The Jackson Laboratory
Leading the search for tomorrow's cures

THE JACKSON LABORATORY OPEN HOUSE

Welcome to the Mouse Research Team!

In our collaborative study on cancer genomics, we will investigate treatments for melanoma cancer using human tumors and mouse models. On this team, you are stepping into the role of a mouse researcher and will be using specific skills and training to evaluate two different melanoma treatments.

When we are talking about biomedical research, research on human diseases, and personalized medicine, using animal models is vitally important. Mouse research means using special laboratory mice that have been bred for generations and generations to be genetically identical. Those "inbred" mouse strains can be used for experiments, to test how genes function, how protein pathways work, or what drugs are most effective. And the reason why mice are used in the first place is because they're more than 80% genetically similar to humans! If you think about it, mice have hearts, and lungs, and muscles, and a lot of the same things that we do. The way their bodies function is very similar to how ours function, down to the very DNA that makes all the proteins and cells and organs in our bodies. Mice can be engineered to mimic a host of human diseases and disorders, from cancers to heart disease.

Before we dive into our research, let's first explore careers in mouse research and learn about what mouse researchers do to contribute to a project like this one.

Careers in Mouse Research

You are now a member of a collaborative group of professionals who work directly or indirectly with mice.

Here's an example of a real mouse research team: there's an animal care technician, a research assistant, and a principal investigator.

1. The **animal care technician** works directly with the animals used in scientific research. They care for the animals daily, including monitoring animal health and maintaining a sanitary environment. They often have a high school diploma and

could have advanced degrees or certificates to gain expertise and responsibilities in their career.

2. The **research assistant** works on a variety of laboratory experiments. They perform experiments and analyze data. Part-time employees often are high school or undergraduate students working towards a degree. Full-time employees often have a bachelor's degree and could have advanced degrees or certificates to gain expertise and responsibilities in their career.
3. The **principal investigator** directs a research program in their own laboratory or research group. They work to propose new research ideas and experiments and manage a group of scientists to drive discoveries in a specific field. Principal Investigators also do a lot of communication, mainly writing, to share their group's findings with the greater scientific community. They have a bachelor's degree and a doctoral degree, plus specific training in their research field.

As you can see, the mouse research team relies on people with all kinds of training and experiences. You can explore these careers further with the resources on our Virtual Open House website.

Your Role as a Mouse Researcher

As mouse researchers for this project, an important first part of our team's job is to design and execute a study using mice to test treatments for melanoma (see **Figure 1**).

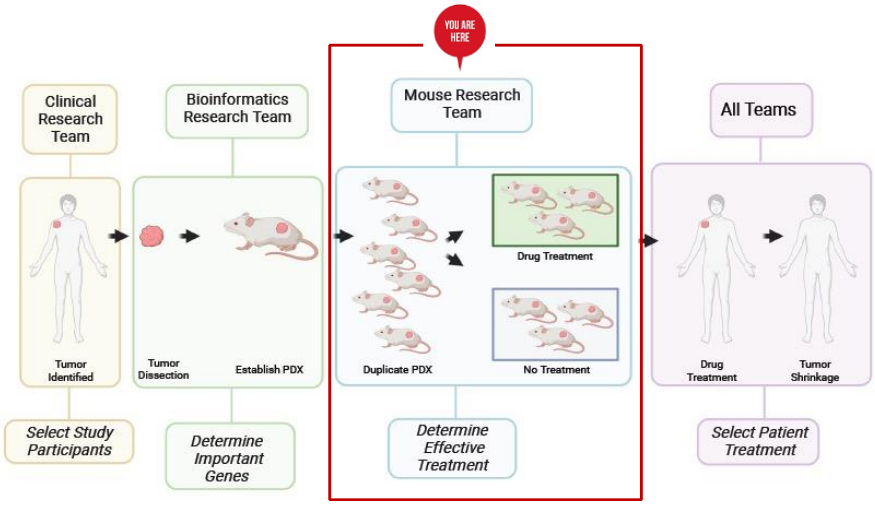

Figure 1. The mouse research team designs experiments to test different treatments on human tumors. In collaboration with the clinical and bioinformatics research teams, the mouse research team plays an important role in this cancer genomics study by collecting valuable data about the efficacy of specific treatments.

When we design a study, some of the questions we ask include: What conditions do we want to test? Which groups of animals will we assign to each condition? How many animals will we need in our study? How do we calculate and interpret the data?

For the purposes of our exercise today we will use data from a mock mouse experiment to determine the efficacy of two potential melanoma treatments. You will be creating *patient-derived xenografts (PDX)* by taking human tumors and studying their growth in mice (see **Figure 2**).

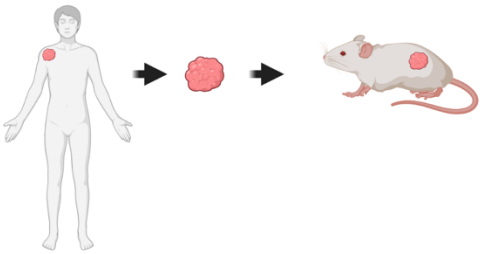

Figure 2. Patient-derived xenograft (PDX) mice. One way to create a mouse model from a specific person's tumor is to place the tumor directly into a mouse by implanting it subcutaneously (under the skin).

You will use tumor size data to compare the effects of two different treatments for melanoma. You'll analyze the data yourself, graph your findings, and interpret the results of the two drug treatments.

Mouse Researcher Activity

For this lab, use the **Activity Spreadsheet** to keep track of your work and document your findings. If you have already completed another lab, you can continue to use the same spreadsheet for this portion.

We are exposing 10 different patient tumors to two different treatments separately. In doing this experiment, we need to take measurements of the tumors prior to and after treatment with each drug. Recall that tumors are balls of cells that are growing out of control. If a treatment is successful, it stops a tumor from growing or it could even shrink the tumor.

Let's look at an example of what the tumors look like on a PDX mouse:

Figure 3. PDX mouse with visible tumor. This is a PDX mouse with a visible tumor growing on its back.

Looking at the image in **Figure 3**, you can get an idea of what a tumor growing in a PDX mouse looks like. Now look at the PDX mice below in **Figure 4**. In order to measure and

compare the size of the different PDX tumors, we would use calipers, a device that can measure the diameter of each tumor down to the millimeter. The caliper is non-invasive and can be used externally on a living mouse.

Figure 4. Calipers are used to measure PDX tumors. Patient-derived tumors are monitored on the mice by measuring their diameters before, during, and after treatments using calipers to measure down to the millimeter.

Part 1. Which tumors responded to which treatments?

1. Now that you have an idea of how we collect the data, look at **Table 1** displaying the data from our mouse study. Remember, we have 10 patient tumors and two different treatments. You need to evaluate the changes in size of the tumors from before (Day 0) and after (Day 30) each treatment.

Table 1. PDX tumor measurement data. Using calipers, mouse researchers measured the diameter of the tumor on each PDX mouse prior to treatment (Day 0) and then 30 days post-treatment (Day 30). The sample numbers are matched to each corresponding patient ID.

Treatment 1			
Patient	Sample	Day 0 Tumor Diameter (mm)	Day 30 Tumor Diameter (mm)
ME001	1	10.5	6.5
	2	7	3.5
	3	9.5	5
ME002	4	9	8.5
	5	8.5	9
	6	8	8
ME007	7	10	10
	8	10.5	10
	9	9.5	10
ME012	10	8	12.5
	11	10	15.5
	12	9	14
ME015	13	9	15
	14	9	16
	15	9	14

Treatment 2			
Patient	Sample	Day 0 Tumor Diameter (mm)	Day 30 Tumor Diameter (mm)
ME001	31	8	12
	32	9	13
	33	8.5	11
ME002	34	10	18
	35	10	17
	36	7	16
ME007	37	9.5	10.5
	38	10.5	11
	39	8.5	8.5
ME012	40	9	6
	41	10.5	5.5
	42	7.5	3.5
ME015	43	9	7
	44	11	4
	45	10	4

Treatment 1			
Patient	Sample	Day 0 Tumor Diameter (mm)	Day 30 Tumor Diameter (mm)
ME021	16	9	10
	17	8	9.5
	18	8.5	10.5
ME029	19	12	13
	20	8.5	12
	21	9.5	14
ME030	22	8.5	4
	23	8	4.5
	24	9	5
ME041	25	10	15
	26	9	13
	27	9.5	15.5
ME045	28	8	13
	29	9	15
	30	10	17

Treatment 2			
Patient	Sample	Day 0 Tumor Diameter (mm)	Day 30 Tumor Diameter (mm)
ME021	46	9	3
	47	9	4
	48	9	2
ME029	49	7	13
	50	11	14
	51	9	15
ME030	52	10	14
	53	9	15
	54	8	16
ME041	55	7	16
	56	11	17
	57	9	12
ME045	58	9	4
	59	8.5	3.5
	60	9.5	4.5

2. What do you notice about the three pieces of data for each patient at each timepoint?

   They are different! Therefore, it's important to have *replicates* in any experiment. We include replicates for several reasons, but mainly because there is natural variation in each sample of an experiment. Therefore, to see the range of possibilities, we include multiple samples.

3. What should we do with the data from these replicates?

   Calculate an average! Go ahead and calculate the average for the three replicates for each patient and each treatment. Once you have calculated the averages, enter them on your spreadsheet under the tab "Mouse (Part 1)". Be sure to enter the data in the correct location and under the corresponding time point for each patient.

   For example, if we look at the data for the tumor from Patient ME001, we can see that those mice had tumor diameters of 10.5 mm, 7 mm, and 9 mm for Treatment 1 on Day 0. If we take the average of those three numbers, we get 9 mm. In the spreadsheet, we have a table for the averages, so we'd put 9 in that table under patient ME001 on Day 0 for Treatment 1.

4. Once you've finished calculating the averages, take a look at the graphs. The averages you calculated are already graphed for you! We have one graph for each

treatment. For each patient tumor, there are two bars: (1) a blue bar on the left representing the average diameter of the tumor in the PDX mice on day 0 before treatment and (2) an orange bar on the right representing the average diameter of the tumor in the PDX mice on day 30 after treatment. The y-axis is average tumor diameter in mm, so a taller bar means a larger tumor!

5. Now, we'll use the data for each of the patients to determine whether the treatment was effective. We can compare the bars for day 0 and day 30 to determine if the tumor grew, shrank, or stayed the same size, and then use that to determine whether the patients' tumors responded to each treatment.

   For example, for patient ME001, the day 30 diameter is smaller than the day 0 diameter for Treatment 1. However, the day 30 diameter is bigger than the day 0 diameter for Treatment 2. So, based on this data, we might say that the tumor from patient ME001 responded to Treatment 1 but not Treatment 2.

   Under "Mouse Research Conclusions" on the Part 1 tab, type the patient ID numbers for the tumors that seemed to respond to each treatment. Remember that we're looking for treatments that reduce tumor size or prevent tumor growth!

6. Next, consider this question: how do we know if the change in size is due to the treatment? What is missing?

   We need controls! Controls in this experiment are PDX mice that are not treated with any drugs. Controls are important to show us what happens to the tumors in the absence of treatment.

Part 2. Which tumors responded to which treatments compared to controls?

1. Navigate to the tab "Mouse (Part 2)" and compare the data from the control samples, which are those without treatment, to the samples that received treatment. For each patient's tumor, does the tumor grow the same amount over 30 days with treatment as it does without treatment? Or do the patients' tumors grow differently with treatment?

2. After analyzing the data including the controls, enter the patient ID numbers for the tumors that responded to each treatment under "Mouse Research Conclusions" on the "Mouse (Part 2)" tab. Remember that we're looking for treatments that reduce size or prevent growth more than when the tumors aren't treated!

3. Then think about the following questions:
   - Did any of the patient tumors not respond to either treatment?
   - What other information would you want to know about these patients that could provide hints as to <u>why</u> some responded to some treatments and others did not?

Conclusions

Awesome work, everyone! Our mouse team really made a lot of research progress.

We learned the types of jobs we could have on mouse research teams, which range from different education paths and different skills.

We learned how to design a study using controls and replicates. We also learned how tumors are sized to indicate their severity, and how to graph and interpret that data.

Reminder: Don't forget to check out the conclusions tab of your spreadsheet! Once you complete all three lab segments, you can see a summary of your combined work.

Extension into Ethics

Is working with mice unique? Are there any special considerations when doing research on laboratory animals such as mice? Can we do any study we want, any time?

Animal research is very powerful and important! But it can be risky to both animals and humans if not done properly. There are ethical standards that guide research practices to make sure the studies are performed responsibly, and that the animals' safety and wellbeing are upheld as much as possible throughout the study.

We'll introduce three case studies about mouse research. What would you do in these scenarios?

Links to resources about working with laboratory animals:
Why the Mouse?

Case study 1: Yolanda the project manager

Yolanda is a project manager for a company that supplies inbred laboratory mice to research groups around the world. She works with scientists to advise them about using mice for their studies. She is currently reviewing a project with Dr. Kukowska, a principal investigator who wants to use mice for a patient-derived xenograft (PDX) experiment to test a new leukemia therapeutic. During their discussion, Dr. Kukowska mentions that she proposed to use 3,000 mice in the study just to make sure her research team has enough. Yolanda stresses the importance of not using more animals than necessary in a research project. She works with Dr. Kukowska to calculate the exact number of mice that will be needed for the study, which came to 240. With that adjusted number of mice, Dr. Kukowska can proceed with her mouse experiment.

Questions to Consider:

- What do you think about the conversation between Yolanda and Dr. Kukowska?
- Why might Dr. Kukowska have wanted to include such an excessive number of mice in the study?

- Why did Yolanda say it was necessary to calculate the exact number of mice needed for the study?
- Thinking about Yolanda's decision to calculate the exact number of mice needed for the study, what would you do similarly, or differently?
- What ethics guidelines should be followed in this case, if any?

Case study 2: Hajime the research technician

Hajime is a research technician at a government laboratory, working to analyze mouse brain samples for genetic mutations in different brain cancers. Hajime's latest experiment reveals a potentially new driver gene that, when mutated, leads to aggressive glioblastoma in mice. They are very excited about this breakthrough because no research group has associated this gene with glioblastoma. When they examine their data more closely, Hajime realizes that one mouse with this mutation develops glioblastoma, but one mouse without this mutation does not. Hajime is so excited by this and suggests to the principal investigator of the study, Dr. Wilson, that they should publish this finding. Dr. Wilson suggests it's too early to publish the finding.

Questions to Consider:

- Information like this, about a new driver mutation, would help clinicians and researchers to better understand glioblastoma, which is a very complicated disease. Why do you think Dr. Wilson feels it's too soon to publish the data?
- What would a delay in publishing the data allow the researchers to do?
- In the long-run, will this help patients with glioblastoma?

Case study 3: Choya the veterinary technician

Choya is a veterinary technician in the animal care unit at a university. She is assigned to work in Animal Room A, a housing unit for rats used in the research studies from several of the labs at the university. During one of her daily rounds performing health checks on the rats, she notices some animals have developed lesions around their eyes. The affected animals all belong to the same laboratory, so Choya reads the research protocol from the lab describing the types of experiments and expected phenotypes in the rats. Choya found that eye lesions were not described in the protocol and therefore are not expected from the study. She decides to call the lab manager and explain that, in order to protect the health of the animals, she will begin treating the rats for their lesions.

Questions to Consider:

- Why is it important that Choya communicate with the laboratory associated with these mice?
- The laboratory doing this study may have to terminate the project due to the lesions if it involves a treatment which may interfere with their results. Should Choya not have treated the mice for the lesions?
- Is it ethical for the laboratory to continue the study even if the mice develop lesions?