A Model Using Yeast to Visualize Immunotherapy

Laura Landry
Memphis University School
6191 Park Ave.
Memphis, TN 38119
laura.mccormick@musowls.org

Mentored by
Dr. Maureen McGargill
St. Jude Children's Research Hospital
Department of Immunology
Memphis, TN 38105

Grant funded by The American Association of Immunologists 2017-2018

Table of Contents:

Introduction

This simulation model, which uses a living cell system, was developed for high school students to set up in the lab to teach them the benefits of removing the brakes from T cells that are fighting cancer. The entire unit can be completed in **four or five** days, does not need expensive or potentially dangerous equipment, and requires only basic lab skills to complete.

Our body uses the immune system to protect us from foreign pathogens that can invade our bodies leading to damage and disease. The immune system also protects the body from abnormally growing cells that can lead to tumors. A basic understanding of the immune system is necessary for the successful completion of this unit. This unit is designed to help students learn how the immune system and specifically T cells are used in **immunotherapy** to fight cancer. Immunotherapy is a treatment that recently has shown great promise in treating many types of cancer. It targets the body's own immune system to attack and eliminate tumors. Checkpoint inhibitor therapy is one type of immunotherapy that works by blocking negative regulatory molecules on T cells, which enables the T cells to clear the tumor.

The lab component of this unit is a hands-on innovative model to demonstrate the principles of cancer immunotherapy. In particular, the project will demonstrate the concepts of checkpoint inhibitor therapy. The model consists of confluent yeast cells growing on an agar plate to represent the tumor. Glass beads will symbolize the T cells. To demonstrate that the T cells have some anti-tumor activity, but become exhausted, the beads will be coated in an antifungal (CaviCide) before they are added to the plate containing the tumor. In this way, the yeast in proximity to the beads will be killed, but will not be sufficient to kill the tumor completely. To demonstrate the injection of a checkpoint inhibitor, an antifungal will be added to the whole plate. This protocol presents an immunotherapy model that can be set up by high school biology students. It is designed to be relatively low cost and require little specialized equipment so that it may be more easily adapted. This model will help students visualize the role of the immune cells combined with immunotherapy drugs in fighting and killing tumor cells.

Student Outcomes and Learning Objectives

Student Outcomes

1. Students will add to their knowledge of the biology of the immune system, including how T cells can attack tumors.
2. Students will learn and apply the basic principles of how immunotherapy is used to fight cancer.
3. Students will use yeast as a tumor model to set up a simulated, controlled experiment to test 'antibody-based drugs'.
4. Students will see the effects of various 'drugs' on cells in culture and analyze the growth of cells in each 'tumor'.
5. Students will simulate removing the checkpoint controls on T cells.
6. Students will model the effect of T cells in the immune system.
7. Students will practice using aseptic technique and micropipetting skills.
8. Students will conduct scientific research on immunotherapy (this can be optional and based on time available)

Learning Objectives

As a result of having completed this unit, students will:

- Grow yeast in culture using aseptic technique.
- Conduct a proper, controlled experiment using yeast as a tumor model.
- Apply the principles of immunotherapy and simulate removing checkpoint inhibitors on T cells
- Observe the range of effects that various treatments have on the 'tumor' and document the observations.
- Collect quantitative data on the growth per plate and compare using a bar or line graph.
- Write up a complete lab report and pass a test

State of Tennessee Educational Standards
Biology I

Embedded Inquiry

CLE 3210.Inq.1 Recognize that science is a progressive endeavor that reevaluates and extends what is already accepted.

CLE 3210.Inq.2 Design and conduct scientific investigations to explore new phenomena, verify previous results, test how well a theory predicts, and compare opposing theories.

- ✓ **3210.Inq.2** Conduct scientific investigations that include testable questions, verifiable hypotheses, and appropriate variables to explore new phenomena or verify the experimental results of others.

CLE 3210.Inq.3 Use appropriate tools and technology to collect precise and accurate data.

CLE 3210.Inq.4 Apply qualitative and quantitative measures to analyze data and draw conclusions that are free of bias.

- ✓ **3210.Inq.4** Determine if data supports or contradicts a hypothesis or conclusion.

SPI 3210 Inq.5 Defend a conclusion based on scientific evidence.

CLE 3210.Inq.5 Compare experimental evidence and conclusions with those drawn by others about the same testable question.

- ✓ **3210.Inq.5** Compare or combine experimental evidence from two or more investigations.
- ✓ **3210.Inq.6** Recognize, analyze, and evaluate alternative explanations for the same set of observations.
- ✓ **3210.Inq.7** Analyze experimental results and identify possible sources of experimental error.
- ✓ **3210.Inq.8** Formulate and revise scientific explanations and models using logic and evidence.

CLE 3210.Inq.6 Communicate and defend scientific findings.

Embedded Math

CLE 3210.Math.2 Utilize appropriate mathematical equations and processes to understand biological concepts.

- ✓ **3210.Math.2** Analyze graphs to interpret biological events.
- ✓ **3210.Math.3** Make decisions about units, scales, and measurement tools that are appropriate for investigations involving measurement.

- ✓ **3210.Math.4** Select and apply an appropriate method to evaluate the reasonableness of results.
- ✓ **3210.Math.5** Apply and interpret rates of change from graphical and numerical data.

Biology II

Cells

CLE 3216.1.5 Investigate how proteins regulate the internal environment of a cell through communication and transport.

Comparative Anatomy and Physiology

CLE 3216.6.1 Investigate the unity and the diversity among living things.

- ✓ **3216.6.1** Describe how the activities of major body systems help to maintain homeostasis.
- ✓ **3216.6.6** Develop a multimedia product for an immune disorder or infectious disease to demonstrate the impact on the individual organism.

Time Requirements

Day 1	Background information	55 min
Day 2	Lab set up	55 min
Day 3	Experiment set up	55 min
Day 4	Analyze results (24-48 hrs later)	40 min
Day 5	Extension Activity	55 min – 2 hours

Teacher Section
Target audience and prior knowledge

This unit is designed for biology high school students grades 9-12.
Students are expected to have some background in the basics of the immune system, proteins and antibodies, cell cycle, controlled experimental set ups, and cancer. It would be best if the students have had practice using aseptic technique and micropipetters as well.

Teacher Background

Please review and cover with your classes the basics of the immune system. Your students should understand the difference between acquired and innate immunity and the difference between T cells (cell-mediated) and B cells (humoral immunity). They should understand the terms major histocompatibility complex (MHC), T cell receptor (TCR), and antigen presenting cell (APC).

When T cells encounter infected or damaged cells, they become activated and differentiate into effector T cells. In order for T cells to become fully active, they must receive at least two signals simultaneously; one through the T cell receptor (TCR), and another through costimulatory molecules. Conversely, in order to prevent excessive T cell activation, T cells also express negative regulatory molecules that function like brakes or checkpoints to prevent unwanted T cell activation. T cells that are found in tumors tend to have high levels of the checkpoint molecules, which hinders their ability to clear the tumor.

Immunotherapy harnesses the body's own immune system to attack and kill cancer cells. Scientists have been working on ways to by-pass the measures that cancer cells use to evade the immune system and grow unchecked in the tissues of the body. General immunotherapies were the first to be developed and included the use of cytokines and interferons to help supercharge the immune system to fight cancer. Today there are four more targeted approaches to immunotherapy:

1. Checkpoint inhibitors- The immune system has many checkpoints to prevent immune cells from attacking the normal, healthy cells of the body. One checkpoint is the expression of negative regulatory molecules on the surface of T cells. These regulatory molecules function as breaks on the T cells by inhibiting activation. Interestingly, T cells that are found in tumors often express high levels of these negative regulatory molecules, which prevent the T cells from attacking the tumor. A checkpoint inhibitor is a new class of drugs that blocks the negative regulatory molecules on T cells, enabling them to attack the tumor.

2. Adoptive T cell immunotherapy – this process requires the removal of T cells from the tumor, isolation of T cells with the ability to attack the tumor, expansion of these cells in culture, and transfer of the expanded T cells back into the patient. An additional step to this approach is to genetically engineer the T cells to express specific surface molecules to enhance the function of the T cells..

3. A non-adoptive T cell immunotherapy that is currently being researched is the use of cancer vaccines to prevent tumors from forming. The vaccines act to prime or train the immune system to recognize a certain protein of a specific cancer type so that in the future when the immune cells are exposed to that protein in a tumor, the immune cells spring into action to take the tumor out before causing disease. An example is Gardasil, which is administered to pre-teens or young teens to provide future protection from Human Papillomavirus (HPV) that has been linked to cancer of the cervix, vulva, anus, and penis.

4. Monoclonal antibodies (MA)- These antibodies are produced in the lab, and can target the cancer cells directly for destruction by the immune system, or be tagged with a chemotherapy molecule or radioactive particle.

This lab will focus on providing the students with a tangible way to visualize the effects of using **two specific checkpoint inhibitor** molecules, **PD-1 and CTLA4**, in killing cancer cells. **PD-1**- (programmed cell death protein 1) is a transmembrane protein expressed on the surface of activated T cells. Signaling through this protein terminates signaling through the TCR, preventing the T cell from becoming fully activated. Many cancer cells express PD-L1, which binds to PD-1, thereby shutting down the T cell response. **CTLA-4**- (Cytotoxic T-Lymphocyte Antigen 4) is a transmembrane protein that also transmits an inhibitory signal to T cells. CTLA-4 is upregulated when T cells become activated and competes with CD28 to bind with CD80 and CD86 to act as a checkpoint inhibitor.

T cell exhaustion is the loss of responsiveness of T cells during a chronic infection or cancer. It is caused by continued encounter with antigen. T cell exhaustion often results from increased expression of negative regulatory molecules such as PD-1 and CTLA-4. Immunotherapy drugs are antibodies that block these checkpoint inhibitors, which enables the T cells to decisively attack the tumor cells.

The list of immunotherapy side effects described on the cancer.gov website is long. The most common side effects are problems related to the site of injection such as pain, swelling, soreness, redness, itchiness, and rash. Flu-like symptoms including fever, chills, weakness, dizziness, nausea, vomiting, muscle or joint aches, fatigue, headache, trouble breathing, and low or high blood pressure are also possible. Other potential side effects include swelling and weight gain, heart palpitations, sinus congestion, diarrhea, and increased risk of infection. Severe or fatal allergic reactions have occurred but are rare.

Resources:

- Dana Farber Cancer Institute, dana-faber.org
- Wherry, JE, T Cell Exhaustion, *Nature Immunology*, May 18, 2011

Pardoll DM, The Blockade of Immune Checkpoints in Cancer Immunotherapy, Nature Reviews Cancer, Mar 22, 2012; 12(4):252-64

- Cancer.gov
- Web MD
- Cancercenter.com
- FDA.gov

Materials, Reagents, and Equipment

Here is a detailed list with the information needed to order the materials for this lab. Substitutions where appropriate may be made with no problem.

Material	Vendor/Cat #	Price	Quantity
Agar, Bacteriological Grade	Flinn Scientific #A0084	$19.65	100g
Yeast Extract	Flinn Scientific #Y0003	$19.25	100g
Petri Dishes, Polystyrene disposable, sterile, 100 x 15 mm	Carolina Biological #741250	$5.95	Pkg of 20
Dextrose, Anhydrous, Powder Reagent grade	Carolina Biological #857450	$8.60	500g
Metrex CaviCide Surface Disinfectant	Amazon	$10.36	24 oz bottle
1.5 ml Microcentrifuge Tubes, Sterile	Amazon	$17.00	Bag of 500
15 ml Falcon Centrifuge Tubes, polypropylene	Amazon	$30.00	Pack of 50
Sharpie Permanent Markers, fine pt. Black, 2/package	Amazon	$1.22	(order 1 marker per group)
80-Well Microtube Rack, (set of 5)	MidSci #HS29025G	$15.00	(order 1 rack per group)
Sterile, Distilled Water	WalMart	$0.98	1 gallon
Fleischmann's Rapid Rise Highly Active Yeast	WalMart	$1.34	3 count- ¼ oz packets
ColiRollers Plating Beads – Sterile (4.5 mm glass beads)	Novagen	$30.80	1 bottle
Benchtop autoclave, refurbished (optional)	Booth Medical Equipment	$1800-3000	1
Micropipetters	Edvotek #591-1 for p20 #592-1 for p1000	$179	1 each size for each group
Micropipette tips	Edvotek #636 p200	$10 and	Per 2 racks of 96

	tips #637 p1000 tips	$20	1 each size for each group
Small wire mesh strainers **Cost effective substitutions:**	Dollar Tree	$1 ea	1/group
Yeast Extract Dextrose Medium (YED) To prepare 2L of media	Carolina Biological	$22.95	1 package
Pre-poured YED plates	Ward's Scientific	$22.99	10/package (each group needs 8 plates)

Teacher Work Flow-

1. Read the entire lab and collect/order materials needed.
2. Up to two weeks prior to the start of the unit, prepare agar plates using aseptic technique and store at 4°C.
3. The day prior to the start of the unit:
    a. Set up lab stations with materials needed
    b. Prepare dilutions and aliquots needed to simulate the immunotherapeutic drugs. For this lab, you will be preparing the students' reagents and it will be important to label all tubes correctly. The solutions will simulate the antibody drugs that block the checkpoint inhibitors, but will not be the antibody drugs. The students DO NOT NEED TO KNOW THIS.
4. **Day 1** Prelab discussion on principles of T cell activation, immunotherapy, and lab instructions
5. **Day 2-4** Conduct the lab

Set up overview-

The students working in **groups of 2 or 3** will set up six Petri dishes that are lightly confluent with yeast (16-20 hours of growth) for this controlled experiment. If they have never worked with sterile Petri dishes, please give them some instruction on how to handle them properly while maintaining sterility and how to label them. They will number the dishes 1-6 which will correspond to the following:

1 Tumor untreated
2 Tumor + T cells only
3 Tumor + T cells + anti-PD1 (αPD1)
4 Tumor + T cells + anti-CTLA4 (αCTLA4)
5 Tumor + T cells + αPD1 + αCTLA4
6 Tumor + αPD1 + αCTLA4

*Volumes for preparation are based on 10 groups for one class so **you should adjust** the quantity of dilutions based on the number of students and classes doing the lab. A 1:3 dilution is made using one part CaviCide and three parts water.

The students will be adding the following to their yeast plates ideally after 16-20 hours of incubation.

Dish 1- Tumor left untreated

Dish 2- Tumor with action of T cells only
12 beads, FS (soaked with full strength (FS) cavicide), no shaking

Dish 3- Tumor with T cells plus αPD-1
12 beads, FS soaked, 200 µl 1:3 dilution cavicide, little shaking

Dish 4- Tumor with T cells plus α CTLA4
12 beads, FS soaked, 200 µl 1:5 dilution cavicide, little shaking

Dish 5- Tumor with T cells plus α PD-1 and CTLA4 (combined drug)
12 beads, FS soaked, 400 µl FS cavicide, lots shaking

Dish 6- Tumor with no T cells plus α PD-1 and CTLA4
No beads and 400 µl water, swirl

Pre-Lab Prep

Explain to the students **how to shake** the petri dish.
Keep the **dish flat on benchtop** and quickly move it left to right then right to left, or use a figure 8 pattern, rotate 90°C and repeat the process to shake side to side again, and repeat turning and shaking two more times.

To **swirl**-
Pick up the dish and swirl the drug over the yeast. The accompanying videos may be helpful for this. Present the shaking action as a T cell process. It is how they are activated and proliferated.

Prepare the Petri dishes-

1. Add the following:
   - 20 g Yeast Extract
   - 40 g Dextrose
   - 20 g Bacto Agar
   - 2 L Distilled water

(I would divide the recipe by 4 and use 4 1L screw top bottles because that is the maximum size my autoclave will accommodate.)

2. Autoclave using the liquids setting
3. Cool to about 60°C and cool enough to touch
4. Pour 20 ml per Petri dish (does not need to be exact)
5. After 30 minutes, stack and store upside down at 4°C covered in plastic wrap until needed
6. Remove from 4°C and unwrap to leave on the benchtop at least 30 minutes before inoculation to let the plates come to room temperature.

**I have found that I can get 20 agar dishes filled with 500 ml of media. Pouring the media can be very time consuming if you measure out the media for each dish plus the media will cool and solidify too quickly by doing this. I recommend that you measure 20 ml of colored water and pour into one dish and use it as a guide to then pour the warm media into each dish more quickly trying to pour an even amount in each. Yes, it could introduce an unwanted variable in a real experiment where the outcome is yet to be determined but in this lab as long as the yeast have a good supply of nutrients provided in the media they will grow at a normal rate for the duration of the lab.

Inoculating the plates with yeast

SPECIAL INSTRUCTIONS TO HELP THE STUDENTS:

1. I found that inoculating with beads produces plates that are more evenly confluent than inoculating with spreaders. The glass plating beads can be tricky to work with though because they move more like a liquid than a solid and they are very easy to spill. They also have the properties of a super bouncy ball and move very quickly in unpredictable directions once they hit the ground. Use with care.

Make a starter culture of yeast about an hour before inoculation. Simply heat a cup of water to about 55°C and add 1 packet of inactive dry yeast and one tablespoon of sugar then stir to mix. Let the culture sit while the yeast activate. The yeast should be ready to use when the foam layer on top is about equal to the volume of the liquid in the beaker. Each group will need 1 ml of yeast starter to inoculate their plates.
The students should label the plates on the tops and bottoms before inoculating. To inoculate:

1. the student will lift the lid up, and add 50µl of yeast starter solution to the middle of a Petri dish with 1% YED.
2. the student should add about 1g of glass beads (about 12 beads) on the dish.
3. the student should put the lid back down and move the dish in a figure 8 pattern to get the beads to spread the yeast across the surface of the dish evenly. They need to rotate the dish 45-90° several times to be sure the entire dish is covered with yeast cells.
4. then carefully and gently transfer the beads to the next dish to inoculate and add 50µl of starter solution to that dish, move the dish in a figure 8 pattern, rotating the dish about every 5 seconds
5. repeat until all agar dishes have been inoculated.
6. Collect all of the used beads in one beaker so they may be washed and autoclaved for reuse at a later date.

Preparation of the reagents:
The students will be best served <u>if they do not know</u> what the reagents really are when they begin the lab.

Dish 1- Tumor. Untreated and nothing added. This is the control plate
Dish 2- Tumor with T cells only. Add 35 ml of CaviCide in a clean container labeled **#2- Activation Solution.**
Needed to soak the sterile glass beads in full strength CaviCide. (The students will add 3ml to one beaker and use the same two beakers and 3ml of CaviCide and strainer for the next three dishes also.)
Dish 3- Tumor with T cells plus αPD-1.
To make 3ml of 1:3 dilution of CaviCide add 750µl of CaviCide to 2250µl of DI water in a Falcon tube and label **#3**
Dish 4- Tumor with T cells plus α CTLA4.
To make 3ml of 1:5 dilution of CaviCide add 500µl of CaviCide to 2500µl of DI water in a Falcon tube and label **#4**
Dish 5- Tumor with T cells plus α PD-1 and CTLA4 (combined drug)
Label a Falcon tube **#5** and add 4.5ml of CaviCide (NO dilution)
Dish 6- Tumor with no T cells plus α PD-1 and CTLA4
Label a Falcon tube **#6** and add 4.5ml of water only

16-20 hours of growth at room temperature works best.

Teacher Protocol

Teacher's Materials
1 bottle of CaviCide
2.5L DI water

Student's Materials
Sterile glass beads
50 ml beakers (x2)
1 small drain strainer
1.5 ml epi tubes (x4)
YED agar dishes (x6)
1.0 ml of Yeast culture
250 µl of αPD1 antibody
250 µl of αCTLA4 antibody
450 µl of αPD1 + αCTLA4 antibodies combined with T cells
450 µl of αPD1 + αCTLA4 antibodies combined without T cells
p200 micropipetter (x1)
1 box of p200 tips
1 epi tube rack
3 ml activation solution in 15 ml conical tube

Day 1
Lecture/discussion on immunotherapy and prelab instructions.

Day 2
Each group will need at their workstation:
Six petri dishes with agar
a gram of glass beads
1 ml of yeast solution to inoculate their plates
1 15 ml conical tube for the activation solution
1 epi tube rack
4 empty epi tubes
1 epi tube with 1 ml of yeast culture solution
1 sharpie
2 small beakers
1 wire mesh strainer (autoclaved if possible)

Students will:

1. Label six agar dishes on the bottom around the edge with your initials, the date, and a number (1-6). Use this key to keep track of what goes on each dish:

    1 Tumor untreated
    2 Tumor + T cells only
    3 Tumor + T cells + αPD1
    4 Tumor + T cells + αCTLA4
    5 Tumor + T cells + αPD1 + αCTLA4
    6 Tumor + αPD1 + αCTLA4

2. Carefully transfer 1 ml of the yeast culture into your epi tube labeled 1.
3. Add 50 µl of yeast solution to the middle of plate 1 being careful to not touch the tip of your pipettor on the agar.
4. Add 10-12 sterile glass beads and shake side to side or in a figure 8 pattern to evenly spread the cells over the surface of the agar.
5. Shake so that all the beads are on one side of the dish and gently tap them from the dish you just inoculated into the dish to inoculate next being careful to transfer them from one dish to the next without spilling them or touching the inside of the plates.
6. Once all 6 plates are inoculated, gently and carefully transfer your glass beads into a large drain strainer in the front of the room.
7. Leave on benchtop to incubate at room temperature until class tomorrow.
8. Each group then prepares for the reagents for tomorrow for the 6 petri dishes in their experimental setup and to organize, they will need 4 epi tubes (labeled 3,4,5,6) and two small beakers as follows:

    1 No tube or aliquot needed for control plate
    2 To aliquot 3ml activation solution into a 15 ml conical tube
    3 Epi tube
    4 Epi tube
    5 Epi tube
    6 Epi tube

Day 3 – Set up controlled experiment

Using the tubes you labeled yesterday, obtain the reagents needed and keep in rack on your bench.

Plate 1 Set Plate 1 aside as this is the tumor left untreated
Plate 2 Place Plate 2 next to the Falcon tube with activation solution. **This is the procedure we use to activate T cells:**

1. Transfer all the activation solution into one small beaker.
2. Add 12 sterile glass beads and swish and swirl them to be sure they are coated with the solution.
3. Place the small strainer over the second beaker and careful pour the activation solution with the beads into the second beaker to isolate the wet beads in the strainer.
4. One student lift and hold the lid for Plate 2 while your lab partner carefully 'pours' the wet beads around on the yeast culture.
5. Replace the lid. (The T cells are activated but NOT working with a drug so they are not supercharged so no shaking this dish.) Leave the beads in place where they fell.
6. Carefully and gently slide the plate to one side of your work space out of the way.

Plate 3 Place Plate 3 next to the beaker with activation solution.

1. One student lift and hold the lid for Plate 3 while your lab partner adds 200 µl of αPD1 antibody from tube 3 directly in the center of the yeast culture.
2. Replace the lid.
3. Now **repeat steps 2-6** from Plate 3 given above to activate the T cells.
4. Shake side to side **ONLY two times**. Leave the beads in place where they come to a rest.
5. Carefully and gently slide the plate to one side of your work space out of the way.

Plate 4 Place Plate 4 next to the beaker with activation solution.

1. One student lift and hold the lid for Plate 4 while your lab partner adds 200 µl of αCTLA4 antibody from tube 4 directly in the center of the yeast culture.
2. Replace the lid.
3. Now **repeat steps 2-6** from Plate 2 given above to activate the T cells.
4. Shake side to side **ONLY two times**. Leave the beads in place where they come to a rest.
5. Carefully and gently slide the plate to one side of your work space out of the way.

Plate 5 Place Plate 5 next to the beaker with activation solution.

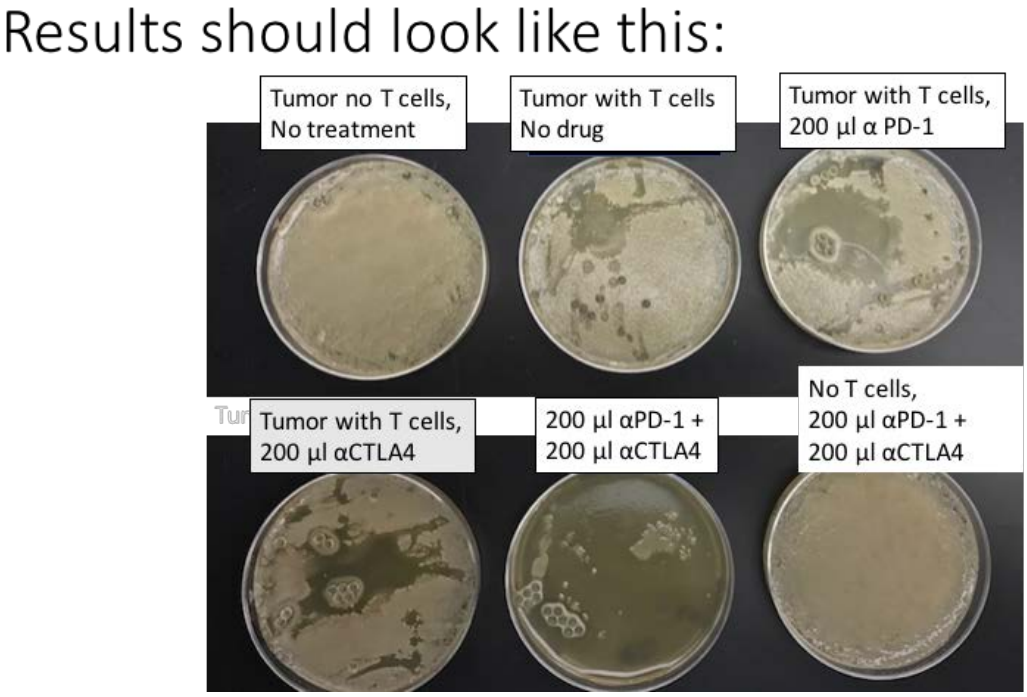
1. One student lift and hold the lid for Plate 5 while your lab partner adds 400 µl of αPD1 + αCTLA4 antibodies combined <u>with T cells</u> from tube 5 directly in the center of the yeast culture.
2. Replace the lid.
3. Now repeat steps 2-6 from Plate 2 given above to activate the T cells.
4. Shake side to side **as you did yesterday to inoculate the plate with yeast.** Leave the beads in place where they come to a rest.
5. Carefully and gently slide the plate to one side of your work space out of the way.

Plate 6 Place Plate 6 next to the beaker with activation solution.

1. One student lift and hold the lid for Plate 6 while your lab partner adds 400 µl of αPD1 + αCTLA4 antibodies combined <u>without T cells</u> from tube 6 directly in the center of the yeast culture.
2. Replace the lid.
3. One student lift the plate and gently swirl (no shaking since there are no T cells) a couple of times but do not shake.
4. Carefully stack all six of your plates (DO NOT SHAKE, SWIRL, DROP, or otherwise compromise your plates) and leave towards the back of the bench so they can incubate at room temperature overnight without being disturbed.

Day 4 Analyze the results

Results should look something like this:
Plates 1,2,3 on the top row
Plates 4,5,6 on the bottom row



M**ore Resources-**
Articles:
Cancer Cell. 2015 April 13; 27(4): 450–461. doi:10.1016/j.ccell.2015.03.001.
Immune checkpoint blockade: a common denominator approach to cancer therapy
Suzanne L. Topalian1, Charles G. Drake2,3, and Drew M. Pardoll3

https://www.immunooncologyhcp.bmsinformation.com/antitumor-immunity/pathways/CTLA-4-pathway

https://www.immunooncologyhcp.bmsinformation.com/antitumor-immunity/pathways/PD1-pathway

https://www.sciencedirect.com/topics/immunology-and-microbiology/ctla-4

Videos explaining cancer biology
Animated Introduction to Cancer Biology (Full Documentary), 12:07 min long
https://youtu.be/46Xh7OFkkCE

Introduction to Cancer Biology (Part 1): Abnormal Signal Transduction; 7:46 min long
https://youtu.be/jjfYQMW_nek

Videos explaining immunotherapy
www.cancercenter.com
in the search box at the top left enter immunotherapy and click on the first link
the video is 3 minutes long and has some great graphics to help the students better understand this type of cancer treatment.

How is Immunotherapy Used to Fight Cancer? | Dana-Farber Cancer Institute | Science Illustrated; 2:43 min long
https://youtu.be/AbmEt_E8kfo

Nature: Cancer Immunotherapy – medical animation; 7:31 min long
https://youtu.be/-9q4c-QRdes

Immunotherapy: A path to a cancer cure- for clinicians; 8:41 min long (really nice animation)
https://youtu.be/UbFjiWOBErA

AstraZeneca
Checkpoint inhibitors PD-1 and CTLA4; 2:55 min long
https://youtu.be/TNOFfSTMOtI

https://www.azimmuno-oncology.com/combination-cancer-therapy.html
and
https://www.azimmuno-oncology.com/immuno-oncology-pathways/ctla-4-inhibition.html?source=ALL_N_H_53&WT.mc_id=ALL_N_H_53&umedium=CPC&uadpub=bing&ucampaign=unbranded%20immuno-oncology&ucreative=ctla-4&uplace=ctla-4

A Model Using Yeast to Visualize Immunotherapy

Student Background

Our body uses the immune system to protect us from foreign pathogens that can invade our bodies leading to damage and disease. The immune system also protects the body from abnormally growing cells that can lead to tumors. Immunotherapy is a treatment that recently has shown great promise in treating many types of cancer. It targets the body's own immune system to attack and eliminate tumors. Checkpoint inhibitor therapy is one type of immunotherapy that works by blocking negative regulatory molecules on T cells, which enables the T cells to clear the tumor.

The protein known as programmed cell death protein-1 or PD-1 is a checkpoint inhibitor on T cells that binds to the ligand on body cells, PD-L1. This recognition between PD-1 and PD-L1 is important in the immune system's self-recognition process. Unfortunately, tumor cells can express high levels of PD-L1 and pass as healthy self cells. The Cytotoxic T-Lymphocyte Antigen 4, or CTLA4, is a surface protein that is upregulated on activated immune cells and acts to dampen or pull back the immune response to prevent an overreaction that may lead to the death of healthy cells. Immunotherapy uses antibodies to block the recognition sites of the surface proteins like PD-1 and CTLA4 causing the T cell to become activated and target the cancer cell for destruction by secreting protein factors or cytokines. Checkpoint inhibitors are one group of immunotherapeutic drugs available today to treat a variety of cancers like melanoma, bladder cancer, and kidney cancer among others.

In this lab, you will simulate the action of immunotherapy drugs on the immune system to kill cancer cells. You will use yeast to represent the growing tumor cells in a petri dish. You will use tiny glass beads to represent the T cells and you will use the antibody drugs, anti-PD-1 and anti-CTLA4 in different combinations with the T cells to determine which is the most effective at killing the tumor cells.

Question: How does immunotherapy work?

Student Work Flow:

Day 1 Lecture/discussion on immunotherapy
Day 2 Prelab instructions, label dishes and inoculate with yeast
Day 3 Set up the controlled experiment
Day 4 Collect data and analyze results

Student Protocol

Do not open any dish until instructed to by your teacher. You will need to use aseptic technique throughout this experiment.

Handle the glass beads as if they are a liquid.

Materials
Sterile glass beads
50 ml beakers (x2)
1 small drain strainer
1.5 ml epi tubes (x6)
YED agar dishes (x6)
1.0 ml of Yeast culture
250 µl of αPD1 antibody
250 µl of αCTLA4 antibody
450 µl of αPD1 + αCTLA4 antibodies combined with T cells
450 µl of αPD1 + αCTLA4 antibodies combined without T cells
p200 micropipetter (x1)
1 box of p200 tips
1 epi tube rack
3 ml activation solution in Falcon tube

Day 1
Lecture/discussion on immunotherapy

Day 2 Pre-lab instructions and set up

1. Label six agar dishes on the bottom around the edge with your initials, the date, and a number (1-6). Use this key to keep track of what goes on each dish:

    1 Tumor untreated
    2 Tumor + T cells only
    3 Tumor + T cells + anti-PD1 (αPD1)
    4 Tumor + T cells + anti-CTLA4 (αCTLA4)
    5 Tumor + T cells + αPD1 + αCTLA4
    6 Tumor + αPD1 + αCTLA4

2. Carefully transfer 1 ml of the yeast culture into your epi tube labeled 1.
3. Add 50 µl of yeast solution to the middle of plate 1 being careful to not touch the tip of your pipettor on the agar.
4. Add 10 sterile glass beads and shake side to side to evenly spread the cells over the surface of the agar.
5. Shake so that all the beads are on one side of the dish and gently tap them from the dish you just inoculated into the dish to inoculate next being careful to transfer them from one dish to the next without spilling them or touching the inside of the plates.
6. Once all 6 plates are inoculated, gently and carefully transfer your glass beads into a large drain strainer in the front of the room.
7. Leave on benchtop to incubate at room temperature until class tomorrow.
8. Label 4 epi tubes as follows:

    3
    4
    5
    6

9. Aliquot 3 ml of Activation Solution into a 15ml conical tube and close tightly to store until class tomorrow.

Day 3 – Set up controlled experiment

Using the tubes you labeled yesterday, obtain the reagents needed and keep in rack on your bench.

Plate 1 Set Plate 1 aside as this is the tumor left untreated
Plate 2 Place Plate 2 next to the Falcon tube with activation solution. **This is the procedure we use to activate T cells:**

1. Transfer all the activation solution into one small beaker.
2. Add 12 sterile glass beads and swish and swirl them to be sure they are coated with the solution.
3. Place the small strainer over the second beaker and careful pour the activation solution with the beads into the second beaker to isolate the wet beads in the strainer.
4. One student lift and hold the lid for Plate 2 while your lab partner carefully 'pours' the wet beads around on the yeast culture.
5. Replace the lid. (The T cells are activated but NOT working with a drug so they are not supercharged so no shaking the dish.) Leave the beads in place where they fell.
6. Carefully and gently slide the plate to one side of your work space out of the way.

Plate 3 Place Plate 3 next to the beaker with activation solution.

7. One student lift and hold the lid for Plate 3 while your lab partner adds 200 µl of αPD1 antibody from tube 3 directly in the center of the yeast culture.
8. Replace the lid.
9. Now **repeat steps 2-6** from Plate 3 given above to activate the T cells.
10. Shake side to side **ONLY two times**. Leave the beads in place where they come to a rest.
11. Carefully and gently slide the plate to one side of your work space out of the way.

Plate 4 Place Plate 4 next to the beaker with activation solution.

12. One student lift and hold the lid for Plate 4 while your lab partner adds 200 µl of αCTLA4 antibody from tube 4 directly in the center of the yeast culture.
13. Replace the lid.
14. Now **repeat steps 2-6** from Plate 2 given above to activate the T cells.
15. Shake side to side **ONLY two times**. Leave the beads in place where they come to a rest.
16. Carefully and gently slide the plate to one side of your work space out of the way.

Plate 5 Place Plate 5 next to the beaker with activation solution.

17. One student lift and hold the lid for Plate 5 while your lab partner adds 400 µl of αPD1 + αCTLA4 antibodies combined <u>with T cells</u> from tube 5 directly in the center of the yeast culture.
18. Replace the lid.
19. Now repeat steps 2-6 from Plate 2 given above to activate the T cells.
20. Shake side to side **as you did yesterday to inoculate the plate with yeast.** Leave the beads in place where they come to a rest.
21. Carefully and gently slide the plate to one side of your work space out of the way.

Plate 6 Place Plate 6 next to the beaker with activation solution.

22. One student lift and hold the lid for Plate 6 while your lab partner adds 400 µl of αPD1 + αCTLA4 antibodies combined <u>without T cells</u> from tube 6 directly in the center of the yeast culture.
23. Replace the lid.
24. One student lift the plate and gently swirl (no shaking since there are no T cells) a couple of times but do not shake.
25. Carefully stack all six of your plates (DO NOT SHAKE, SWIRL, DROP, or otherwise compromise your plates) and leave towards the back of the bench so they can incubate at room temperature overnight without being disturbed.

Day 4 Analyze the results

Data to graph:

<u>Counting colonies</u> will likely **not** be the best way to go since the plates will be covered by colonies that are tightly packed and indistinguishable. I had my students estimate clear areas by percent of the total plate (which represents drug effectiveness) at each observation and graph this data at the end of the experiment.

I also have my students document their plates at different time points during the experiment with their cell phone camera so they can include the pictures in their data section of the lab write up. They should place a piece of paper next to each dish to clearly label and identify the conditions in each dish.

Alternative Data Representations:

1. Students can measure the clear areas (using close estimations) on each plate to then calculate total area of clear zones. They can use a ruler to measure areas of growth in cm to the nearest mm. Students can calculate the area of the petri dish using the formula $A = \pi r^2$ in centimeters Recording this data for each of the 6 plates at two time points could be very useful. They can then use this data to make a graph (Bar or Line graph) to represent their results.
2. Students can use graphing paper to draw representations (simply shade in lightly areas covered with yeast) of the yeast growth for each of the six plates then count the number of squares that are **not touching** any yeast growth or colonies. This clear zone data can be used to represent the killing effectiveness of the drug and graphed. To do this, I would have the students take a sheet of graphing paper and the bottom of a clean, empty dish then trace the dish six times on the paper in three sets of two leaving space between dishes to label appropriately and number the circles 1-6 to represent their cultures. This would give the students a good way to quantitatively analyze their data as well as provide clear documentation of the experimental results if downloading and printing pictures is not an option.

Name _____ Period _____

Questions:

1. What is cancer?

2. What is immunotherapy?

3. How does immunotherapy work?

4. How are cancer cells able to evade the immune system?

5. What is a checkpoint inhibitor? What do they act as in the immune system?

6. Explain why we included controls in our experiment.

7. Which plates served as controls (both positive or negative) and which kind was each?

8. What did you expect your results to be when you set up your plates? Did your results turn out like you expected?

9. What is aseptic technique? Why is it so important in this lab?

10. Identify two advantages to using immunotherapy to fight cancer.

11. What disadvantage/s can you think of for using immunotherapy?

12. Identify the molecules' actions this experiment simulated and explain what their job is in the immune system.

13. Which of your 6 plates had the most growth? Which plate had the least growth?

14. Identify at least 3 errors that you could have possibly made during this experiment.

15. What is the one thing that you will remember the longest about this lab experience?

16. Do you think it will be possible to cure cancer for everyone in the future? Why or why not?

Formal Lab Write Up sections:
(I use the AP Bio Format)

- Title
- Question
- Background
- Materials
- Procedure
- Results/Data
- Conclusion/Discussion
- Literature citations

My grading rubric:

Immunotherapy Lab Report
Grading Rubric

Electronic copy and hard copy	☐	
Title	(5 pts)	_____
Introduction	(25 pts)	_____
Materials and Procedures	(10 pts)	_____
Results/Data/Analysis	(25 pts)	_____
Discussion/Conclusion	(20 pts)	_____
Literature Citation	(5 pts)	_____
Questions	(10 pts)	_____

Lab Grade _____

Extension Activity

Have the students choose an immunotherapeutic cancer drug to research. They can answer a set of questions like:

How does this drug affect the immune cell or cancer cell?
What molecule does it target?
What types of cancers does it treat?
What are the possible side effects?
When was it discovered or when was it approved by the FDA for use?

The students would then have a presentation day where they each present to class the information they have learned about their researched drug.

A small list of current immunotherapy drugs from the FDA website

Pembrolizumab (Keytruda®)	Melanoma, lung cancer, head and neck cancer, Hodgkin lymphoma
Nivolumab (Opdivo®)	Hodgkin lymphoma, lung cancer, kidney cancer, melanoma, bladder cancer
Ipilimumab (Yervoy®)	Melanoma
Atelizumab (Tecentriq®)	Bladder cancer
Avelumab (Bavencio®)	Merkel cell carcinoma