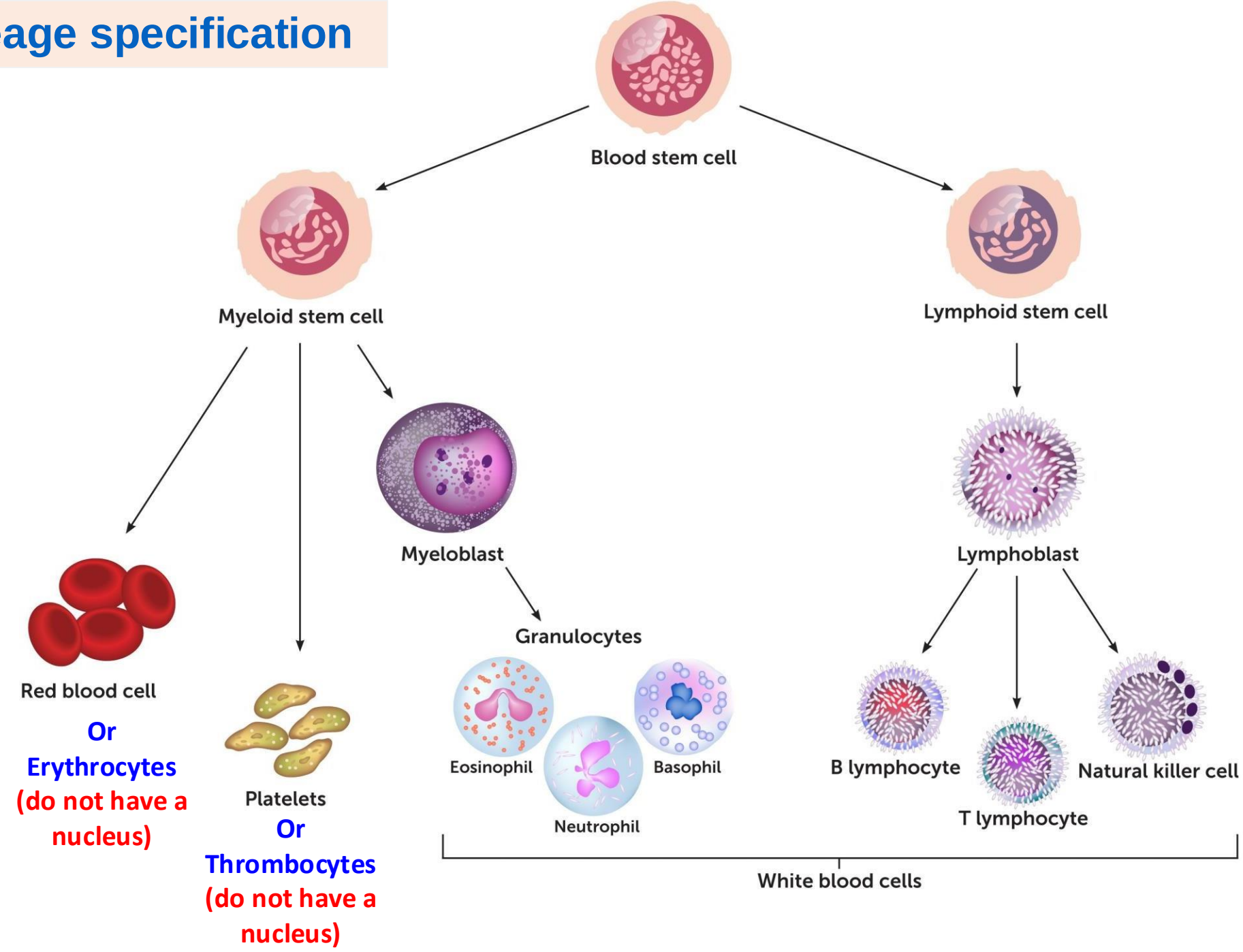
- The immune system consists of many parts that work together to defend the body against invaders.
- The primary parts of the immune system include the bone marrow and thymus.
- The bone marrow is extremely important to the immune system because all the body's blood cells (including T and B lymphocytes) originate in the bone marrow.
- B lymphocytes remain in the marrow to mature, while T lymphocytes travel to the thymus.
- The thymus is responsible for producing the hormone thymosin, which in turn aids in the production of T cells. While in the thymus, T cells multiply, acquire different antigen receptors, and differentiate into helper T cells and cytotoxic T cells. Various proteins (e.g., CD4, CD8) are expressed on the T cell surface. The thymus will have produced all the T cells an individual needs by puberty.
- After the T and B lymphocytes have matured in the thymus and bone marrow, they then travel to the lymph nodes and spleen where they remain until the immune system is activated. Lymph nodes are located throughout the body.
- The spleen is located in the upper left area of the abdomen, behind the stomach, and under the diaphragm. The main function of the spleen is to filter the blood. Healthy red blood cells easily pass through the spleen; however, damaged red blood cells are broken down by macrophages (large white blood cells specialized in engulfing and digesting cellular debris, pathogens and other foreign substances in the body) in the spleen. The spleen serves as a storage unit for platelets and white blood cells. The spleen aids the immune system by identifying microorganisms that may cause infection.
- In addition to the lymph nodes and spleen, mucosal associated lymphoid tissues (MALTs) and gut associated lymphoid tissues (GALTs) play a vital role in the immune system, although they are considered to be part of the lymphatic system. MALTs are lymphoid tissues found in parts of the body where mucosa is present, such as the intestines, eyes, nose, skin and mouth. They contain lymphocytes and macrophages that defend against pathogens attempting to enter from outside the body. GALTs are lymphoid tissues found in the mucosa and submucosa of the gastrointestinal tract, tonsils, appendix and Peyer's patches in the small intestine.

Blood lineage specification

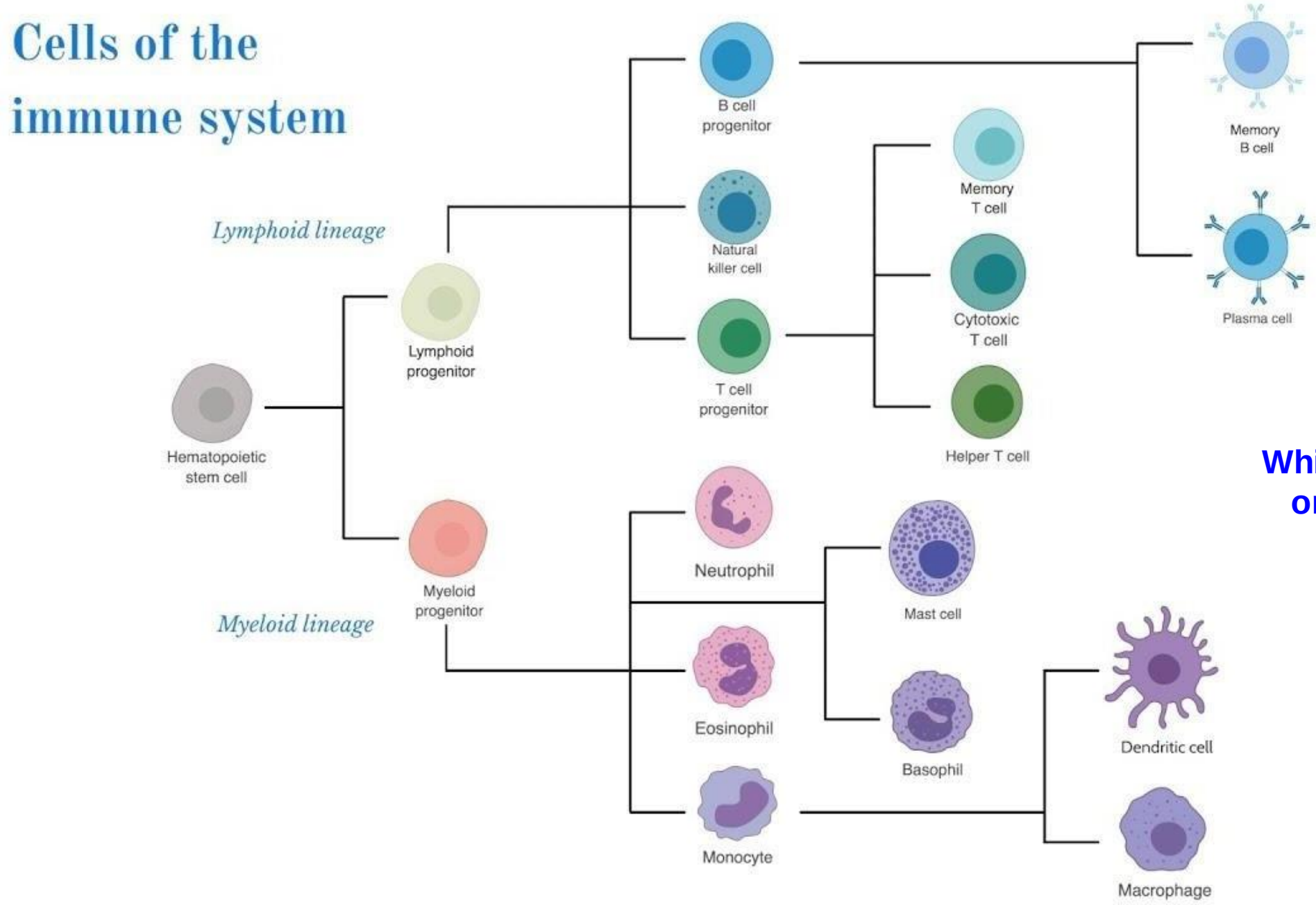
Blood lineage specification



Blood lineage specification

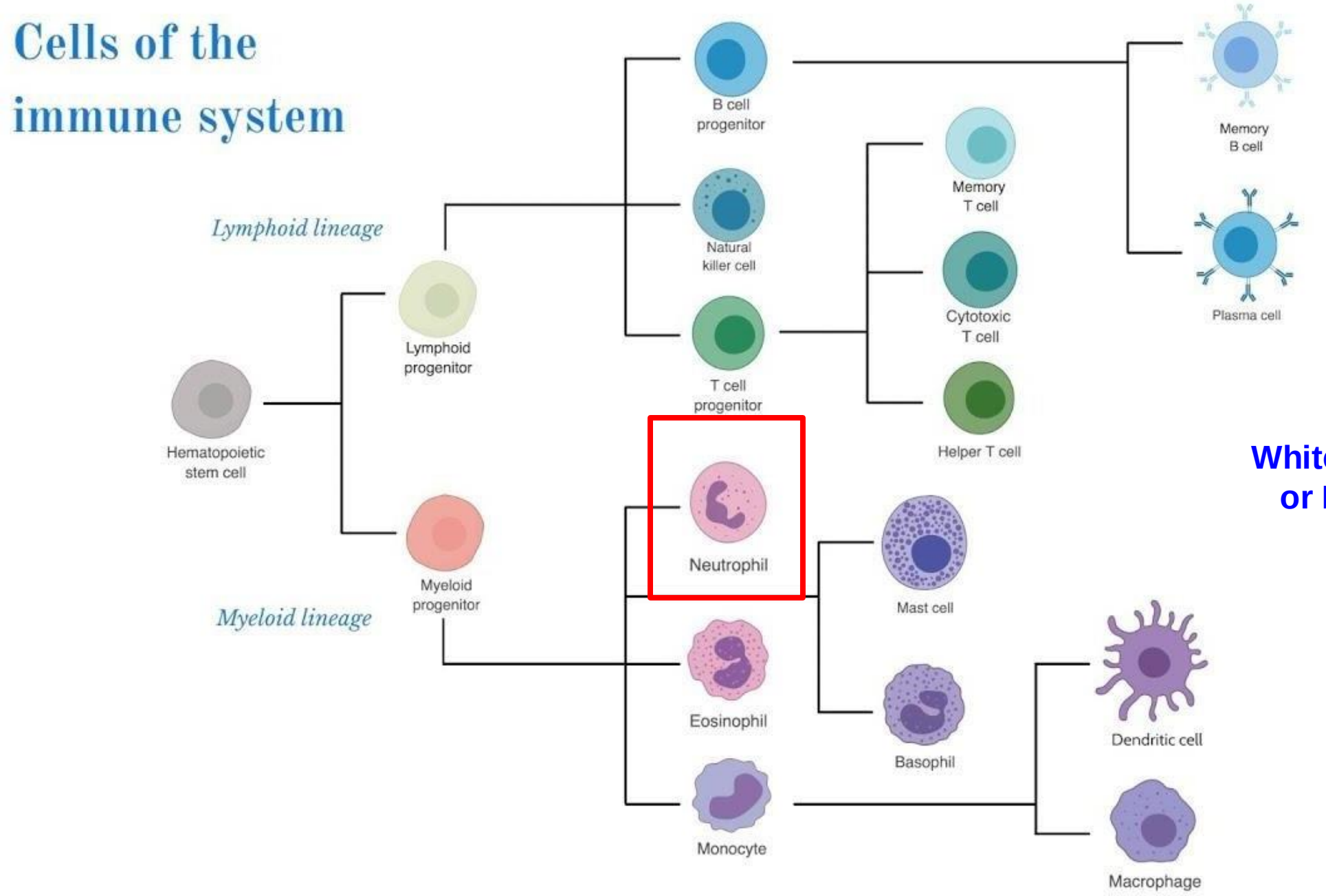


Components of the Immune System – Immune Cells



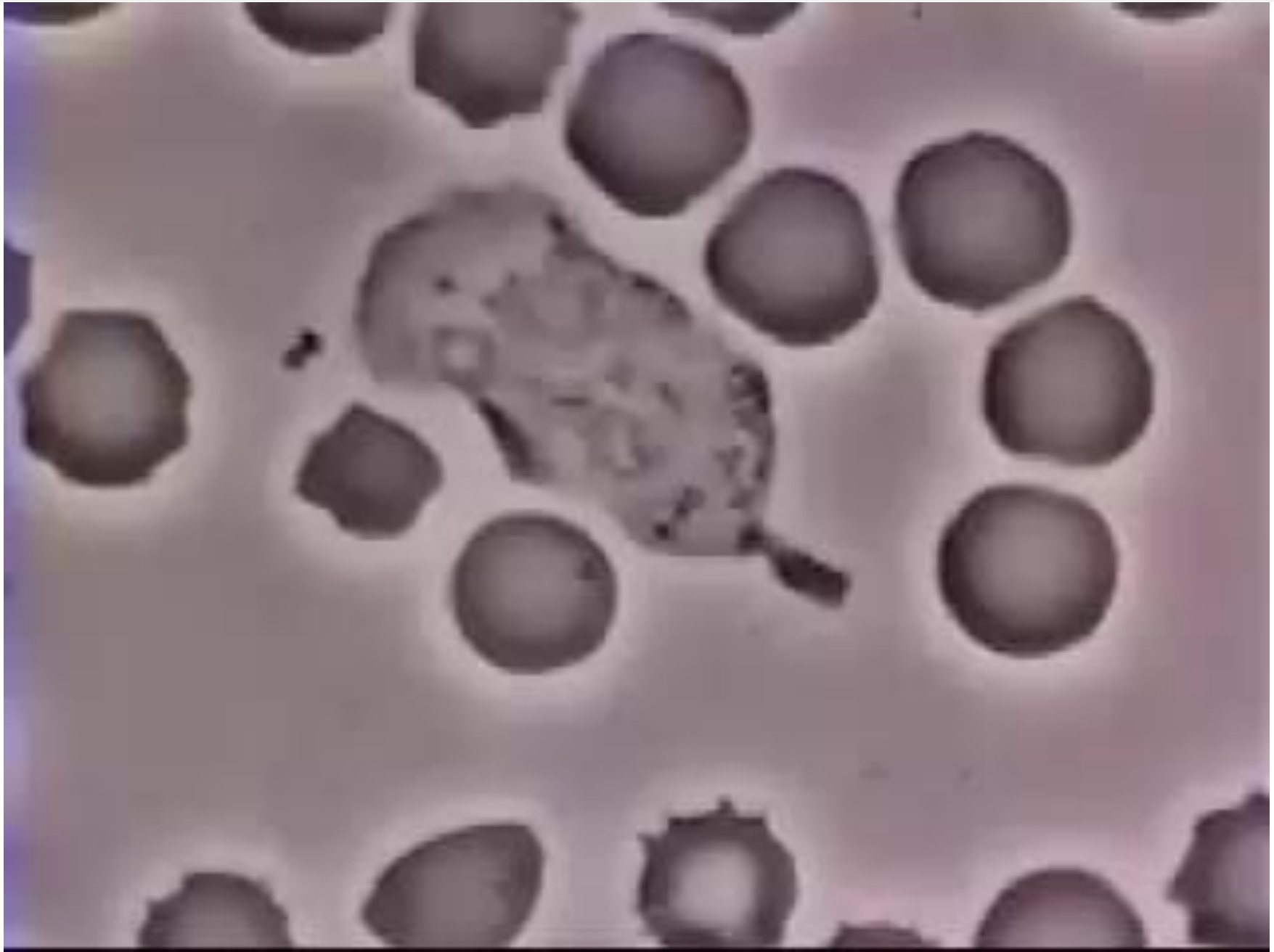
White Blood Cells or Leuckocyte

Components of the Immune System – Immune Cells



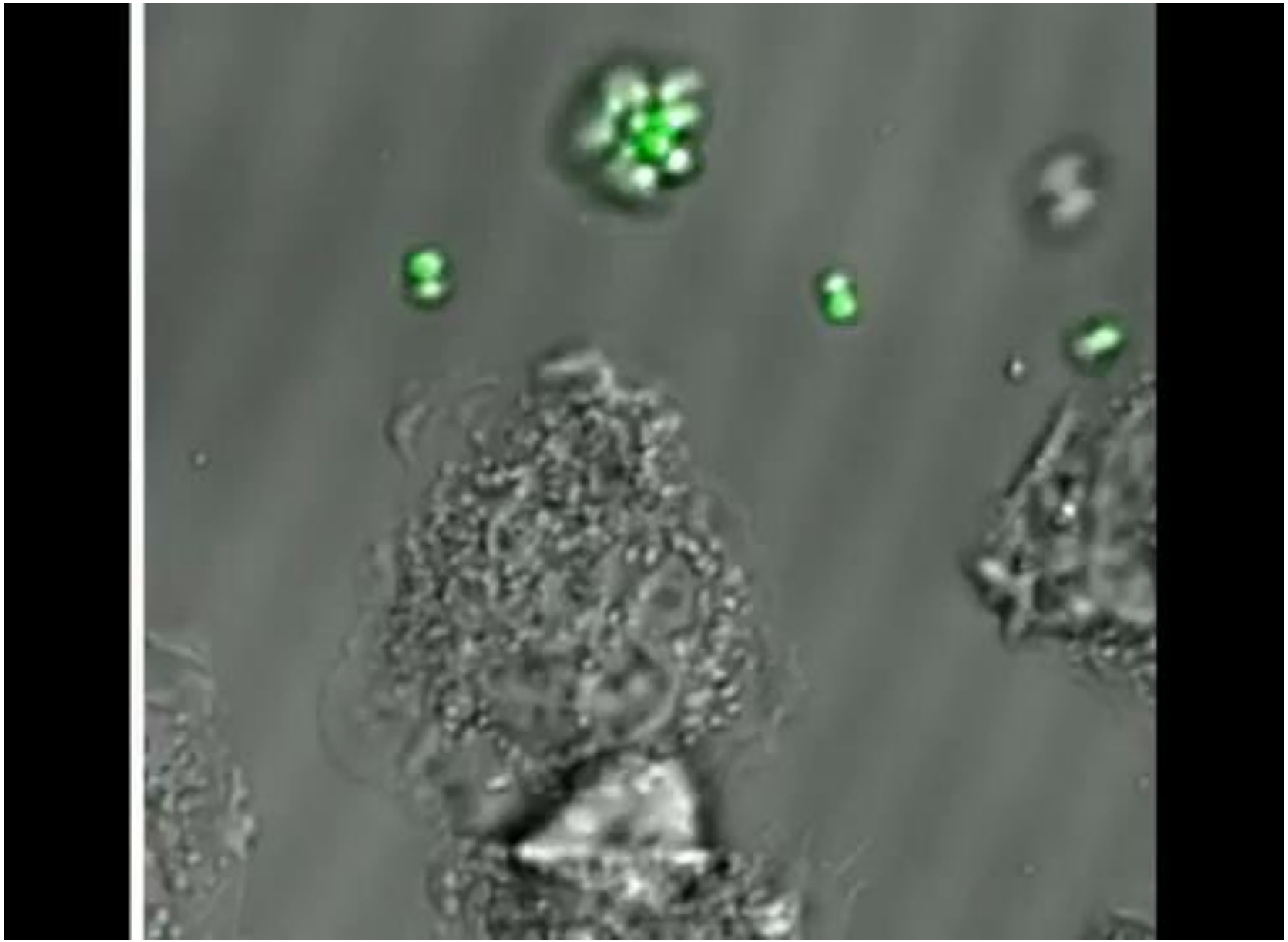
White Blood Cells or Leuckocyte

White Blood Cell (Neutrophil) Chases Bacteria

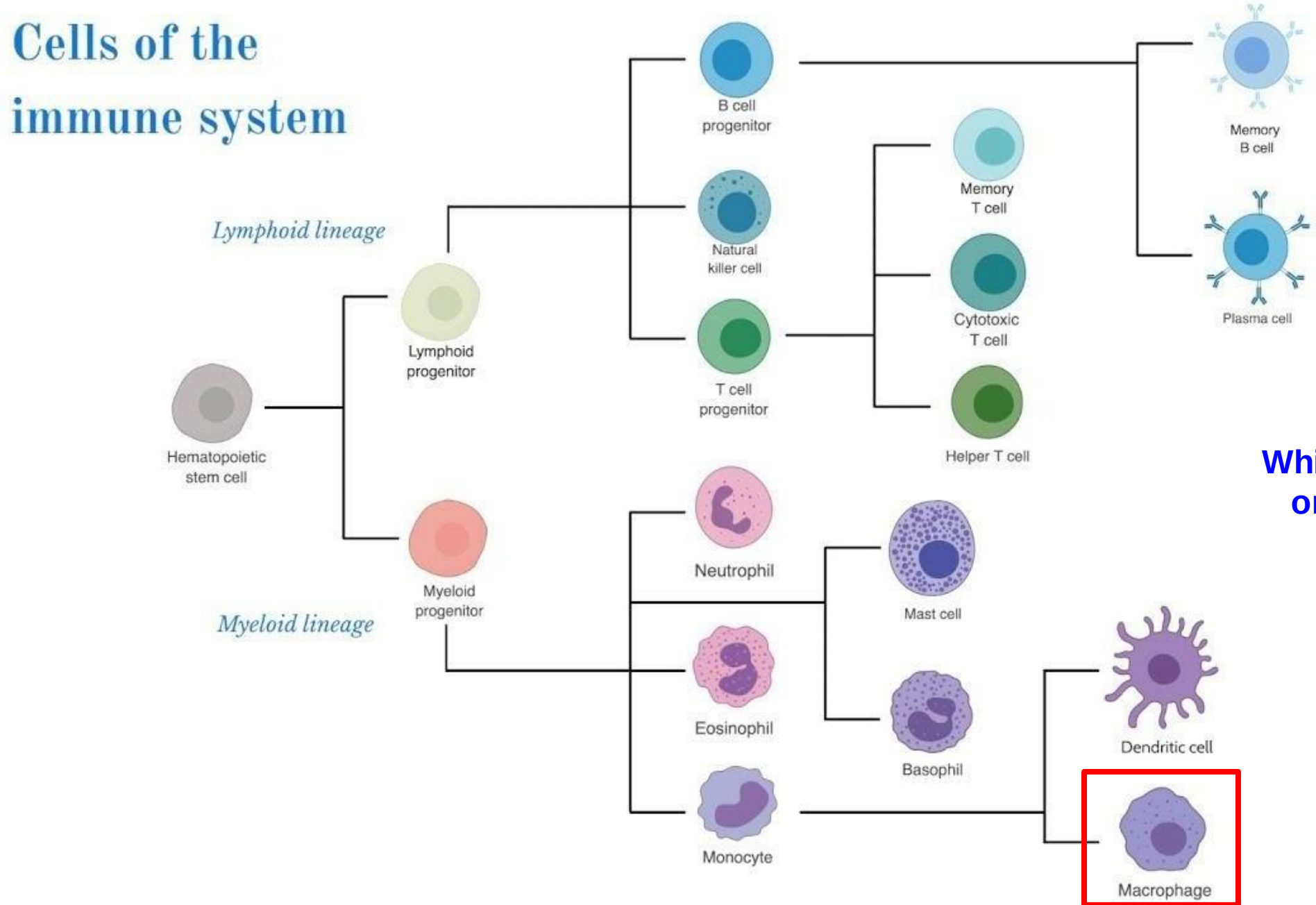


Phagocytosis of MRSA by a human neutrophil

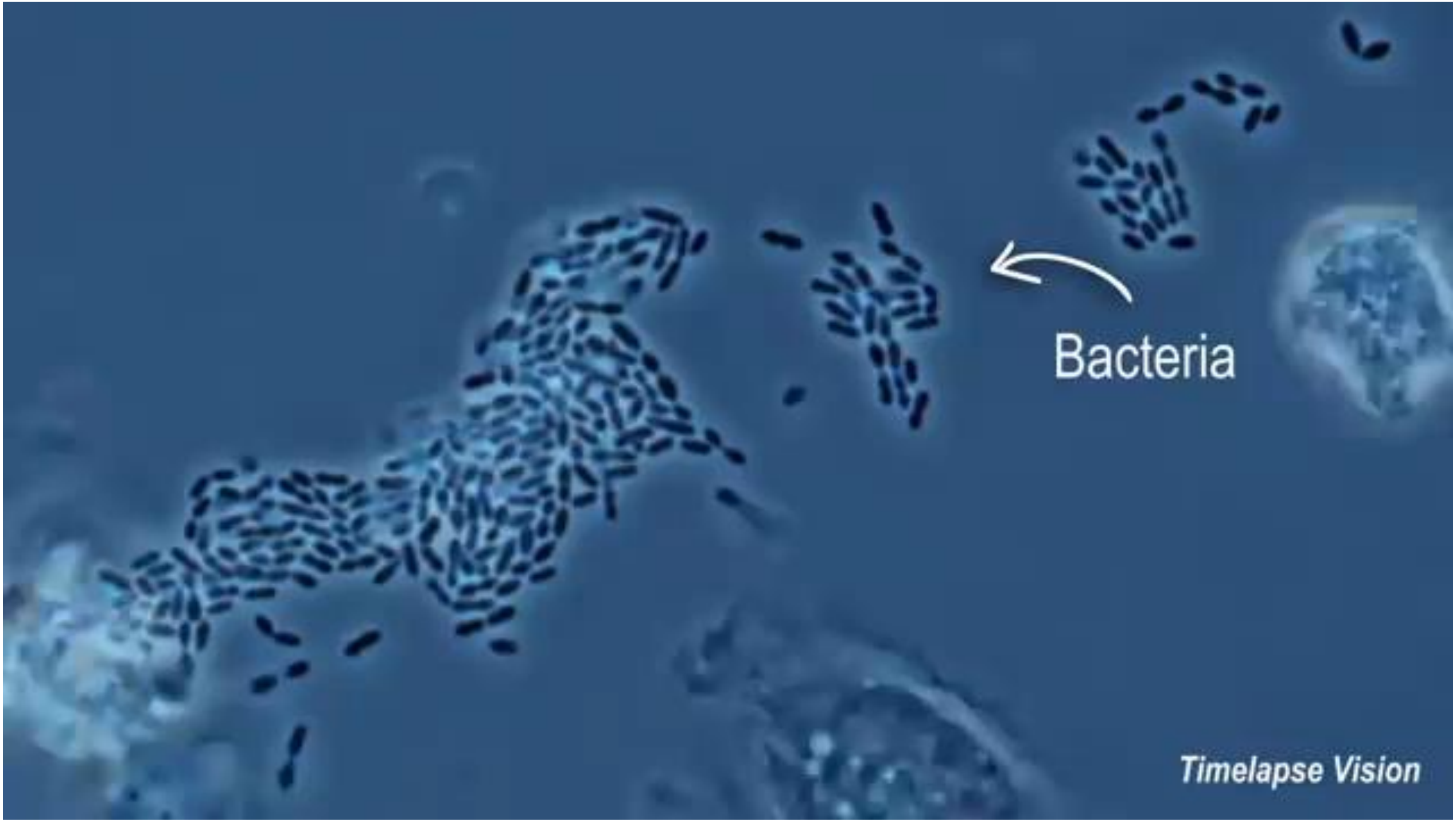
Methicillin-resistant Staphylococcus aureus (MRSA) is a bacterium that causes infections in different parts of the body.



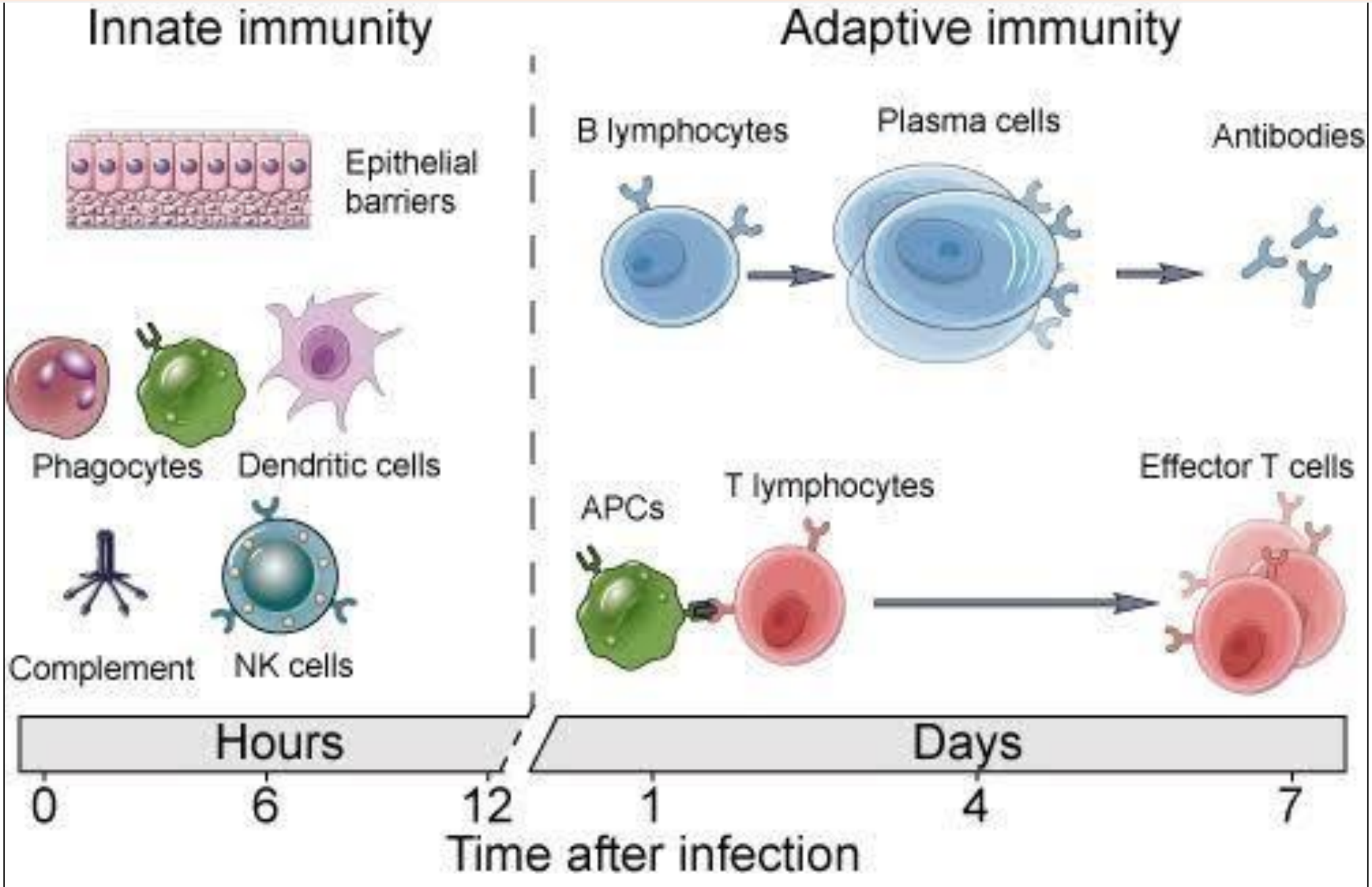
Components of the Immune System – Immune Cells



White Blood Cells or Leuckocyte


Bacteria
Timelapse Vision

Adaptive Immunity



Innate vs Adaptive Immunity

INNATE IMMUNITY:

- The first line of defense against non-self pathogens is the innate, or non-specific, immune response. The innate immune response consists of physical, chemical and cellular defenses against pathogens. The main purpose of the innate immune response is to immediately prevent the spread and movement of foreign pathogens throughout the body.
- Cells involved: Natural killer cells, macrophages, neutrophils, dendritic cells, mast cells, basophils, eosinophils

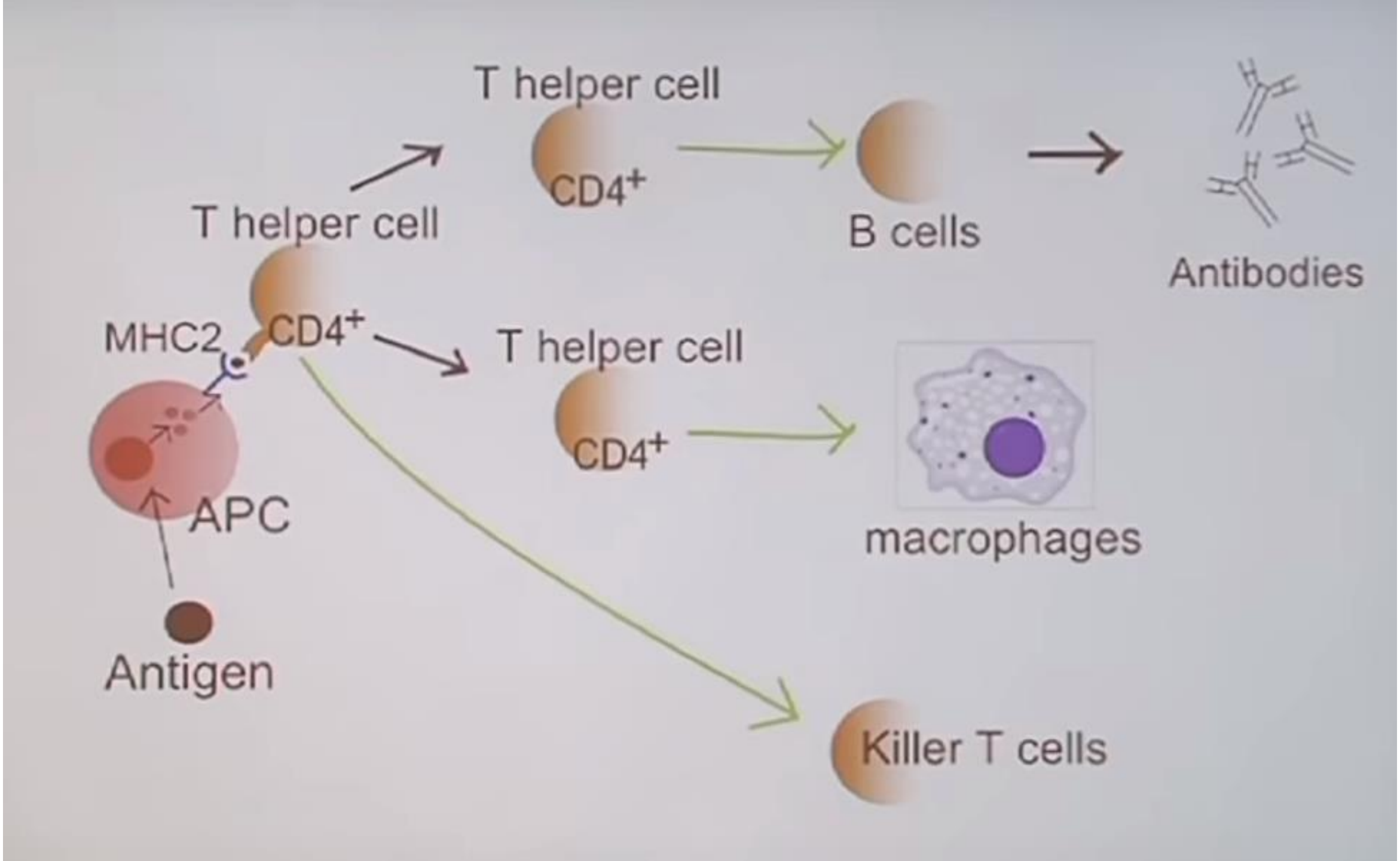
ADAPTIVE or ACQUIRED IMMUNITY:

- The second line of defense against non-self pathogens is called adaptive immune response. Adaptive immunity is also referred to as acquired immunity or specific immunity and is only found in vertebrates. The adaptive immune response is specific to the pathogen presented. The adaptive immune response is meant to attack non-self pathogens but can sometimes make errors and attack itself. When this happens, autoimmune diseases can develop (e.g., Type 1 Diabetes Mellitus, Rheumatoid arthritis, etc.).

- The hallmark of the adaptive immune system is clonal expansion of lymphocytes. Clonal expansion is the rapid increase of T and B lymphocytes from one or a few cells to millions. Each clone that originates from the original T or B lymphocyte has the same antigen receptor as the original and fights the same pathogen.

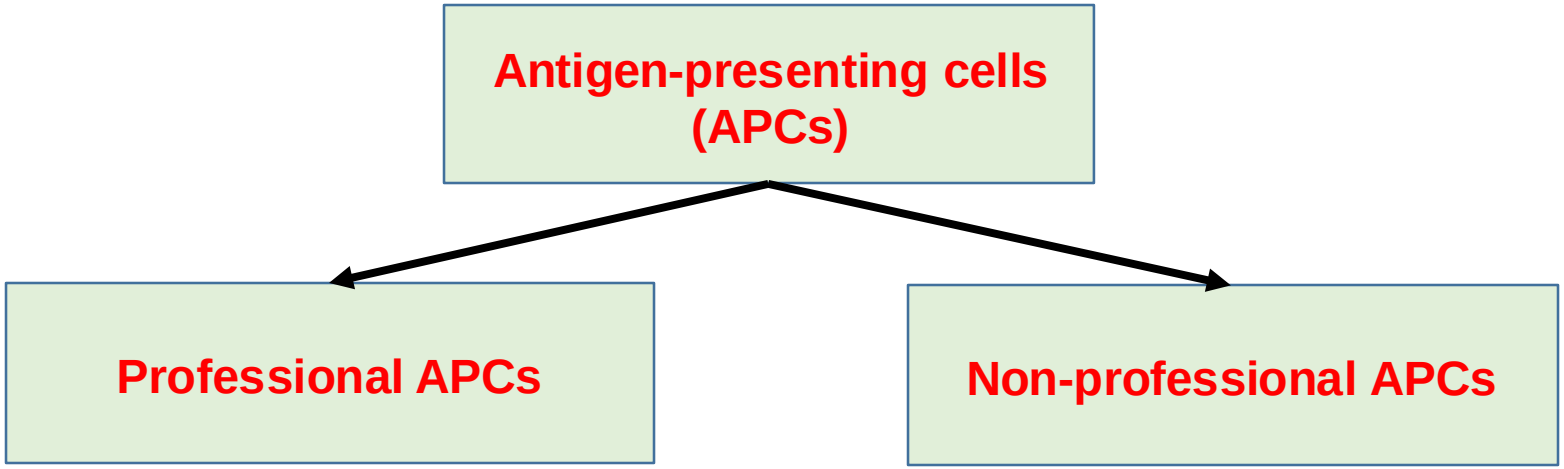
- While the innate immune response is immediate, the adaptive immune response is not. However, the effect of the adaptive immune response is long-lasting, highly specific, and is sustained long-term by memory T cells.

- Humoral immunity and cell mediated immunity are two types of adaptive immunity in which a specific immune. response is produced for a particular pathogen.
- Antibodies are produced by the plasma T cells in the humoral immunity.
- In cell mediated immunity, T cells induce the apoptosis of the infected cells.

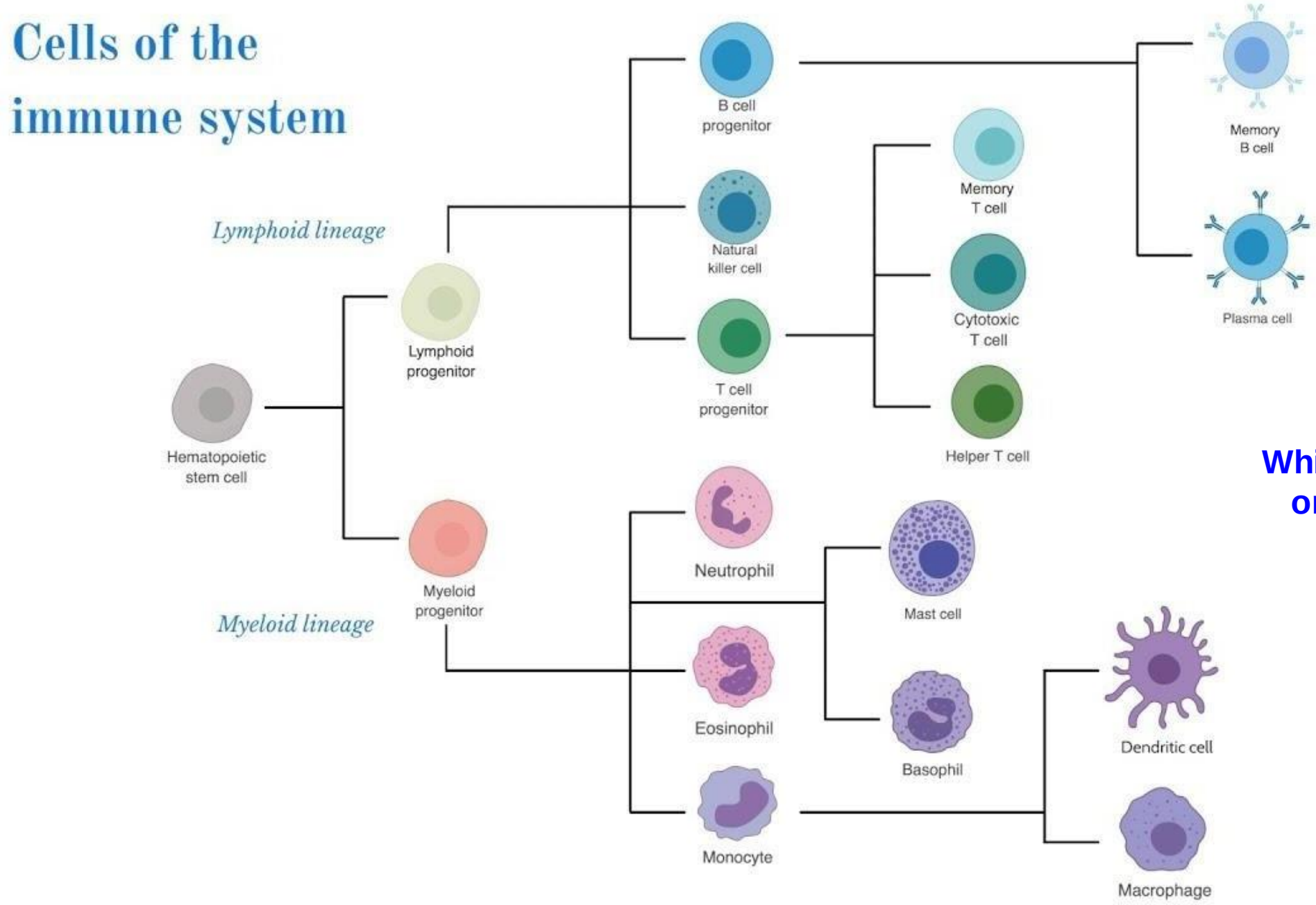

T helper cell
CD4+
B cells
Antibodies
T helper cell
MHC2
CD4+
T helper cell
CD4+
APC
macrophages
Antigen
Killer T cells

Antigen presenting cells (APC)

- Macrophages, Dendritic cells and B-cells are termed professional antigen-presenting cells (APCs).
- These cells can process protein antigens into peptides.
- These peptides can then be presented (along with major histocompatibility complex) to T-cell receptors on the surface of the cell.
- APCs are equipped with special "co-stimulatory" ligands recognized by co-stimulatory receptors on T cells.
- Adaptive immune response would be inefficient without those costimulatory signals as the T cells would become anergic (fail to respond to their specific antigen).



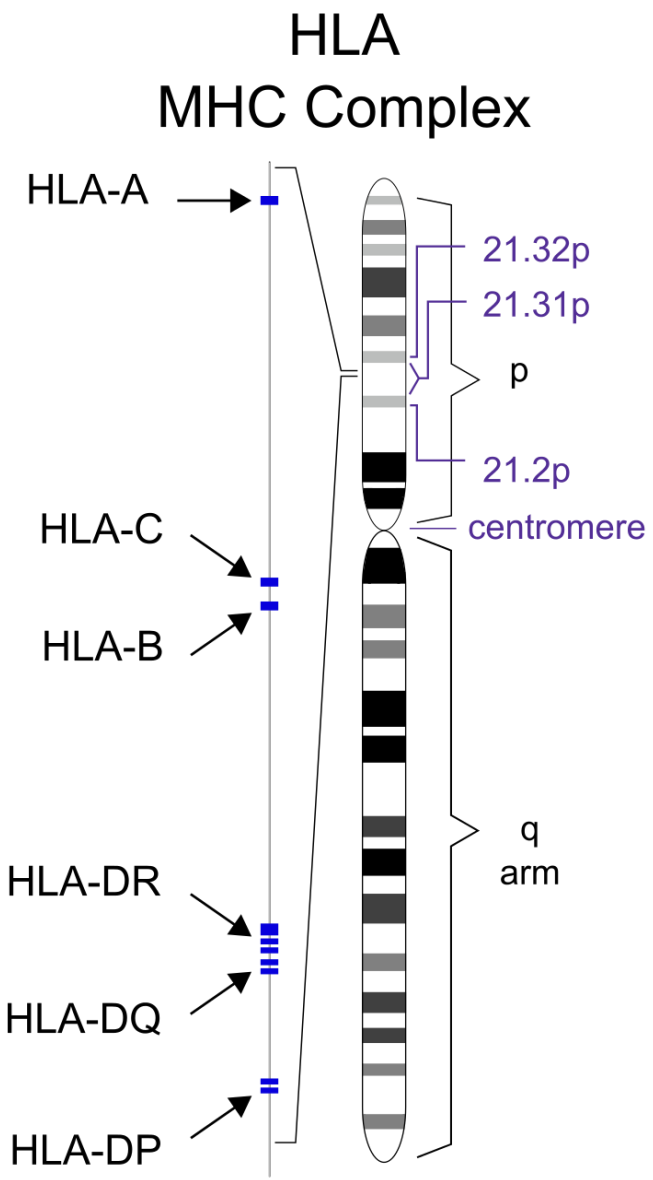
Components of the Immune System – Immune Cells



White Blood Cells or Leuckocyte

Antigen presenting cells (APC) - HLA system

- The Human Leukocyte Antigen system or complex is a gene complex encoding the major histocompatibility complex (MHC) proteins in humans.
- These cell-surface proteins are responsible for the regulation of the immune system in humans.
- The MHC complex is a family of 200+ genes categorized into three classes: I, II, III.
- Class I genes make proteins that are located on the surface of almost all cells.
- Class II genes are located on the surface of immune cells.
- Class III genes are also involved with the immune system and inflammation.



human chromosome 6

Antigen presenting cells (APC) - HLA system

- **HLAs corresponding to MHC class I (A, B, and C) are present in all the cells and present peptides from inside the cell.** For example, if the cell is infected by a virus, the HLA system brings fragments of the virus to the surface of the cell so that the cell can be destroyed by the immune system. These peptides are produced from digested proteins that are broken down in the proteasomes. In general, these particular peptides are small polymers, about 9 amino acids in length. Foreign antigens presented by MHC class I attract killer T-cells (also called CD8 positive- or cytotoxic T-cells) that destroy cells. MHC class I proteins associate with β2-microglobulin, which unlike the HLA proteins is encoded by a gene on chromosome 15.

- **HLAs corresponding to MHC class II** (DP, DM, DO, DQ, and DR) present antigens from outside of the cell to T-lymphocytes. These particular antigens **stimulate the multiplication of T-helper cells** (also called CD4 positive T cells), which in turn stimulate antibody-producing B-cells to produce antibodies to that specific antigen. Self-antigens are suppressed by regulatory T cells.

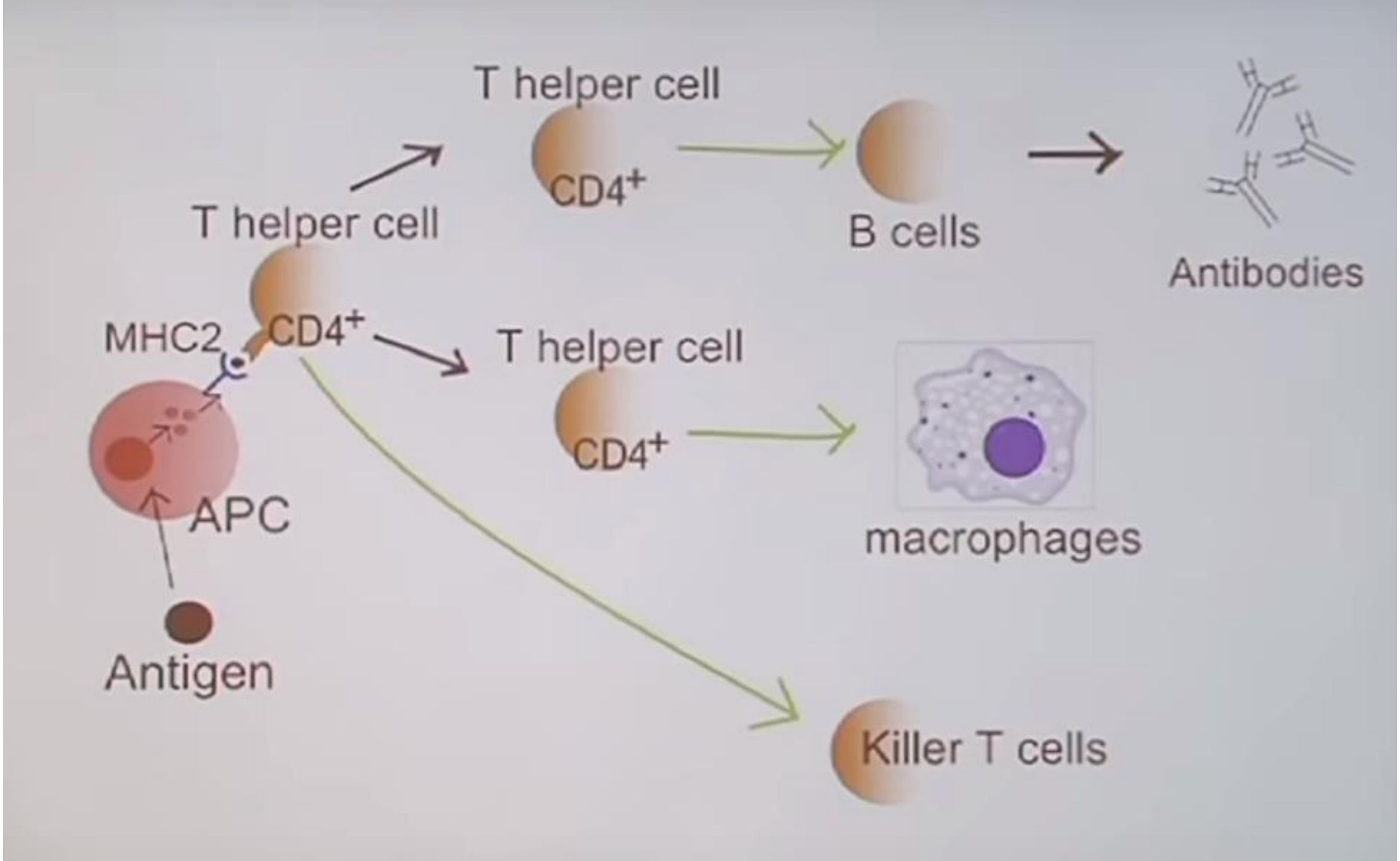
- **HLAs corresponding to MHC class III encode components of the complement system**. The complement system is a part of the immune system that enhances (complements) the ability of antibodies and phagocytic cells to clear microbes and damaged cells from an organism, promote inflammation, and attack the pathogen's cell membrane. It is **part of the innate immune system**.

- HLAs have other roles.
- They are important in disease defense.
- They are the major cause of organ transplant rejections.

CART (Chimeric Antigen Receptor T cell) therapy

CART (Chimeric Antigen Receptor T cell) therapy

T cells are cells of the immune system that fight against infections


T helper cell
CD4+
B cells
Antibodies
T helper cell
MHC2
CD4+
T helper cell
CD4+
APC
macrophages
Antigen
Killer T cells

CART (Chimeric Antigen Receptor T cell) therapy

- **Chimeric antigen (artificial) receptor T cells (also known as CAR T cells) are T cells that have been genetically engineered to produce an artificial T-cell receptor for use in immunotherapy.**

- **CARs are receptor proteins that have been engineered to give T cells the new ability to target a specific protein.**

- **The receptors are chimeric because they combine both antigen-binding and T-cell activating functions into a single receptor.**

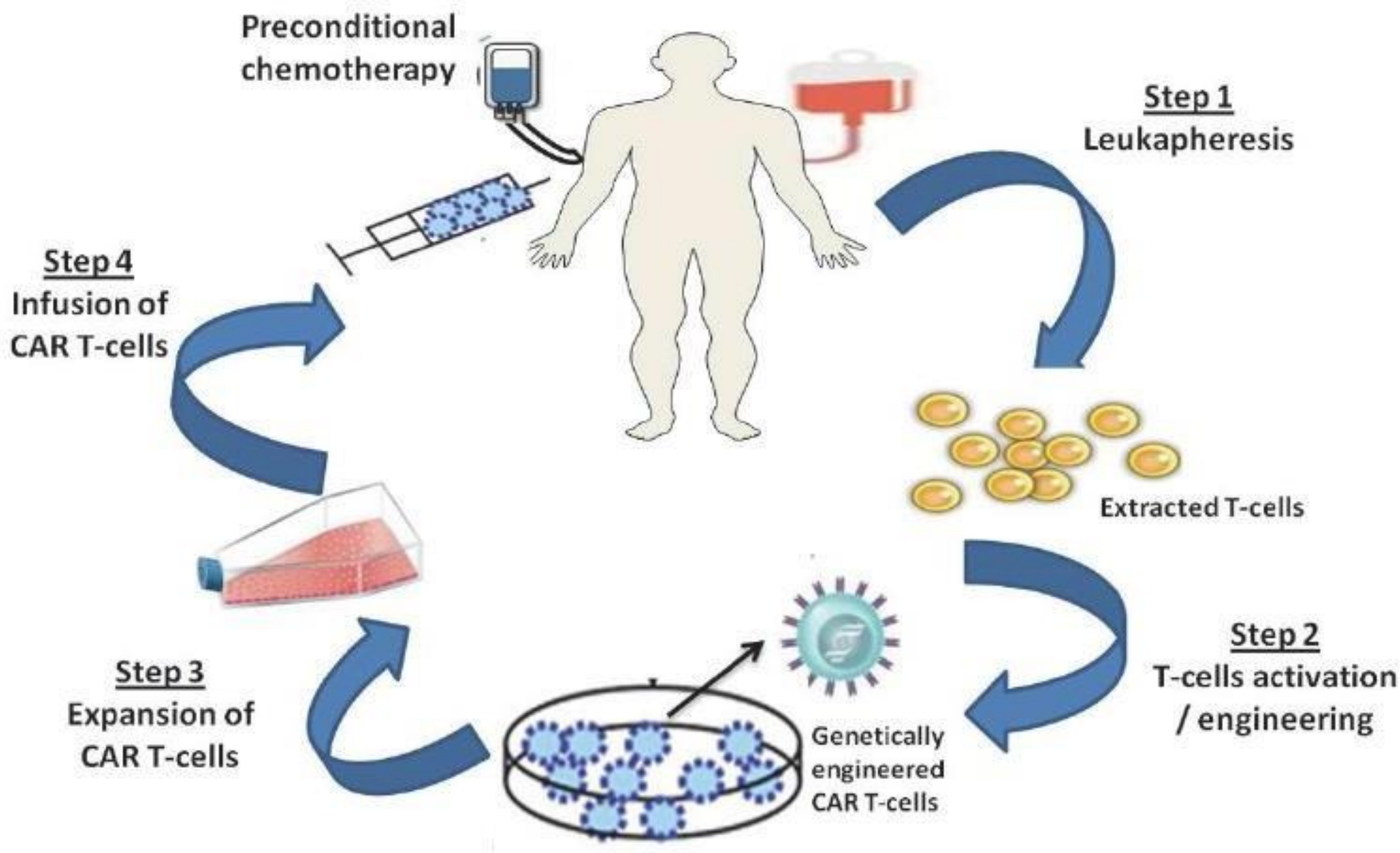
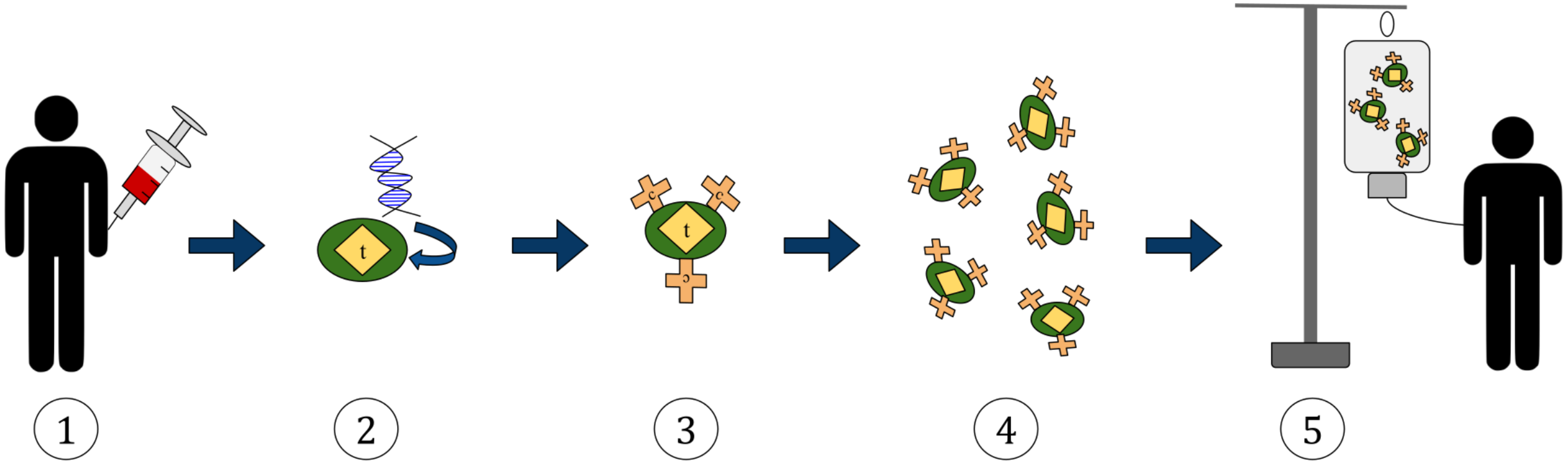
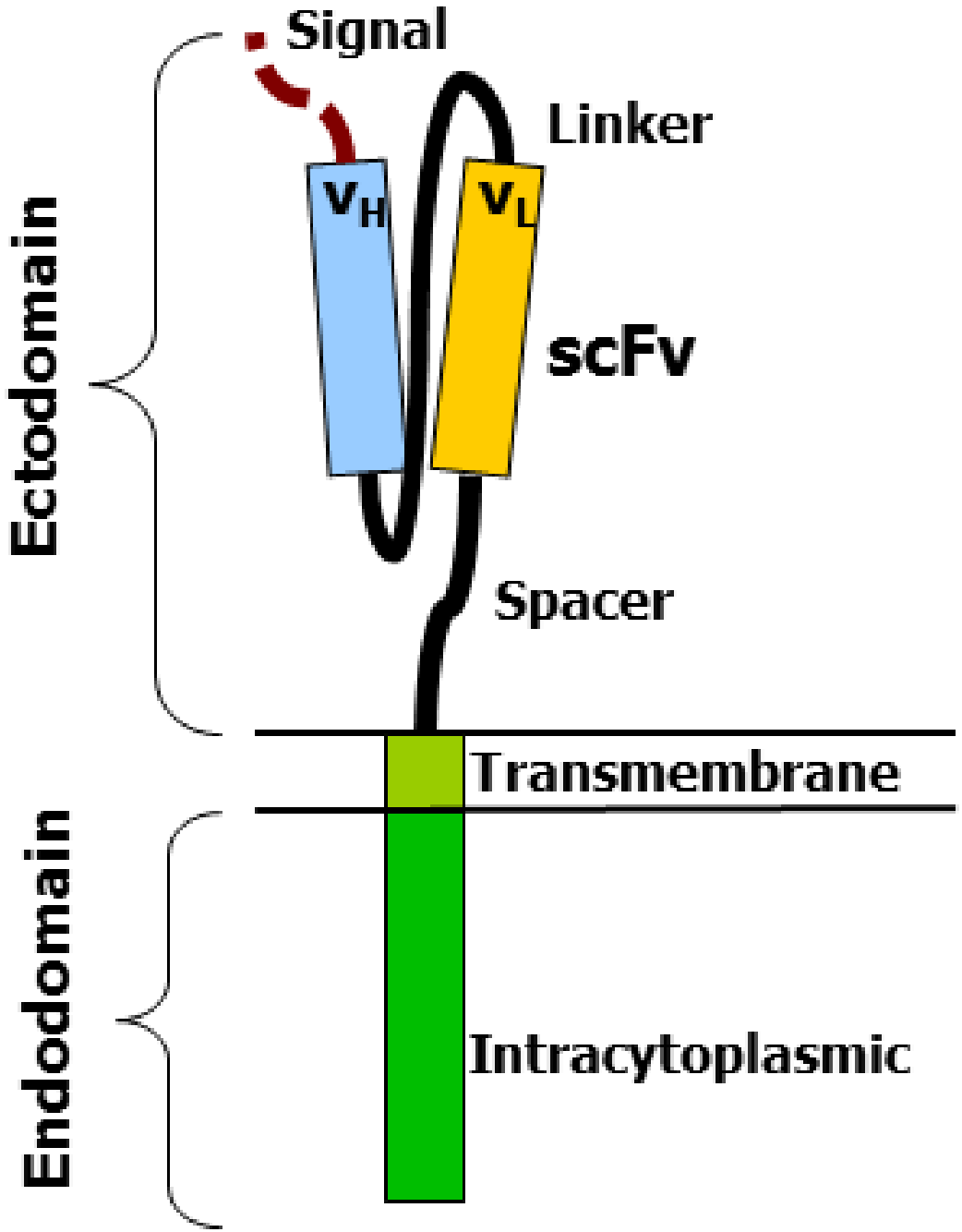


Fig. 3. A diagram presenting the steps of CAR T-cell therapy procedure in clinic

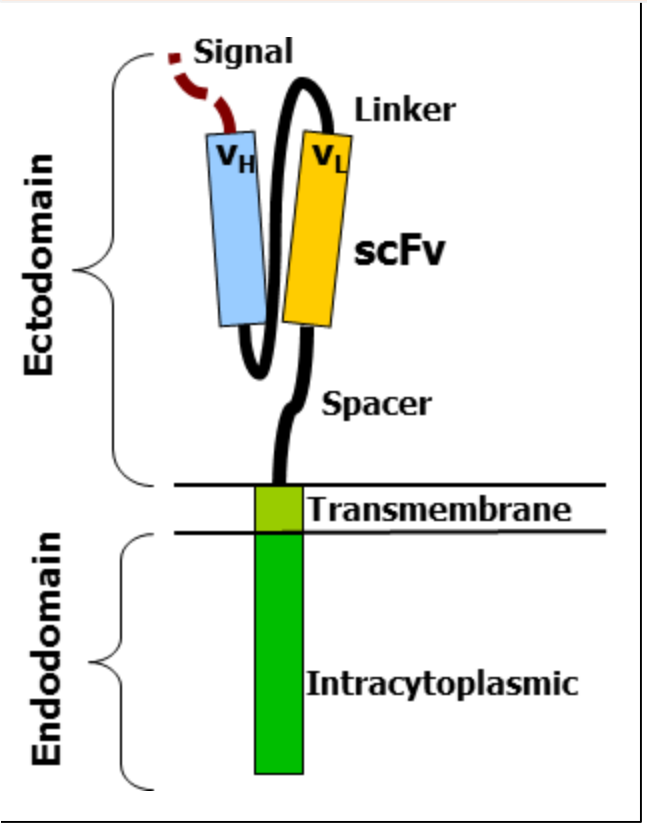


- The diagram above represents the process of chimeric antigen receptor T-cell therapy (CAR), this is a method of immunotherapy, which is a growing practice in the treatment of cancer. The final result should be a production of equipped T-cells that can recognize and fight the infected cancer cells in the body.

1. T-cells (represented by objects labeled as ’t’) are removed from the patient's blood.
2. Then in a lab setting the gene that encodes for the specific antigen receptors are incorporated into the T-cells.
3. Thus producing the CAR receptors (labeled as c) on the surface of the cells.
4. The newly modified T-cells are then further harvested and grown in the lab.
5. After a certain time period, the engineered T-cells are infused back into the patient.


Signal
Linker
V_H
V_L
scFv
Ectodomain
Spacer
Transmembrane
Endodomain
Intracytoplasmic

Structure of receptor of a CAR T cell



- Chimeric antigen receptors combine many facets of normal T cell activation into a single protein. They link an extracellular antigen recognition domain to an intracellular signalling domain, which activates the T cell when an antigen is bound.
- CARs are composed of four regions:
- **an antigen recognition domain**
- **an extracellular hinge region**
- **a transmembrane domain**
- **an intracellular T-cell signaling domain.**

Antigen recognition domain:

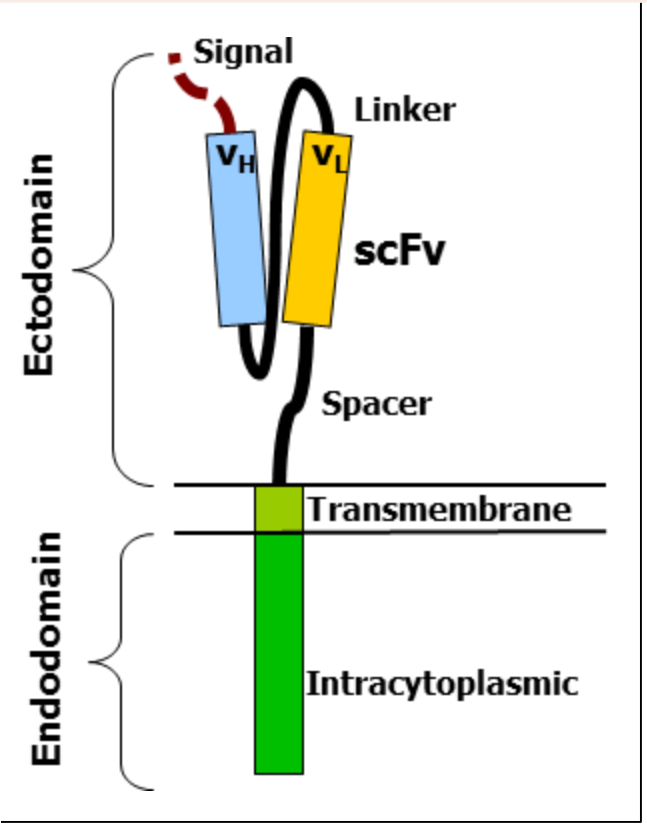
- The antigen recognition domain is exposed to the outside of the cell, in the ectodomain portion of the receptor. It interacts with potential target molecules and is responsible for targeting the CAR-T cell to any cell expressing a matching molecule.

- The antigen recognition domain is typically derived from the variable regions of a monoclonal antibody linked together as a single-chain variable fragment (scFv).

- An scFv is a chimeric protein made up of the light (VL) and heavy (VH) chains of immunoglobins, connected with a short linker peptide.

- These VL and VH regions are selected in advance for their binding ability to the target antigen (**such as CD19**). The linker between the two chains consists of hydrophilic residues with stretches of glycine and serine in it for flexibility as well as stretches of glutamate and lysine for added solubility.

Structure of receptor of a CAR T cell



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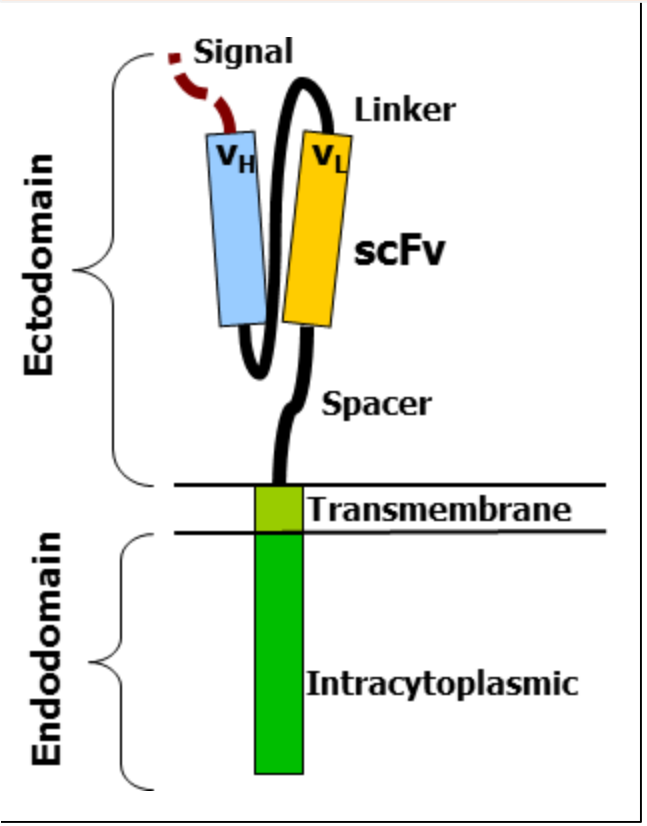
Hinge region (or spacer):

- The hinge, also called a spacer, is a small structural domain that sits between the antigen recognition region and the cell's outer membrane.

- An ideal hinge enhances the flexibility of the scFv receptor head, reducing the spatial constraints between the CAR and its target antigen.

- This promotes antigen binding and synapse formation between the CAR-T cells and target cells.

Structure of receptor of a CAR T cell



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- **a transmembrane domain**
- **an intracellular T-cell signaling domain.**

Transmembrane domain:

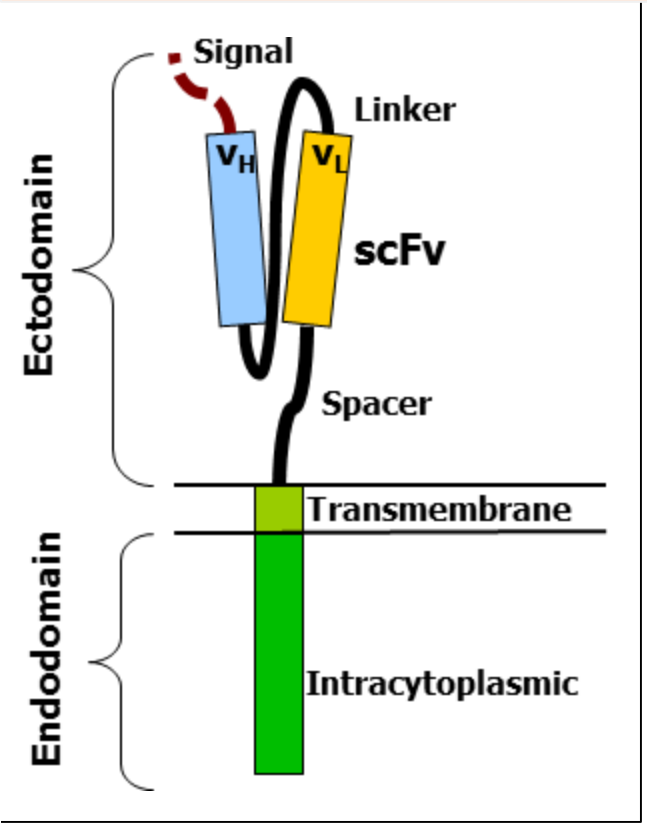
- The transmembrane domain is a structural component, consisting of a hydrophobic alpha helix that spans the cell membrane.

- It anchors the CAR to the plasma membrane, bridging the extracellular hinge and antigen recognition domains with the intracellular signaling region.

- This domain is essential for the stability of the receptor as a whole.

- The CD28 transmembrane domain is known to result in a highly expressed, stable receptor.

Structure of receptor of a CAR T cell



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- CARs are composed of four regions:
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- **an extracellular hinge region**
- **a transmembrane domain**
- **an intracellular T-cell signaling domain.**

Intracellular T-cell signaling domain:

- The intracellular T-cell signaling domain lies in the receptor's endodomain, inside the cell.
- After an antigen is bound to the external antigen recognition domain, CAR receptors cluster together and transmit an activation signal.
- Then the internal cytoplasmic end of the receptor perpetuates signaling inside the T cell.

Evolution of CAR design

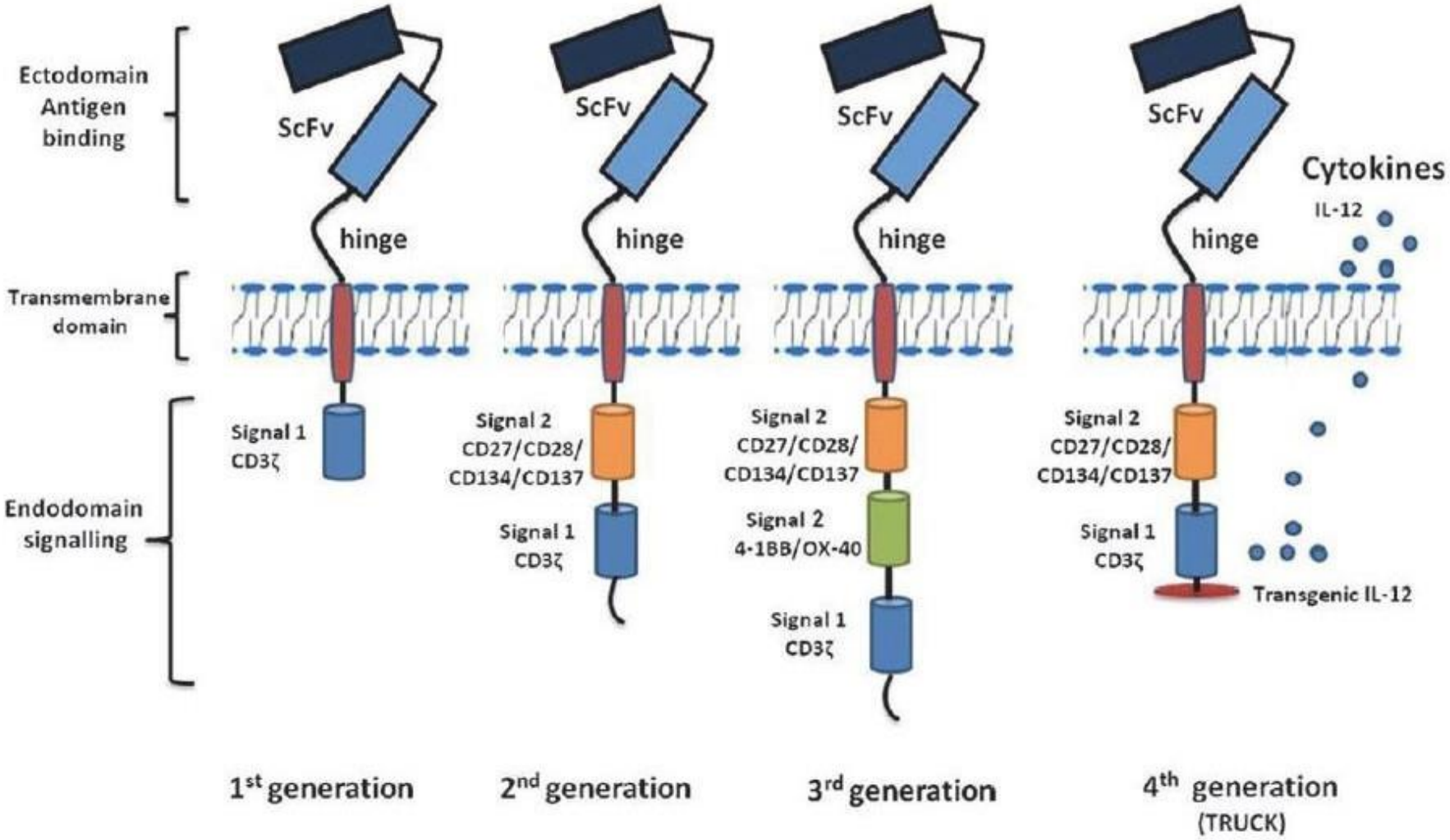
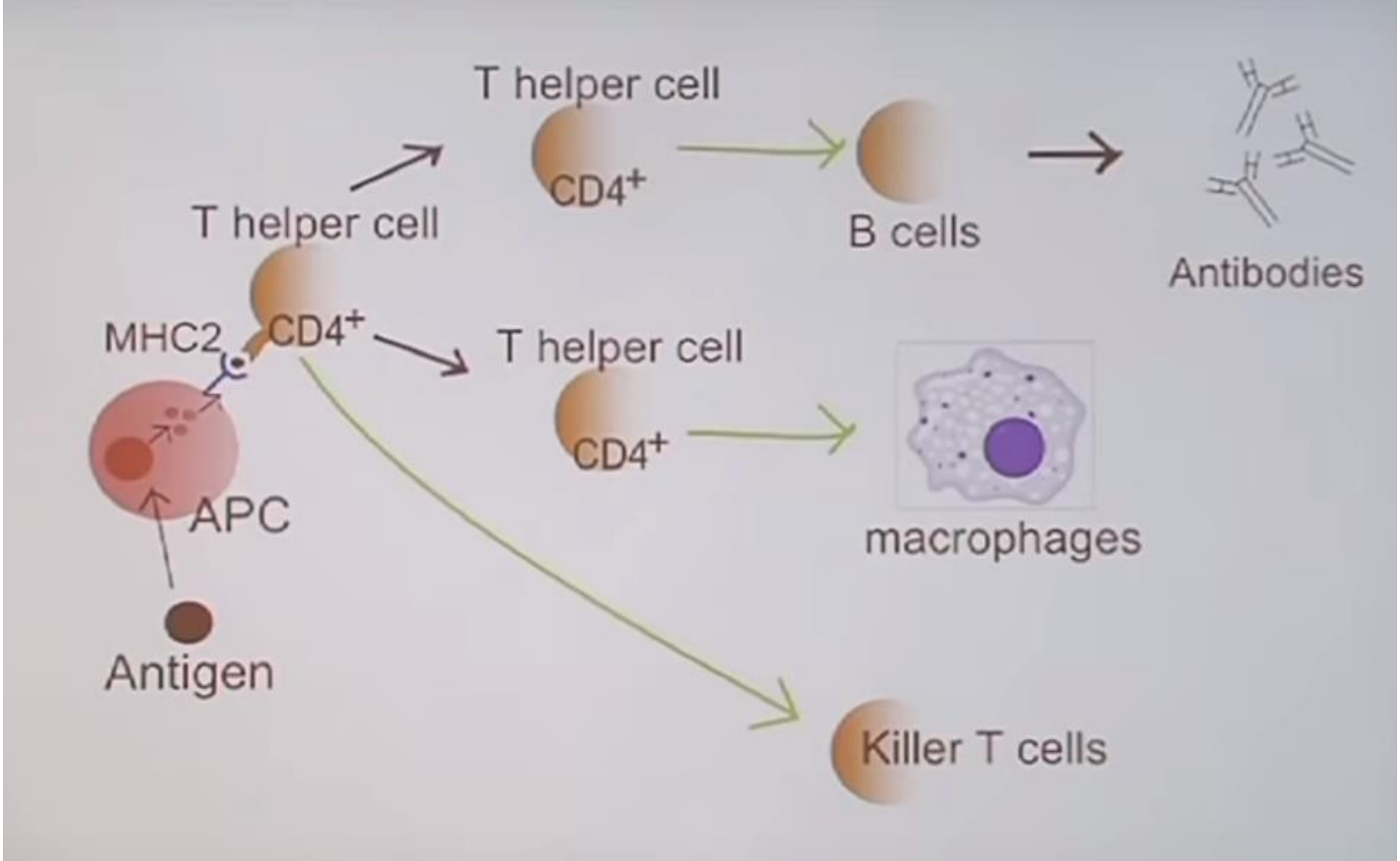


Fig. 2. Schematic representation elucidating the different generations of CAR T-cells

First generation of CAR T-cells contains single signaling module CD3ζ, while second and third generations contain one or two additional co-stimulatory domains to enhance the survival as well as proliferation and persistence of activated CAR T-cells. The 4th generation (TRUCK) is the newest approach in adoptive T-cell therapy, where CAR T-cells are genetically modified with an inducible cytokine gene cassette to immediately secrete cytokines such as IL-12 upon CAR T-cell activation.

Table 3 Characteristics of first to fourth generation CAR-engineered T cells

Properties	First generation	Second generation	Third generation	Fourth generation
Intracellular domains	CD3ζ	CD3ζ and CD28/CD137/CD134/ICOS	CD3ζ and CD28 and CD137	CD3ζ and CD28/CD137/CD134/ICOS
Additional transgenes	–	–	–	IL-12 or co-stimulatory ligands
Signaling and function	T cell activation; modest IL-2 secretion; adequate target cell lysis	Increased proliferation, IL-2 secretion and target cell lysis; increased resistance to apoptosis; increased *in vivo* persistence	High proliferation, cytokine secretion and cytotoxicity; high resistance to apoptosis; high *in vivo* persistence	Same as third generation CARs; additional pro-inflammatory cytokine secretion that reforms the tumor microenvironment
Application in clinical trials	Yes	Yes	Yes	Pending


T helper cell
CD4+
B cells
Antibodies
T helper cell
MHC2
CD4+
T helper cell
CD4+
APC
macrophages
Antigen
Killer T cells

Production of CAR T cells

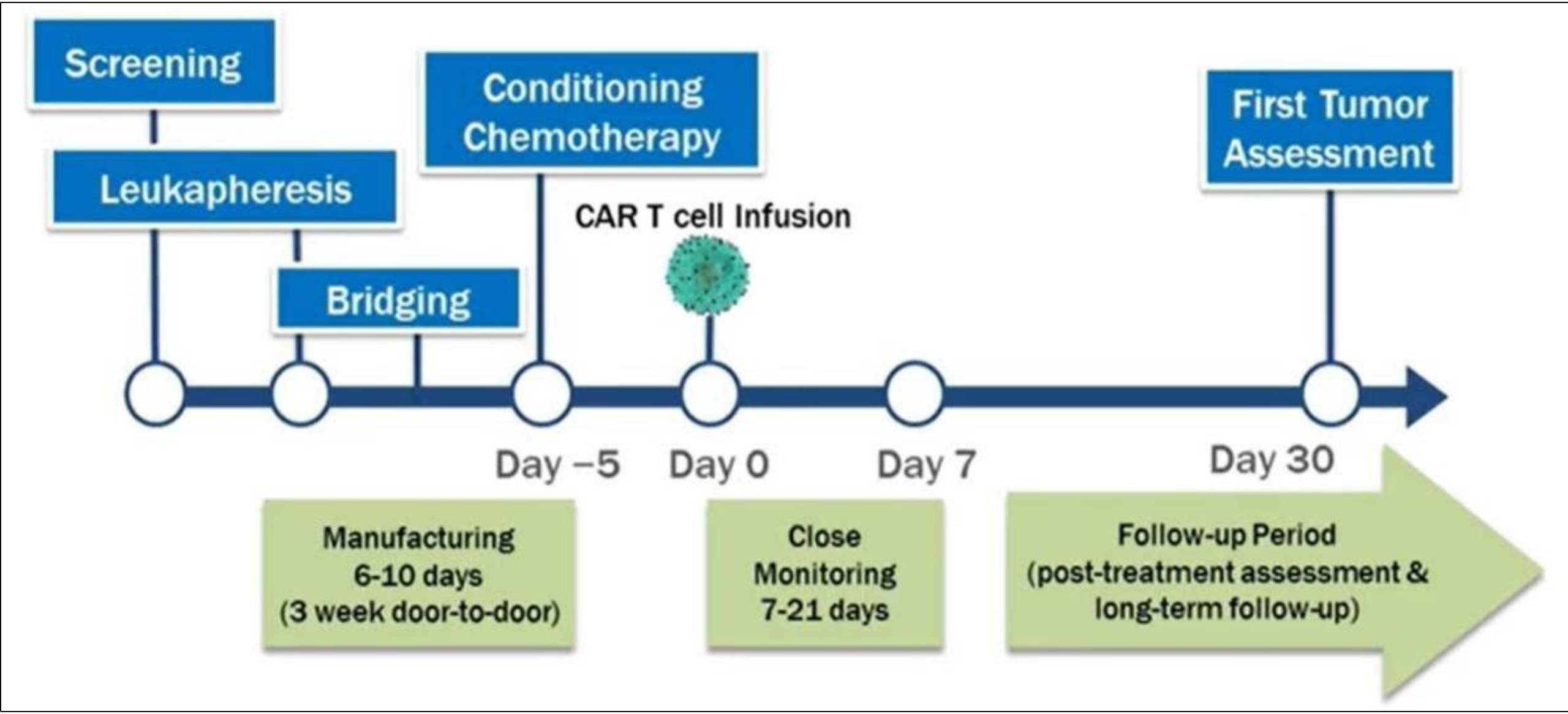
- The first step in the production of CAR-T cells is the isolation of T cells from human blood.

- CAR-T cells may be manufactured either from the patient's own blood, known as an autologous treatment, or from the blood of a healthy donor, known as an allogeneic treatment.
- The manufacturing process is the same in both cases; only the choice of initial blood donor is different.

- First, leukocytes are isolated using a blood cell separator in a process known as leukocyte apheresis. Peripheral blood mononuclear cells (PBMC) are then separated and collected. The products of leukocyte apheresis are then transferred to a cell-processing center.
- In the cell processing center, specific T cells are stimulated so that they will actively proliferate and expand to large numbers. To drive their expansion, T cells are typically treated with the cytokine interleukin 2 (IL-2) and anti-CD3 antibodies.

- The expanded T cells are purified and then transduced with a gene encoding the engineered CAR via a retroviral vector, typically either an integrating gammaretrovirus (RV) or a lentiviral (LV) vector. These vectors are very safe in modern times due to a partial deletion of the U3 region.
- The new gene editing tool CRISPR/Cas9 has recently been used instead of retroviral vectors to integrate the CAR gene into specific sites in the genome.

- The patient undergoes lymphodepletion chemotherapy prior to the introduction of the engineered CAR-T cells. The depletion of the number of circulating leukocytes in the patient upregulates the number of cytokines that are produced and reduces competition for resources, which helps to promote the expansion of the engineered CAR-T cells.


Screening
Leukapheresis
Bridging
Conditioning Chemotherapy
CAR T cell Infusion
First Tumor Assessment
Day −5
Day 0
Day 7
Day 30
Manufacturing
6-10 days
(3 week door-to-door)
Close
Monitoring
7-21 days
Follow-up Period
(post-treatment assessment &
long-term follow-up)

CART therapy for cancer

- CAR-T cell therapy uses T cells engineered with CARs for cancer therapy.

- The premise of CAR-T immunotherapy is to modify T cells to recognize cancer cells in order to more effectively target and destroy them.
- Scientists harvest T cells from people, genetically alter them, then infuse the resulting CAR-T cells into patients to attack their tumors.

- CAR-T cells can be either derived from T cells in a patient's own blood (autologous) or derived from the T cells of another healthy donor (allogeneic).
- Once isolated from a person, these T cells are genetically engineered to express a specific CAR, which programs them to target an antigen that is present on the surface of tumors.

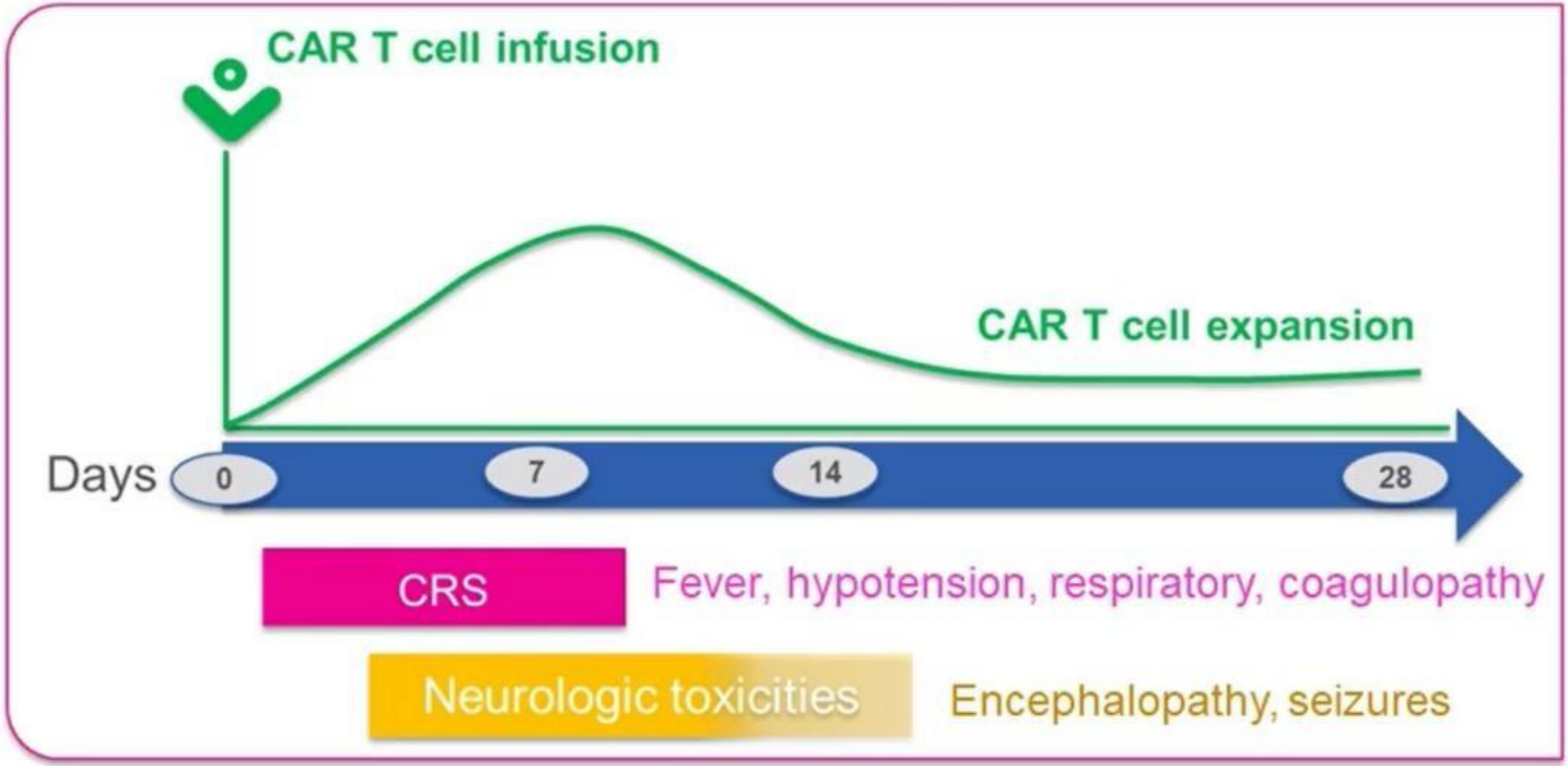
- For safety, CAR-T cells are engineered to be specific to an antigen expressed on a tumor that is not expressed on healthy cells.

- After CAR-T cells are infused into a patient, they act as a "living drug" against cancer cells.
- When they come in contact with their targeted antigen on a cell, CAR-T cells bind to it and become activated, then proceed to proliferate and become cytotoxic.
- CAR-T cells destroy cells through several mechanisms, including extensive stimulated cell proliferation, increasing the degree to which they are toxic to other living cells (cytotoxicity) and by causing the increased secretion of factors that can affect other cells such as cytokines, interleukins and growth factors.

CART therapy for cancer

- Early CAR-T cell research has focused on blood cancers.
- The first approved treatments use CARs that target the antigen CD19, present in B-cell-derived cancers such as acute lymphoblastic leukemia (ALL) and diffuse large B-cell lymphoma (DLBCL).
- Tisagenlecleucel (Kymriah) is approved to treat relapsed/refractory B-cell precursor acute lymphoblastic leukemia (ALL), while axicabtagene ciloleucel (Yescarta) is approved to treat relapsed/refractory diffuse large B-cell lymphoma (DLBCL).
- There are also efforts underway to engineer CARs targeting many other blood cancer antigens, including CD30 in refractory Hodgkin's lymphoma; CD33, CD123, and FLT3 in acute myeloid leukemia (AML); and BCMA in multiple myeloma.
- Preclinical studies developing CAR-T cells with dual targeting of CD19 plus CD22 OR CD19 plus CD20 have demonstrated promise, and trials studying bispecific targeting to circumvent CD19 down-regulation are ongoing.
- Solid tumors have presented a more difficult target.
- Identification of good antigens has been challenging: such antigens must be highly expressed on the majority of cancer cells, but largely absent on normal tissues. CAR-T cells are also not trafficked efficiently into the center of solid tumor masses, and the hostile tumor microenvironment suppresses T cell activity.
- As of March 2019, there were around 364 ongoing clinical trials happening globally involving CAR-T cells. The majority of those trials target blood cancers: CAR-T therapies account for more than half of all trials for hematological malignancies.

Side effects of CART therapy



Side effects of CART therapy

- CAR-T cells are undoubtedly a major breakthrough in cancer treatment. However, there are serious side effects that result from CAR-T cells being introduced into the body, including cytokine release syndrome and neurological toxicity.

- The most common issue after treatment with CAR-T cells is cytokine release syndrome (CRS), a condition in which the immune system is activated and releases an increased number of inflammatory cytokines. The clinical manifestation of this syndrome resembles sepsis with high fever, fatigue, myalgia, nausea, capillary leakages, tachycardia and other cardiac dysfunction, liver failure, and kidney impairment. CRS occurs in almost all patients treated with CAR-T cell therapy; in fact, the presence of CRS is a diagnostic marker that indicates the CAR-T cells are working as intended to kill the cancer cells. Note, however, that a higher grade of CRS severity does not correlate with an increased response to the treatment, but rather higher disease burden.

- Neurological toxicity is also often associated with CAR-T cell treatment. The underlying mechanism is poorly understood, and may or may not be related to CRS. Clinical manifestations include delirium, the partial loss of the ability to speak coherently while still having the ability to interpret language (expressive aphasia), lowered alertness (obtundation), and seizures. During some clinical trials deaths caused by neurotoxicity have occurred. The main cause of death from neurotoxicity is cerebral edema. In a study carried out by Juno Therapeutics, Inc., five patients enrolled in the trial died as a result of cerebral edema. Two of the patients were treated with cyclophosphamide alone and the remaining three were treated with a combination of cyclophosphamide and fludarabine. In another clinical trial sponsored by the Fred Hutchinson Cancer Research Center, there was one reported case of irreversible and fatal neurological toxicity 122 days after the administration of CAR-T cells.

- There is also the possibility that the engineered CAR-T cells will themselves become transformed into cancerous cells through insertional mutagenesis, due to the viral vector inserting the CAR gene into a tumor suppressor or oncogene in the host T cell's genome. Some retroviral (RV) vectors carry a lower risk than lentiviral (LV) vectors. However, both have the potential to be oncogenic.

Other control mechanisms

- Adding a synthetic control mechanism to engineered T cells allows doctors to precisely control the persistence or activity of the T cells in the patient's body, with the goal of reducing toxic side effects.

- **Suicide genes:**
- Genetically modified T cells are engineered to include one or more genes that can induce apoptosis when activated by an extracellular molecule. Herpes simplex virus thymidine kinase (HSV-TK) and inducible caspase 9 (iCasp9) are two types of suicide genes that have been integrated into CAR-T cells. In the iCasp9 system, the suicide gene complex has two elements: a mutated FK506-binding protein with high specificity to the small molecule rimiducid/AP1903, and a gene encoding a pro-domain-deleted human caspase 9. Dosing the patient with rimiducid activates the suicide system, leading to rapid apoptosis of the genetically modified T cells. Although both the HSV-TK and iCasp9 systems demonstrate a noticeable function as a safety switch in clinical trials, some defects limit their application. HSV-TK is virus-derived and may be immunogenic to humans. It is also currently unclear whether the suicide gene strategies will act quickly enough in all situations to halt dangerous off-tumor cytotoxicity.

- **Dual-antigen receptor:**
- CAR-T cells are engineered to express two tumor-associated antigen receptors at the same time, reducing the likelihood that the T cells will attack non-tumor cells. Dual-antigen receptor CAR-T cells have been reported to have less intense side effects. An in vivo study in mice shows that dual-receptor CAR-T cells effectively eradicated prostate cancer and achieved complete long-term survival.

- **ON-switch:**
- In this system, CAR-T cells can only function in the presence of both tumor antigen and a benign exogenous molecule. To achieve this, the CAR-T cell's engineered chimeric antigen receptor is split into two separate proteins that must come together in order to function. The first receptor protein typically contains the extracellular antigen binding domain, while the second protein contains the downstream signaling elements and co-stimulatory molecules (such as CD3ζ and 4-1BB). In the presence of an exogenous molecule (such as a rapamycin analog), the binding and signaling proteins dimerize together, allowing the CAR-T cells to attack the tumor.

- **Bispecific molecules as switches:**
- Bispecific molecules target both a tumor-associated antigen and the CD3 molecule on the surface of T cells. This ensures that the T cells cannot become activated unless they are in close physical proximity to a tumor cell. The anti-CD20/CD3 bispecific molecule shows high specificity to both malignant B cells and cancer cells in mice. FITC is another bifunctional molecule used in this strategy. FITC can redirect and regulate the activity of the FITC-specific CAR-T cells toward tumor cells with folate receptors.

- **Small molecule drug conjugates adaptor technology**

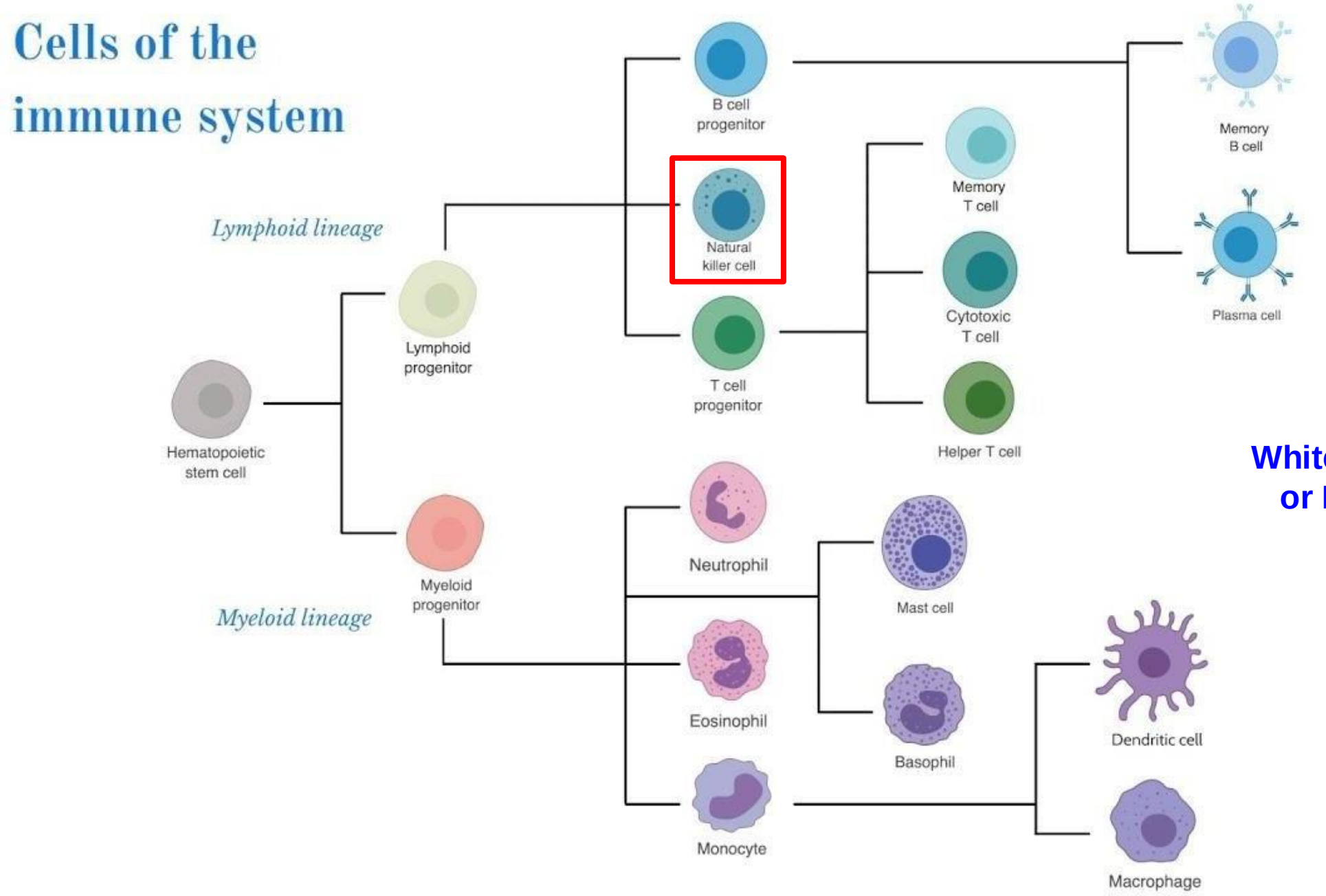
Tumor control via targeting PD-L1 with chimeric antigen receptor modified NK cells

Yvette Robbins[1], Sarah Greene[1], Jay Friedman[1], Paul E Clavijo[1], Carter Van Waes[2], Kellsye P Fabian[3], Michelle R Padget[3], Houssein Abdul Sater[4], John H Lee[5], Patrick Soon-Shiong[5], James Gulley[4], Jeffrey Schlom[3], James W Hodge[3], Clint T Allen[1,6*]

Programmed cell death protein 1, also known as PD-1

Programmed death-ligand 1 (PD-L1)

Components of the Immune System – Immune Cells



White Blood Cells or Leuckocyte

CART therapy

- Genome editing can be used to modify T cells ex vivo to improve their functionality in favor of tumor killing.

- Adoptive cell therapy with genetically modified T-cells aims to:

  - i) redirect specificity to tumor cell antigens,
  - ii) increase the number and persistence of antigen-specific T-cells,
  - iii) improve T-cell effector functions a,
  - iv) overcome suppression of T-cells by disrupting inhibitory molecules, and
  - v) guide T-cells to the tumor site through the interaction between chemokines and their receptors.

- T-cells can be also equipped with suicide genes for their conditional elimination if they induce an unintended immune response or transform to a malignant phenotype.