Stem Cell Therapy: Modeling the application of renewable stem cells on bodies affected by cancer

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Executive Summary

Stem cell therapy is some of the newest and most innovate medicinal sciences to date. Due to this, the full potential of stem cells has not been fully tapped. This was our team's goal going into the 2018-2019 challenge. Stem cells are fundamentally blank canvases in the forms of cells that can be used to other rehabilitate old, diseased, and dying cells. There are already stem cell infusion treatments for certain orthopedic injuries. However, we hypothesized that stem cells can also be used to treat cancerous tumors as well as a multitude of other diseases.

Our team used a tumor model from the NetLogo library to serve as the foundation of our code. It, along with a variety of other models from the library, has all of the parameters we need to simulate a real-life scenario. We then made a model of the production of daughter cells that can then become any specialized cell that the body needs.

In the case of a tumor, the body is producing too many new cells at too fast of a rate. When this happens, the daughter cells come in to help break down the tumor and potentially completely eradicate it without having to do surgery. Such is the case with multiple myeloma, a cancer affecting the bone marrow. Recently one of our team member's family has been diagnosed with this disease, which provided the inspiration for this project. Our team hopes to continue working on this project across all aspects.

Research Fast Facts

Amyloplast: an organelle in some plant cells that stores starch

ATP: Adenosine Triphosphate; a high-energy molecule used for energy storage by organisms

Cancer: a disease caused by an uncontrolled division of abnormal cells in a part of the body

Cell Cycle: Series of events that take place in a cell leading to its division and duplication of its DNA to produce two daughter cells

Cell Growth: An irreversible increase in the size of a cell; it can be caused by a change in the osmotic potential within the cell, or by a reduction of the pressure exerted by the cell wall

Cell Membrane: the thin layer of protein and fat that surrounds the cell

Cell Signaling: A chemical response method in which one cell sends information to another cell through potassium positive and sodium positive pathways

Cellular Respiration: A set of metabolic reactions and processes that take place in the cells of organisms to convert biochemical energy from nutrients into adenosine triphosphate (ATP), and then release waste products such as carbon dioxide

Centrosome: a small body located near the nucleus - it has a dense center and radiating tubules

Cristae: the multiply-folded inner membrane of a cell's mitochondria that are finger-like projections. (it is where ATP is generated)

Cytoplasm: the jelly like material outside the cell nucleus in which the organelles are located.

Epidermal Cells: The cells that are on the surface of the skin, the outer organ that encases the rest of the bodies' organs

Golgi body: a flattened, layered, sac-like organelle that looks like a stack of pancakes and is located near the nucleus

Mitochondria: A double membrane-bound organelle found in all eukaryotic organisms

Mitochondria generate most of the cell's supply of adenosine triphosphate (ATP), used as a source of chemical energy

Mitochondrion: spherical to rod-shaped organelles with a double membrane. The mitochondrion converts the energy stored in glucose into ATP for the cell

Multiple Myeloma: a cancer that forms in plasma cells that causes cancer cells to accumulate in the bone marrow, where they crowd out healthy blood cells

Nuclear Membrane: the membrane that surrounds the nucleus

Nucleolus: an organelle within the nucleus - it is where ribosomal RNA is produced.

Nucleus: spherical body containing many organelles, including the nucleolus. The nucleus controls many of the functions of the cell and contains DNA. The nucleus is surrounded by the nuclear membrane

Programmed Cell Death: There are three types of cell death including, apoptosis, autophagisis, and necrosis. Each has a varying degree of “idleness”

Ribosome: small organelles composed of RNA-rich cytoplasmic granules that are sites of protein synthesis

Rough Endoplasmic Reticulum: (rough ER) a vast system of interconnected, membranous, infolded and convoluted sacks that are located in the cell's cytoplasm Rough ER transport materials through the cell and produces proteins in sacs called cisternae

Smooth Endoplasmic Reticulum: (smooth ER) a vast system of interconnected, membranous, infolded and convoluted tubes that are located in the cell's cytoplasm. Smooth ER transport materials through the cell. It contains enzymes and produces and digests lipids (fats) and membrane proteins

Stem Cells: an unspecialized cell that gives rise to differentiated cells

Stem Cell Therapy: the use of stem cells to treat or prevent a disease or condition

Tumor: a swelling of a part of the body, generally without inflammation, caused by an abnormal growth of tissue, whether benign or malignant

Vacuole: a large, membrane-bound space within a plant cell that is filled with fluid. Most plant cells have a single vacuole that takes up much of the cell. It helps maintain the shape of the cell

In-Depth Research

Stem Cells:

Stem cells have the remarkable potential to develop into many different cell types in the body during early life and growth. In addition, in many tissues they serve as a sort of internal repair system, dividing essentially without limit to replenish other cells as long as the person or animal is still alive. When a stem cell divides, each new cell has the potential either to remain a stem cell or become another type of cell with a more specialized function, such as a muscle cell, a red blood cell, or a brain cell (NIH Stem Cell Information Home Page).

Stem cells are distinguished from other cell types by two important characteristics. First, they are unspecialized cells capable of renewing themselves through cell division, sometimes after long periods of inactivity. Second, under certain physiologic or experimental conditions, they can be induced to become tissue- or organ-specific cells with special functions. In some organs, such as the gut and bone marrow, stem cells regularly divide to repair and replace worn out or damaged tissues. In other organs, however, such as the pancreas and the heart, stem cells only divide under special conditions (NIH Stem Cell Information Home Page).

Until recently, scientists primarily worked with two kinds of stem cells from animals and humans: embryonic stem cells and non-embryonic "somatic" or "adult" stem cells. The functions and characteristics of these cells will be explained in this document. Scientists discovered ways to derive embryonic stem cells from early mouse embryos more than 30 years ago, in 1981. The detailed study of the biology of mouse stem cells led to the discovery, in 1998, of a method to derive stem cells from human embryos and grow the cells in the laboratory. These cells are called human embryonic stem cells. The embryos used in these studies were created for reproductive purposes through *in vitro* fertilization procedures. When they were no longer

needed for that purpose, they were donated for research with the informed consent of the donor. In 2006, researchers made another breakthrough by identifying conditions that would allow some specialized adult cells to be "reprogrammed" genetically to assume a stem cell-like state. This new type of stem cell, called induced pluripotent stem cells (iPSCs), will be discussed in a later section of this document (NIH Stem Cell Information Home Page).

Stem cells are important for living organisms for many reasons. In the 3- to 5-day-old embryo, called a blastocyst, the inner cells give rise to the entire body of the organism, including all of the many specialized cell types and organs such as the heart, lungs, skin, sperm, eggs and other tissues. In some adult tissues, such as bone marrow, muscle, and brain, discrete populations of adult stem cells generate replacements for cells that are lost through normal wear and tear, injury, or disease (NIH Stem Cell Information Home Page).

Given their unique regenerative abilities, stem cells offer new potentials for treating diseases such as diabetes, and heart disease. However, much work remains to be done in the laboratory and the clinic to understand how to use these cells for cell-based therapies to treat disease, which is also referred to as regenerative or reparative medicine (NIH Stem Cell Information Home Page).

Laboratory studies of stem cells enable scientists to learn about the cells' essential properties and what makes them different from specialized cell types. Scientists are already using stem cells in the laboratory to screen new drugs and to develop model systems to study normal growth and identify the causes of birth defects (NIH Stem Cell Information Home Page).

Research on stem cells continues to advance knowledge about how an organism develops from a single cell and how healthy cells replace damaged cells in adult organisms. Stem cell research is one of the most fascinating areas of contemporary biology, but, as with many

expanding fields of scientific inquiry, research on stem cells raises scientific questions as rapidly as it generates new discoveries (NIH Stem Cell Information Home Page).

Mitochondria

The mitochondrion (plural mitochondria) is a double membrane-bound organelle found in all eukaryotic organisms. Some cells in some multicellular organisms may lack them (mature mammalian red blood cells). A number of unicellular organisms, such as microsporidia, parabasalids, and diplomonads, have also reduced or transformed their mitochondria into other structures. Mitochondria generate most of the cell's supply of adenosine triphosphate (ATP), used as a source of chemical energy. Mitochondria are commonly between 0.75 and 3 μm in diameter but vary considerably in size and structure. Unless specifically stained, they are not visible. In addition to supplying cellular energy, mitochondria are involved in other tasks, such as signaling, cellular differentiation, and cell death, as well as maintaining control of the cell cycle and cell growth. Mitochondrial biogenesis is in turn temporally coordinated with these cellular processes. Mitochondria have been implicated in several human diseases, including mitochondrial disorders, cardiac dysfunction, heart failure and autism. The number of mitochondria in a cell can vary widely by organism, tissue, and cell type. For instance, red blood cells have no mitochondria, where liver cells can have more than 2000. The organelle is composed of compartments that carry out specialized functions. These compartments or regions include the outer membrane, the intermembrane space, the inner membrane, and the cristae and matrix. Although most of a cell's DNA is contained in the cell nucleus, the mitochondrion has its own independent genome that shows substantial similarity to bacterial genomes. Mitochondrial proteins (proteins transcribed from mitochondrial DNA) vary depending on the tissue and the species. In humans, 615 distinct types of protein have been identified from cardiac mitochondria,

whereas in rats, 940 proteins have been reported. The mitochondrial proteome is thought to be dynamically regulated (Alberts, Johnson, Lewis, et. al., 2002).

Epidermal Cells

Human epidermal cells are the cells that are on the surface of the skin, the outer organ that encases the rest of the bodies' organs. Together with the dermis these cells form the cutis or skin. The epidermis is a stratified squamous epithelium, composed of proliferating basal and differentiated supra-basal keratinocytes. The epidermis is ectodermal (primary germ cell) in origin. The epidermis is avascular (not directly supplied by blood cells) nourished by diffusion from the dermis. Blood capillaries are found beneath the epidermis. The epidermis serves as a barrier to protect the body against microbial pathogens, oxidant stress and chemical compounds and provides mechanical resistance. Most of that function is played by the stratum corneum (Alberts, et. al., 2002).

The epidermis is composed of approximately 95% keratinocytes but also containing melanocytes, Langerhans cells, Merkel cells, and inflammatory cells. Rete ridges are epidermal thickenings that extend downward between dermal sensory organs. The epidermis is composed of 4 to 5 layers depending on the region of skin. In humans, the epidermis is thinnest on the eyelids at 0.05 mm and thickest on the palms of the hands and soles of the feet at 1.5 mm. The layers of the epidermis in descending order are: 1) the cornified layer (stratum corneum) which is composed of 10 to 30 layers of polyhedral, anucleated (without nucleus) corneocytes which are cells that are in the final step of keratinocyte differentiation. This is the skin that you see, and the palms of the hands and the soles of the feet have the most layers of this particular layer. Corneocytes are surrounded by a protein envelope filled with water-retaining keratin proteins, attached together through corneodesmosomes and surrounded in the extracellular space by

stacked layers of lipids. The extra layer is called the stratum lucidum, and is found only on the palms and soles, where an extra layer is apparent where the skin is the thickest. 2) The granular layer (stratum granulosum) is where keratinocytes lose nuclei and their cytoplasm appears granular. Lipids, contained in the keratinocytes within lamellar bodies, are released into the extracellular space through exocytosis to form a lipid barrier. Polar lipids are then converted into non-polar lipids and arranged parallel to the cell surface. 3) spinous layer (stratum spinosum) in this layer, Keratinocytes become connected through desmosomes and start to produce lamellar bodies from within the Golgi, enriched in polar lipids, glycosphingolipids, free sterols, phospholipids and catabolic enzymes. Langerhans cells, (immunologically active cells), are located in the middle of this layer. 4) basal/germinal layer (stratum basale/germinativum), this traditionally last layer before the dermis is composed mainly of proliferating and non-proliferating keratinocytes, attached to the basement membrane by hemidesmosomes. Melanocytes are present, connected to numerous keratinocytes through dendrites. Merkel cells are also found with large numbers in touch-sensitive sites such as the fingertips and lips. They are closely associated with cutaneous nerves and seem to be involved in light touch sensation. The epidermis is separated from the dermis, its underlying tissue, by a basement membrane (Alberts, et. al., 2002).

The stratified squamous epithelium is maintained by cell division within the stratum basale. Differentiating cells delaminate from the basement membrane and are displaced outwards through the epidermal layers, undergoing multiple stages of differentiation until, in the stratum corneum, losing their nucleus and fusing to squamous sheets, which are eventually shed from the surface. Differentiated keratinocytes secrete keratin proteins which contribute to the formation of an extracellular matrix and are an integral part of the skin barrier function. In normal skin, the

rate of keratinocyte production equals the rate of loss, taking about two weeks for a cell to journey from the stratum basale to the top of the stratum granulosum, and an additional four weeks to cross the stratum corneum. The entire epidermis is replaced by new cell growth over a period of approximately 48 days(Alberts, et. al., 2002).

Keratinocytes act as the body's major barrier against the environment by preventing pathogens from entering, making the skin a natural barrier to infection. The epidermis also regulates the amount of water released from the body into the atmosphere through trans-epidermal water loss. Keratinocyte differentiation throughout the epidermis is partially mediated by a calcium gradient, increasing from the stratum basale until the outer stratum granulosum, where it reaches maximum, and expires in the stratum corneum. Calcium concentration in the stratum corneum is very low in part because these relatively dry cells are not able to dissolve ions. This calcium gradient parallels keratinocyte differentiation and is considered a key regulator in the formation of epidermal layers. Laboratory culture of keratinocytes to form artificial skin mimics most of the properties of the epidermis and is routinely used as a tool for drug development and testing (Alberts, et. al., 2002).

Keratinocytes are formed by differentiation from epidermal stem cells residing in the lower part of the stratum basale of the epidermis, attached to the basement membrane through hemidesmosomes. Those stem cells and their differentiated progeny are organized into columns named epidermal proliferation units. Keratinocytes in the stratum basale layer of the epidermis are attached together through desmosomes and will proliferate through a few rounds of cell divisions within the stratum basale before moving up through the epidermis as they differentiate. During this differentiation process, keratinocytes permanently withdraw from the cell cycle, initiate expression of epidermal differentiation markers, and move suprabasally as they become

part of the stratum spinosum, stratum granulosum and eventually become corneocytes in the stratum corneum. At each stage of differentiation, keratinocytes express specific keratins (keratin 1, keratin 5, etc.) and other markers such as involucrin, loricrin, transglutaminase, filaggrin and caspase 14 (Alberts, et. al., 2002).

Calcium and Vitamin D3 (cholecalciferol) regulates keratinocyte proliferation and differentiation mostly by modulating calcium concentrations and regulating the expression of genes involved in keratinocyte differentiation. Keratinocytes are the only cells in the body with the entire vitamin D metabolic pathway from vitamin D production to catabolism and Vitamin D receptor expression. Other factors that regulate keratinocyte proliferation include Cathepsin E, TALE homeodomain transcription factors and Hydrocortisone. Since keratinocyte differentiation stops keratinocyte proliferation, factors that promote keratinocyte proliferation should be considered as preventing differentiation (Alberts, et. al., 2002).

Keratinocytes form tight junctions with the nerves of the skin and hold the Langerhans cells and intra-dermal lymphocytes in position within the epidermis. Keratinocytes also modulate the immune system. Keratinocytes contribute to protecting the body from ultraviolet radiation (UVR) by taking up melanosomes, vesicles containing the endogenous photo protectant melanin, from epidermal melanocytes. Each melanocyte in the epidermis has several dendrites that stretch out to connect it with many keratinocytes. The melanin is then stored within keratinocytes and melanocytes in the perinuclear area as supranuclear “caps”, where it protects the DNA from UVR-induced damage. Keratinocytes play a vital role in wound healing and reparation by the migration of keratinocytes to fill in the gap created by the wound. The first set of keratinocytes to participate in that repair come from the bulge region of the hair follicle and have limited survival. Within the healed epidermis they will be replaced by keratinocytes originating from the

epidermis. Epidermal keratinocytes can contribute to hair follicle formation during the healing of large wounds (Alberts, et. al., 2002).

Epidermal organogenesis, the formation of the epidermis, begins in cells covering the embryo after neurulation; the formation of the central nervous system. In most vertebrates, this original one-layered structure quickly transforms into a two-layered tissue; a temporary outer layer, the periderm, which is disposed once the stratum germinativum has formed. This inner layer is a germinal epithelium that gives rise to all epidermal cells. It divides to form the outer spinous layer (stratum spinosum). The cells of these two layers, (the Malpighian layer) divide to form the stratum granulosum of the epidermis. The cells in the stratum granulosum do not divide, but instead form skin cells called keratinocytes from the granules of keratin. These skin cells finally become the stratum corneum, where the cells become flattened sacks with their nuclei located at one end of the cell. After birth these outermost cells are replaced by new cells from the stratum granulosum and throughout life they are shed at a rate of 1.5 g per day. Epidermal development is a product of several growth factors, including transforming growth factor Alpha (TGFα); an autocrine growth factor by which basal cells stimulate their own division. Another growth factor that allows for epidermal development is Keratinocyte growth factor (KGF or FGF7) which is a paracrine growth factor produced by the underlying dermal fibroblasts in which the proliferation of basal cells is regulated (Alberts, et. al., 2002).

The barrier portion of the skin have several characteristics including: the physical barrier through keratinocytes attached together via cell–cell junctions and associated to cytoskeletal proteins, which gives the epidermis its mechanical strength; the chemical barrier, which acts through the presence of highly organized lipids, acids, hydrolytic enzymes and antimicrobial peptides. The immunologically active barrier acts through humoral and cellular constituents of

the immune system. Water content of the stratum corneum drops towards the surface, creating hostile conditions for pathogenic microorganism growth. An acidic pH (around 5.0) and low amounts of water make it hostile to many micro-organic pathogens. As the skin layers progress towards the dermis, the pH integrates closer to the homeostatic internal pH of 7.2-7.6. The presence of non-pathogenic microorganisms on the surface helps defend against pathogens by limiting food availability and through chemical secretions. Things that can alter the barrier include psychological stress, through an increase in glucocorticoids, and sudden and large shifts in humidity alter stratum corneum hydration in a way that could allow entry of pathogenic microorganisms. The ability of the skin to hold water is primarily due to the stratum corneum and is critical for maintaining healthy skin. Lipids arranged through a gradient and in an organized manner between the cells of the stratum corneum form a barrier to transepidermal water loss. The outer layer of skin is traditionally considered 'dead', but still possesses osmotic transference abilities and viability in limited form. A number of structural proteins (filaggrin, keratin), enzymes (proteases), lipids and antimicrobial peptides (defensins) contribute to maintain the important barrier function of the skin. Keratinization is part of the physical barrier formation (cornification), in which the keratinocytes produce more and more keratin and eventually undergo programmed cell death. The fully cornified keratinocytes that form the outermost layer are constantly shed off and replaced by new cells (Alberts, et. al., 2002).

The amount and distribution of melanin pigment in the epidermis is the main reason for variation in skin color in Homo sapiens. Melanin is found in the small melanosomes, particles formed in melanocytes from where they are transferred to the surrounding keratinocytes. The size, number, and arrangement of the melanosomes vary between racial groups, but while the number of melanocytes can vary between different body regions, their numbers remain the same in

individual body regions in all human beings. In white and oriental skin, the melanosomes are packed in "aggregates", but in black skin they are larger and distributed more evenly. The number of melanosomes in the keratinocytes increases with UV radiation exposure, while their distribution remains largely unaffected (Alberts, et. al., 2002).

Cell Signaling/Cell voltage change

Cell Signaling is a chemical response method in which one cell sends information to another cell through K+ Na+ pathways. This is also how cells communicate with each other, particularly when they are going through cell cycle changes or environmental stimuli changes. The mitochondria in their ATP production process communicate to other cells through cell membrane potential, a chemically reactive positive or negative charge that goes through one membrane to another. This also produces the noticeable cell voltage, through energy production from the electrolytes produced with ATP production. A higher level of resistance indicates homeostasis within the cell pathways and the stability of glycolysis, while a higher rate of conductivity shows instability in the cell cycle and pathways, therefore an interruption in the conductivity, through an excess of Na+ processing (Alberts, Johnson, Lewis, et. al., 2002).

Cell Cycle

The human body relies on DNA within the nucleus of a cell to send out instructions for body functions and body energy usage. A huge amount of information resides within the DNA and through cellular reproduction processes can separate genetic material and share it via cell division. These methods are called mitosis and meiosis. Mitosis is a process that produces two cells, each of which is identical to the original parent cell. Mitosis is preceded by replication of the cell's DNA so each 'daughter cell' will have a full amount of genetic material. Traditionally in animals, mitosis is used for growth and repair of somatic body cells. This generally leads to

asexual reproduction. Meiosis produces four cells from an original parent cell that is not identical to the parent cell, and only has half the parent cell's genetic matter. This is traditionally known as sexual reproduction. (Alters and Alters, 2008)

The cell cycle is the life cycle of the cell. These include prophase, prometaphase metaphase, anaphase telophase, interphase and cytokinesis. The cell cycle spends the most amount of time in interphase. This is the part of the cell cycle that includes cell growth, replication of cell organelles, replication of DNA, assembly of the parts of mitosis and the condensation of DNA. This phase is subdivided into stages G_1, S and G_2. The G_1 stage is the time gap between the last cell division and the start of DNA replication, during which time the cell is growing. This growth period occupies the major portion of the cell's lifespan, where the cell doubles its size and carries out its normal life functions. S (Synthesis) stage produces a complete replica of the cell's DNA for cell division. By the end of this stage, the cell contains two complete, identical copies of the hereditary information. The G_2 stage signifies the time gap between the end of DNA replication and the beginning of cell division. The coils of DNA condense into tightly compacted masses that become visible chromosomes during mitosis. Each chromosome contains two copies of hereditary information in sister chromatids, connected by a centromere. Mitosis is a continuous sequence of events that occurs just after interphase, resulting in the separation of the sister chromatids. (Alters and Alters, 2008)

The first phase of mitosis, prophase is when the chromosomes have condensed. As prophase continues, the chromosomes continue to shorten and thicken. The nucleolus disappears because the cell is no longer capable of producing ribosomal RNA (rRNA). The microtubules (thin tubes of protein structures) are formed. The centrosome is the area in which these microtubules are organized. The centrosomes of animal cells begin to move away from each

other in the beginning and by the end, each member of the pair has moved to an opposite end or pole of the cell. As the spindle fibers formulate, the nuclear membrane breaks down and the spindle fibers build a bridge between centrosomes from one pole to another. The spindle fibers then attach to kinetochores. This connection is critical to the separation and movement of sister chromatids during later stages of mitosis. The next stage of animal cell mitosis is metaphase. This occurs when the pairs of sister chromatids align in one plane at the center of the cell. This will indicate where the future plane of the cell division will be. After metaphase comes anaphase. In this phase, the sister chromatids are pulled in two different directions simultaneously. The chromatids separate into chromosomes and move rapidly toward opposite poles of the cell. They are pulled by the kinetochore by attached and shortening microtubules. This separation produces duplicate sets of hereditary material. The final phase, telophase is the preparation of the cell for cytokinesis. The spindle fibers are chemically disassembled and disappear. The nuclear envelope reforms around each set of chromosomes, which begin to uncoil, and the nucleolus reappears as rRNA. This phase is like prophase in reverse order. (Alters and Alters, 2008)

Mitosis is complete after telophase. The process of cell division is not. The portion of the cell outside the nucleus; cytoplasm is divided starting in telophase and completed in cytokinesis, where the separation of one cell into two takes place. In animal cells, this occurs by pinching the cell in two by a belt of microfilaments encircling the cell at the metaphase stage. As the microfilaments contract, a cleavage furrow appears around the circumference of the cell. As the contraction proceeds the furrow deepens until the opposing edges of the cell membrane make contact with one another. The membranes fuse, producing the cell separation. (Alters and Alters, 2008)

Programmed Cell Death

Cancer and cellular toxicity causes mitochondria to be ‘reprogrammed’, and in the case of tumorigenic activity, the cells are encapsulated and oxygen is cut off, therefore forcing the mitochondria to work in anaerobic conditions, forcing glycolysis. Glycolysis is a series of biochemical reactions from which one molecule of glucose is oxidized to two molecules of pyruvic acid and a small amount of ATP. Increased activity in the glycolytic pathway is an indicator of disease in humans. Malignant, rapidly-growing tumor cells have glycolytic rates that are up to 200 times higher than those of their normal tissues of origin (NCI, 2014).

Mitochondria are also suspected to play a role in the aging process. Once the mitochondria are forced into glycolysis, they continue to feed cancerous mutations. If these reprogrammed mitochondria are shut off or eliminated, then glycolysis can be shut down, and the surrounding cells can either escape the cancerous mutation cycle, or can be remediated. The mitochondria can also be re-programmed to return to the production of ATP as a primary energy source, which cancerous material is not as prone to reproduce under. Cancerous material feeds off of sugar based chemicals for their energy source and relies heavily on carbohydrates, to reproduce biochemically. Certain chemicals interrupt these carbohydrate and anaerobic cycles, forcing oxygen into the reaction, and balancing off any free radical reactions (American Cancer Society, 2012).

Genetically programmed cell death (apoptosis, PCD), is one of the many concepts that is used to treat cancer. Some cells of the human body self-destruct after a limited lifespan, while others are programmed to last the lifetime of the organism. Those that self-destruct are replaced by the body with new cells produced from cell division of the survivors. Cancer is an indicator of a disruption of the cell’s life cycle, where cells that should die do not and develop from failure of programmed cell death and divide uncontrollably. Apoptosis is a type of PCD in which cell

suicide is pre-programmed; the cell membrane remains intact as the cell dies so that it does not release its contents and trigger a local inflammatory reaction. The dying cell splits into small membrane-bound bodies that are engulfed and digested by white blood cells. Many compounds have been tested as apoptosis agents (Alters and Alters, 2008).

Programmed Cell Death or PCD has had three main pathways identified: Type I: Apoptosis, Type II: Autophagisis and Type III: Necrosis. Apoptosis, as described above, is cell death produced by biochemical changes that lead to morphology changes and death. These morphology changes include blebbing, (irregular bulge in the plasma membrane caused by localized decoupling of the cytoskeleton from the plasma membrane) membrane cell shrinkage, nuclear fragmentation, chromatin condensation, and chromosomal DNA fragmentation.

Apoptosis occurs naturally in the cell cycle, including in the development of body parts. Apoptosis is responsible for appendages such as fingers and toes to be separated, and can either lead to mutagenic qualities or death of the cell. This process produces cell fragments (apoptotic bodies) that phagocytic cells engulf and remove before the contents of the cell can spill out and cause damage in surrounding cells. The process of apoptosis can be controlled by a large and diverse range of cell signals that can originate extracellularly (extrinsic inducers) which include toxins, hormones, growth factors, nitric oxide or cytokines or intracellularly (intrinsic inducers). These inducers must either cross the plasma membrane or transducer to produce a response; the positive or negative triggering of apoptosis. The cell will initiate intracellular apoptotic signaling in response to stresses, which can potentially lead to a ‘cell suicide’. The cell death is precipitated by enzymes, produced by the apoptotic signals that cause regulatory proteins to initiate apoptosis, which allows the selection of apoptosis in certain cells, or can stop the process should the cell no longer need to die. These two main methods of regulation are targeting

mitochondria functionality, or transducing the signal through adaptor proteins to apoptotic mechanisms. Apoptosis occurs very quickly (NCI, 2014).

Viral proteins can cause intracellular stress which induces apoptosis. This response to internal stimuli causes a caspase cascade which in turn triggers a series of morphological changes within the cell. Once the caspase cascade has started, it is an irreversible process resulting in certain cell death. Apoptosis in HeLa cells is inhibited by proteins produced by the cell. Inhibitory proteins target retinoblastoma tumor suppressing proteins. These tumor suppressing proteins regulate the cell cycle, but are rendered inactive when bound to an inhibitory protein (Oyagbemi, et. al., 2010).

Apoptotic morphology is as follows:

1. **Cell shrinkage and rounding because of the breakdown of the proteinaceous cytoskeleton by caspases (executioner proteins). Cell swelling can also be noted.**
2. **The cytoplasm appears dense, and the organelles appear tightly packed.**
3. **Chromatin undergoes condensation into compact patches against the nuclear envelope (pyknosis)**
4. **The nuclear envelope becomes discontinuous and the DNA inside it is fragmented. (karyorrhexis) The nucleus breaks into several discrete chromatin bodies or nucleosomal units due to the degradation of DNA.**
5. **The cell membrane shows blebs.**
6. **The cell breaks apart into several vesicles called apoptotic bodies, which are then phagocytosed (NCI, 2014).**

Type II PCD is autophagisis; a catabolic process that involves the degradation of a cell's components through lysosomal machinery, or the bursting of the cells' components through cellular damage. This is a tightly regulated process that is generally involved normally in cell growth, development and homeostasis. It helps to maintain a balance between the synthesis, degradation and subsequent recycling of cellular products. This is a mechanism where a starving cell can reallocate nutrients from unnecessary processes to necessary ones (NCI, 2014).

Type III PCD is necrosis, which is the premature death of cells in living tissue, caused by external factors including infections, toxins or trauma. Necrosis is almost always detrimental. Cells that die due to necrosis do not send chemical signals to the immune system, so phagocytes do not locate and engulf the dead cells which in turn lead to a build-up of dead tissue and cell debris. Necrotic tissue generally has to be removed surgically in a process called debridement (NCI, 2014).

Other pathways of PCD are being discovered including necroptosis, the opposite of apoptosis, with less necrotic final result, anoikis is a form of apoptosis which is induced by anchor-dependent cells detaching from the surrounding extracellular matrix. Excitotoxicity is a pathological process by which nerve cells are damaged and killed through excessive stimulation by neurotransmitters (NCI, 2014). There is the regeneration or reconstitution of cells that have undergone apoptosis, but then the cell matter regenerates in another location, and can even go so far as to reproduce a cell layer. A cell layer is a group of cells that attach to one another to produce a larger field of cells. Cancerous cell layers lead to tumors, but healthy cell layers lead to tissue. Certain chemicals have been found to have this characteristic, where the actual cell components are not destroyed, and allowed to in essence be 'reprogrammed' to regenerate.

Healthy cells reconstitute their own parts, and resume the cellular cycle, albeit temporarily as these cells are structurally unsound (Alberts, et. al., 2002).

Cell swelling is an indicator of sub-lethal cell damage, where the cell's membranes swell outward as certain substances enter the cell through osmosis, and cause an imbalance of the cell structure. The cell can be recovered, or it can enter apoptosis from this stage. The swelling is an indicator of the accumulation of electrolytes or inducers within the cell beyond the cell's normal capacity or functioning point. Apoptosis is generally signaled by cell shrinkage or cell swelling (NCI, 2014).

Cytotoxicity

Cytotoxicity is the quality of being toxic to cells. Treating cells with the cytotoxic compound can result in a variety of cell fates. The cells may undergo necrosis, in which they lose membrane integrity and die rapidly as a result of cell lysis. The cells can stop actively growing and dividing (a decrease in cell viability), or the cells can activate a genetic program of controlled cell death (apoptosis). Cells undergoing necrosis typically exhibit rapid swelling, lose membrane integrity, shut down metabolism and release their contents into the environment. Cells that undergo rapid necrosis in vitro do not have sufficient time or energy to activate apoptotic machinery and will not express apoptotic markers. Apoptosis is characterized by well-defined cytological and molecular events including a change in the refractive index of the cell, cytoplasmic shrinkage, nuclear condensation and cleavage of DNA into regularly sized fragments. Cells in culture that are undergoing apoptosis eventually undergo secondary necrosis. They will shut down metabolism, lose membrane integrity and lyse. Assessing cell membrane integrity is one of the most common ways to measure cell viability and cytotoxic effects. Compounds that have cytotoxic effects often compromise cell membrane integrity. Vital dyes,

such as trypan blue or propidium iodide are normally excluded from the inside of healthy cells; however, if the cell membrane has been compromised, they freely cross the membrane and stain intracellular components. Alternatively, membrane integrity can be assessed by monitoring the passage of substances that are normally sequestered inside cells to the outside. One molecule, lactate dehydrogenase (LDH), is commonly measured using LDH assay. LDH reduces NAD to NADH which elicits a color change by interaction with a specific probe. Protease biomarkers have been identified that allow researchers to measure relative numbers of live and dead cells within the same cell population. The live-cell protease is only active in cells that have a healthy cell membrane, and loses activity once the cell is compromised and the protease is exposed to the external environment. The dead-cell protease cannot cross the cell membrane, and can only be measured in culture media after cells have lost their membrane integrity. The toxicity of cells can lead to disease implications in the form of dysfunction and carcinogen (Alberts, et. al, 2002).

Adenosine Triphosphate (ATP)

ATP is considered to be the energy currency of life. It is the high-energy molecule that stores the energy we need for just about everything in life. ATP is present in the cytoplasm and nucleoplasm of every cell. Essentially all of the physiological mechanisms that require energy use the stored ATP in the cell. As food in the cells is gradually oxidized (combined chemically with oxygen) the energy that is released is used to restore the ATP so that the cell always has a supply of this very important molecule. (In animal systems the ATP is formed in the mitochondria.) Living things can use ATP as a sort of "battery". It can power needed reactions by losing one of its phosphorous groups to form ADP, but use the energy from food to then convert it back to ATP so that it can go back to its work as life's battery.

Krebs Cycle

In plants sunlight energy can be used to convert the less active compound back to the highly energetic form. But for animals you use the energy from your high energy storage molecules to do what you need to stay alive, after that you “recharge” them to put them back in the high energy state. This process is called the TCA cycle or Krebs Cycle. Krebs Cycle is the sequence of reactions by which most living cells generate energy during the process of respiration. Like ATP it takes place in the mitochondria. During that process there is consuming of oxygen, producing carbon dioxide and water as a waste product. This all leads up to the conversion of ADP to energy-rich ATP.

Problem Definition:

Stem cell therapy is some of the newest and most innovate medicinal sciences to date. Due to this, the full potential of stem cells has not been fully tapped. Essentially, stem cells are the body's building blocks- the basic and unprocessed materials that construct a variety of human life forms. Stem cells can be categorized into two types based off of where they are harvested in the body- Embryonic and Adult. Embryonic stem cells are collected from Embryos, while Adult stem cells are collected from tissues within the body. Despite the fact that these are both viable forms of stem cells, adult stem cells are more difficult to use for regeneration and give rise to a lesser variety of how they can be utilized. However, Embryonic stem cells have a high versatility, making them favorable to use for stem cell therapy.

When a human is developing, stem cells regenerate and form daughter cells. These daughter cells can then either be used to generate more stem cells or generate new cells with specialized functions. This process happens on a regular basis within the human body. For instance, when someone suffers from a cut, the cut typically bleeds. After the bleeding has stopped, a scab forms. In a matter of days, the scab fades and the injured body is back to normal. This process occurs because the body and its' supply of stem cells work together to heal itself. Based off of this premise, stem cells can therefore be harvested, manipulated, and specialized depending on what is needed on a case-by-case basis, and therefore reduce recovery time. These fundamental basics behind stem cell therapy can then be utilized at an increased rate, contributing to the reduction of fatal diseases and injuries.

Problem Solution:

Using NetLogo, we have been able to model what stem cells are and how they can be harnessed to combat different types of cancer and diseases within the body. Particularly, we have modeled the regeneration of stem cells, and how they can be used to combat cancerous tumors. We hope this model, and all of its variations, can highlight the true potential of stem cells and stem cell therapy, while continuing to advance the application and widespread implementation of it. Essentially, we just wish to see this project go beyond the challenge and into the future of applied medicinal science.

Expected Results:

The expected results of the model are that after new stem cells are introduced to the tumor, the daughter cells that are created turn into white blood cells and kill the tumor. We expect that our stem cell code overlaid onto the NetLogo tumor model should give us these results. If not, the tumor will not die and our model will be incorrect. We also expect that after the addition of new variables and our other intended stem cell methodologies, that this model will represent what is currently being tested in clinical trials with both qualitative and quantitative data.

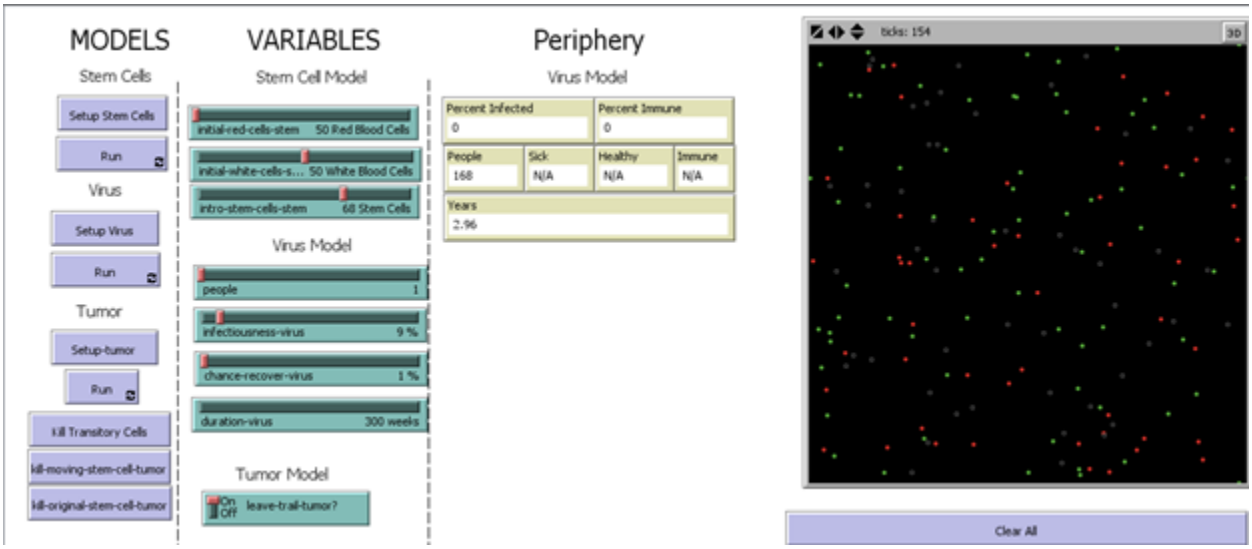
Software and Code (validation of model):

Our supercomputing code references models in the model library provided by NetLogo. We referenced the following models: virus, tumor, enzyme kinetics, aids, disease solo, and plant growth. These models are made by Uri Wilensky. We did this because we felt as if each of these generic models had some aspect of something we wanted in our model. We then compiled the code using NetLogo version 6.0.4.

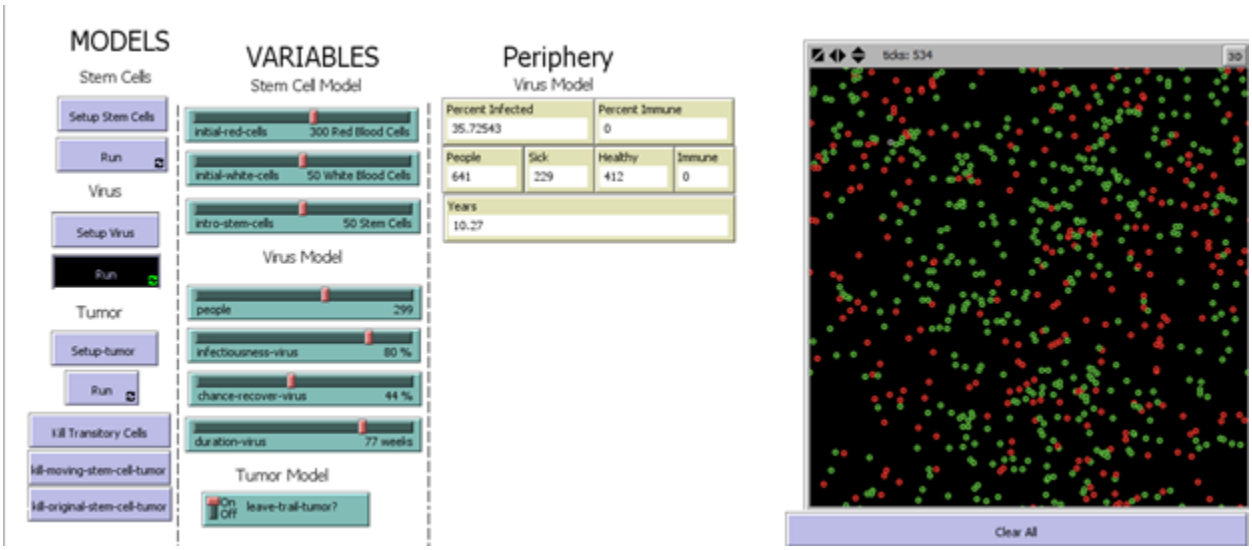
The first step coding or model was to integrate the references into one working NetLogo program allowing us to run the program using continuous abstractions of the different models. Part of this was done by renaming each procedure and its variables with unique identifiers to eliminate errors. The code was also divided into sections to comprehensively convey the associated model. We commented out the name of each model to be the header for the following code. Uri Wilensky commented the supplemented code that was implemented into the program.

The interface section of the code was separated into four categories: the individual models, variables, periphery, and then the model output. Each model had a “setup” procedure that would clear the outputs and prepare it for the associated model’s “run” procedure. Each model would uniquely decide the mechanisms the turtles would use to interact in the model. The variables section would allow you to access parameters that would impact the model output. Additionally, the periphery section displayed auxiliary outputs for the associated running model. If running a different model than the periphery anticipated then the results would be skewed. After we assimilated the code the expansion displays the benefits of stem cell utilization in medical practices.

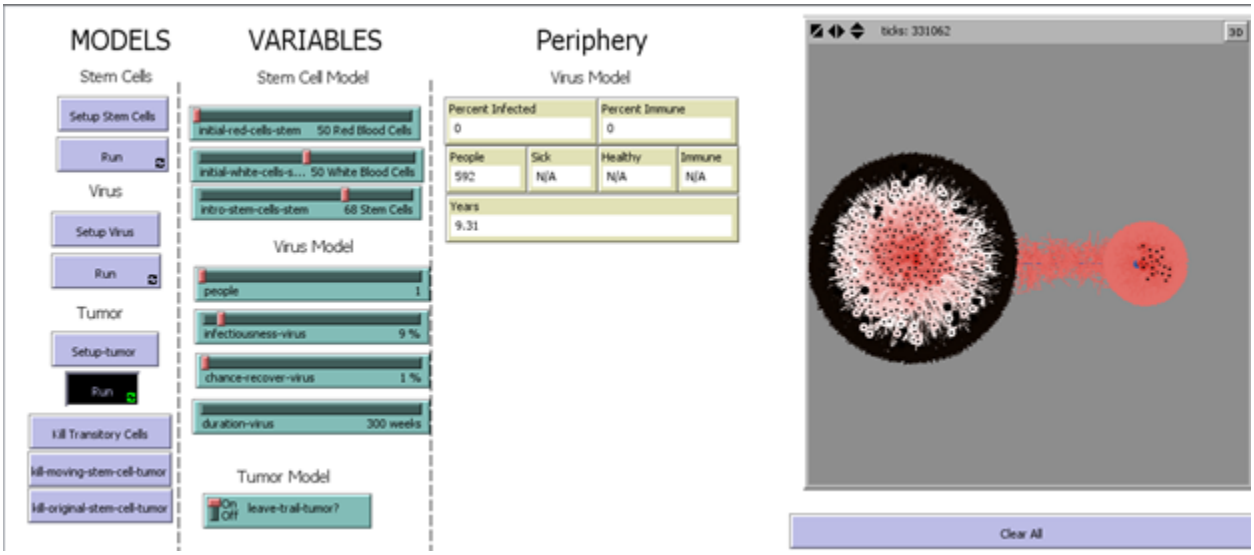
Stem cell model:



Virus model:



Tumor model:


MODELS
VARIABLES
Periphery
Stem Cells
Stem Cell Model
Virus Model
Setup Stem Cells
Run
initial-red-cells-stem 50 Red Blood Cells
initial-white-cells-s... 50 White Blood Cells
intro-stem-cells-stem 68 Stem Cells
Percent Infected
0
Percent Immune
0
People
592
Sick
N/A
Healthy
N/A
Immune
N/A
Years
9.31
Virus
Setup Virus
Run
Virus Model
people 1
infectiousness-virus 9 %
chance-recover-virus 1 %
duration-virus 300 weeks
Tumor
Setup-tumor
Run
Kill Transitory Cells
kill-moving-stem-cell-tumor
kill-original-stem-cell-tumor
Tumor Model
On Off leave-trail-tumor?
ticks: 331062
3D
Clear All

```
 ;;Supercomputing Challenge 2019
 ;;Delaney Galligan
 ;;Cyrus O'Hern
 ;;Kyle Totman

turtles-own
[ ;;; Stem Cell Code

  rejuvenated-stem? ;; If true, the cell is rejuvenated.  It may be known or unknown.

  ;;; Virus Code

  sickness-virus?        ;; if true, the turtle is infectious
  immune-virus?      ;; if true, the turtle can't be infected
  sickness-virus-count   ;; how long the turtle has been infectious
  age-virus        ;; how many weeks old the turtle is

  ;;; Tumor Code

  stem-tumor?     ;; true for stem cells, false for transitory cells
  age-tumor  ;; age of cell. changes color with age
  metastatic-tumor?  ;; false for progeny of stem cell 0, true for progeny of stem cell 1
]
globals
[
  ;;;Virus Code

  %infected-virus                 ;; what % of the population is infectious
  %immune-virus                   ;; what % of the population is immune-virus
  lifespan-virus                  ;; the average-virus lifespan-virus of a turtle
  average-virus-offspring-virus   ;; the average-virus number of offspring a turtle could have
  carrying-capacity-virus         ;; the number of turtles that can be in the world at one time

  ;;; Tumor Code

  cell-count-tumor
]
;;; Stem Code ;;; ;;;      ;;;      ;;;      ;;;      ;;;      ;;;      ;;;      ;;;      ;;;      ;;
;;; Stem Code ;;; ;;;      ;;;      ;;;      ;;;      ;;;      ;;;      ;;;      ;;;      ;;;      ;;
;;; Stem Code ;;; ;;;      ;;;      ;;;      ;;;      ;;;      ;;;      ;;;      ;;;
to setup-stem
  clear-all
  setup-cells-stem
  setup-white-cells-stem
  setup-stem-cells-stem
  move-stem
  reset-ticks
end

;; Different cells are displayed in 3 different colors
;; green are stem cells, white are white blood cells, red are red blood cells

to setup-cells-stem
  crt initial-red-cells-stem
    [ setxy random-xcor random-ycor
        set shape "circle"
        set color red
    ]
end
```

```
to setup-white-cells-stem
  crt initial-white-cells-stem
  [setxy random-xcor random-ycor
        set shape "circle 2"
        set color white
        set heading (random 360)
        repeat 5 [
        rt random 100
        lt random 100
        forward 1
        ]
  ]
  end

to setup-stem-cells-stem
  crt intro-stem-cells-stem
  [setxy random-xcor random-ycor
    set shape "circle"
    set color green
  ]
end

to move-stem  ;; cells move about at random.
  ask turtles
  [
    rt random 100
    lt random 100
    forward 1
    ]
end

to go-stem
  move-stem
  tick
end
;;; Virus Code ;;; ;;;      ;;;      ;;;      ;;;      ;;;
;;; Virus Code ;;; ;;;      ;;;      ;;;      ;;;      ;;;
;;; Virus Code ;;; ;;;      ;;;      ;;;      ;;;      ;;;


to setup-virus
  clear-all
  setup-constants-virus
  setup-turtles-virus
  update-global-variables-virus
  update-display-virus
  reset-ticks
end
```

```
;; We create a variable number of turtles of which 10 are infectious,
;; and distribute them randomly
to setup-turtles-virus
  create-turtles number-people-virus
    [ setxy random-xcor random-ycor
      set age-virus random lifespan-virus
      set sick-time-virus 0
      set remaining-immunity-virus 0
      set size 1.5  ;; easier to see
      get-healthy-virus ]
  ask n-of 10 turtles
    [ get-sick-virus ]
end

to get-sick-virus ;; turtle procedure
  set sick-virus? true
  set remaining-immunity-virus 0
end

to get-healthy-virus ;; turtle procedure
  set sick-virus? false
  set remaining-immunity-virus 0
  set sick-time-virus 0
end

to become-immune-virus ;; turtle procedure
  set sick-virus? false
  set sick-time-virus 0
  set remaining-immunity-virus immunity-duration-virus
end

;; This sets up basic constants of the model.
to setup-constants-virus
  set lifespan-virus 50 * 52      ;; 50 times 52 weeks = 50 years = 2600 weeks old
  set carrying-capacity-virus 300
  set chance-reproduce-virus 1
  set immunity-duration-virus 52
end

to go-virus
  ask turtles [
    get-older-virus
    move-virus
    if sick-virus? [ recover-or-die-virus ]
    ifelse sick-virus? [ infect-virus ] [ reproduce-virus ]
  ]
  update-global-variables-virus
  update-display-virus
  tick
end
```

```
to update-display-virus
  ask turtles
    [ if shape != turtle-shape-virus [ set shape turtle-shape-virus ]
      set color ifelse-value sick-virus? [ red ] [ ifelse-value immune-virus? [ grey ] [ green ] ] ]
end

;;Turtle counting variables are advanced.
to get-older-virus ;; turtle procedure
  ;; Turtles die of old age once their age exceeds the
  ;; lifespan (set at 50 years in this model).
  set age-virus age-virus + 1
  if age-virus > lifespan-virus [ die ]
  if immune-virus? [ set remaining-immunity-virus remaining-immunity-virus - 1 ]
  if sick-virus? [ set sick-time-virus sick-time-virus + 1 ]
end

;; Turtles move about at random.
to move-virus ;; turtle procedure
  rt random 100
  lt random 100
  fd 1
end

;; If a turtle is sick, it infects other turtles on the same patch.
;; Immune turtles don't get sick.
to infect-virus ;; turtle procedure
  ask other turtles-here with [ not sick-virus? and not immune-virus? ]
    [ if random-float 100 < infectiousness-virus
      [ get-sick-virus ] ]
end

;; Once the turtle has been sick long enough, it
;; either recovers (and becomes immune) or it dies.
to recover-or-die-virus ;; turtle procedure
  if sick-time-virus > duration-virus                    ;; If the turtle has survived past the virus' duration, then
    [ ifelse random-float 100 < chance-recover   ;; either recover or die
      [ become-immune-virus ]
      [ die ] ]
end

;; If there are less turtles than the carrying-capacity
;; then turtles can reproduce.
to reproduce-virus
  if count turtles < carrying-capacity-virus and random-float 100 < chance-reproduce-virus
    [ hatch 1
      [ set age-virus 1
        lt 45 fd 1
        get-healthy-virus ] ]
end

to-report immune-virus?
  report remaining-immunity-virus > 0
end

to startup-virus
  setup-constants-virus ;; so that carrying-capacity can be used as upper bound of number-people slider
end
```

```
;;; Tumor Code ;;; ;;;      ;;;      ;;;      ;;;      ;;;      ;;;
;;; Tumor Code ;;; ;;;      ;;;      ;;;      ;;;      ;;;      ;;;
;;; Tumor Code ;;; ;;;      ;;;      ;;;      ;;;      ;;;      ;;;

to setup-tumor
  clear-all
  set-default-shape turtles "circle 3"
  ask patches
    [ set pcolor gray ]
  set-stem-tumor
  evaluate-params-tumor
  reset-ticks
end

to set-stem-tumor   ;;create two stem cells
  create-turtles 2
  [
    set size 2   ; easier to see
    setxy (min-pxcor / 2) 0
    set stem-tumor? true
    set metastatic-tumor? false
    set color blue
    set age-tumor 0
  ]
  ask turtle 1
  [
    set metastatic-tumor? true
    set heading 90            ;; stem cell 1 will move away
  ]
  set cell-count-tumor 2
end

to go-tumor
  ask turtles
  [
    ifelse leave-trail-tumor?
      [ pen-down ]
      [ pen-up ]
    if (who = 1) and (xcor < 25)
    [ fd 1 ]  ;stem cell movement
    set age-tumor age-tumor + 1
    move-transitional-cells-tumor
    mitosis-tumor
    death-tumor
  ]
  tick
  evaluate-params-tumor
end
```

```
;;transitional cells move and hatch more. Turtle proc.
to move-transitional-cells-tumor
  if (not stem-tumor?)
  [
    set color ( red + 0.25 * age-tumor )
    fd 1
    if (age-tumor < 6)
    [
      hatch 1
      [  ;amplification
        rt random-float 360
        fd 1
      ]
    ]
  ]
end

to mitosis-tumor ;; turtle proc. - stem cells only
  if stem-tumor?
  [
    hatch 1
    [
      fd 1
      set color red
      set stem-tumor? false
      ifelse (who = 1)
        [ set age-tumor 16 ]
        [ set age-tumor 0 ]
    ]
  ]
end

to death-tumor   ;; turtle proc.
  if (not stem-tumor?) and (not metastatic-tumor?) and (age-tumor > 20)
    [ die ]
  if (not stem-tumor?) and metastatic-tumor? and (age-tumor > 4)
    [ die ]
end

to evaluate-params-tumor
  set cell-count-tumor count turtles  ;cell count
  if (cell-count-tumor <= 0)
    [ stop ]
end

to kill-original-stem-cell-tumor
  ask turtle 0
    [ die ]
end

to kill-moving-stem-cell-tumor
  ask turtle 1
    [ die ]
end

to kill-transitory-cells-tumor
  ask turtles with [ age-tumor < 10 and not stem-tumor? ]
    [ die ]
end
```

Results and Conclusion:

Qualitatively, our model works exactly as designed. The tumor spawns and grows as in real life. All of the colors stay as they should be, even after the stem cells are introduced. The stem cells also do their job by creating daughter cells that turn into white blood cells to help kill the tumor. However, at this point in time, we do not have the model producing very useful quantitative data. Although this may be the case right now, our team will be continuing work on our model up until the expo in hopes of completing a closer version of a model to the one that we are striving for. We hope to have also added in the modeling of the specialization and manipulation of stem cells, how stem cells are harvested, how stem cells work together in the body, and how they can be used to combat a variety of disease other than cancerous tumors.

Achievements

This year, the biggest achievement we have accomplished is being able to say that we have completed the challenge for our final year as members. As it was our senior year this year, we struggled managing our time between honors and AP classes, college classes, jobs, scholarship and college applications, and activities like the Challenge. Additionally, our school suffered from a ransomware attack that left our personal Z-Drives, filled with research, code, and important information pertaining to the challenge, unrecoverable. This ransomware attack left our computer lab at school shut down for over a week and our Z-Drives have still yet to be recovered.

Despite the struggles, we were still able to learn and comprehend a lot about NetLogo and the topic of our project as a whole. Finally, we can say that we've achieved pride in what we have produced this year with the model, PowerPoint presentation, and presentation board. We hope to do very well at the Supercomputing Expo in late April and bring some satisfaction and congratulations to a rather challenging year.

Acknowledgements

Our team would like to thank the following for everything they did to help us throughout the Challenge this year:

- Tracy Galligan
- Taos Municipal Schools
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- Sandia National Labs
- The entire Supercomputing Challenge Consult and Staff
- Judges
- Volunteers
- Our valued friends, family, and loved ones

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