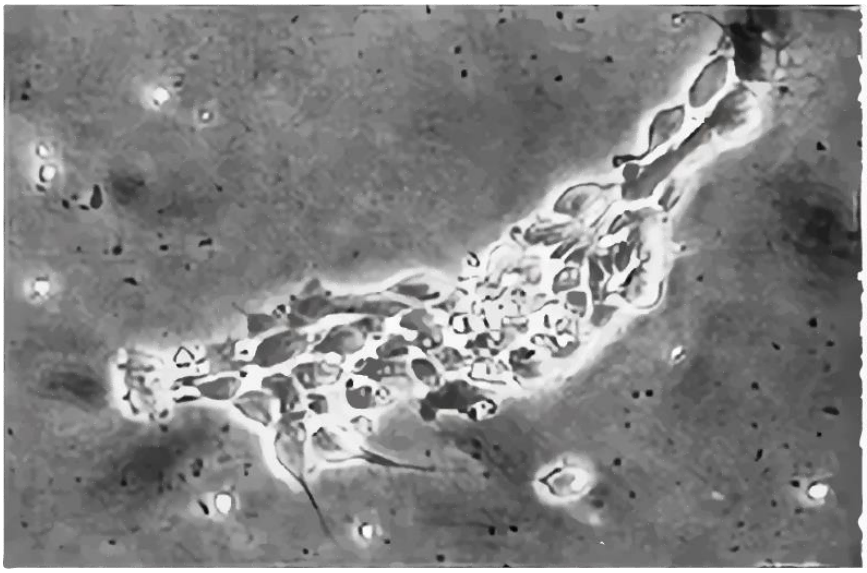
This is the way: From Stem Cells to neuronal progenitors



This course provides an overview of the concept of stem cells and their pathway to becoming neurons. The course covers the theoretical understanding of stem cells, their differentiation process, and the approaches used to produce neuronal progenitors. Students will also have the opportunity to analyze real-time experiments and observe the morphological changes during the differentiation process.

Recommended grade level

Highly motivated high school students (10 - 12th grades)

Resource Structure:
The course resources include slides used by the instructor, videos capturing the differentiation process, and additional images and illustrations to enhance understanding. These resources are designed to provide both theoretical knowledge and practical insights into stem cell differentiation.

Learning Goals:
By the end of the course, students are expected to achieve the following learning goals:

1. Understand the concept of stem cells: Students will gain knowledge about the different levels (morphological, functional, etc.) of stem cells and their significance in development and regeneration.
2. Explore the differentiation process: Students will review the key concepts and pathways involved in the differentiation of stem cells with a neuronal destiny. They will learn about the molecular factors and signaling pathways that influence stem cell differentiation.
3. Identify approaches to produce neuronal progenitors: Students will be introduced to various methods and techniques used to generate neuronal progenitors from stem cells. They will

understand the importance of different conditions, culture mediums, and specific molecules in directing stem cells towards the neural lineage.

4. Analyze real-time experiments: Through the examination of videos obtained from a real-time experiment, students will observe and interpret the morphological changes that occur during the differentiation process. They will learn to relate the effects of different molecules and conditions on stem cell differentiation.

Student Knowledge:

To benefit from this course, students should have a basic understanding of cell biology and molecular biology. Familiarity with concepts such as cell differentiation, signaling pathways, and gene expression will be advantageous. Some prior knowledge of stem cells and their general properties would also be helpful but is not required, as the course provides an introductory overview of stem cells and their differentiation process.

Section 1: Introduction to Stem Cells and their pathway to Become a neuron

Introduction:

- In this section, you will review the concept of a stem cell at different levels (morphological, functional, etc.).
- You will review key concepts and pathways in the differentiation process of a stem cell with a neuronal destiny.
- Finally, you will identify different approaches to produce neuronal progenitors.

This section corresponds to the theoretical part of the current course. Pay attention to the instructions provided by the instructor, who will use audiovisual material to provide you with all the necessary knowledge for the following activities.

Use the following link to download the slides used by your teacher.

[insert some slides screenshots?}

Section 2: Through the differentiation process

Introduction:

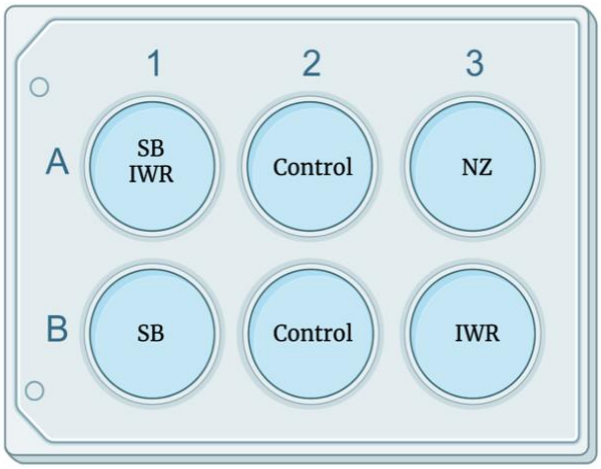
- In this section, you will be able to visualize the morphological changes during the differentiation process from a stem cell to a neuronal progenitor
- You will explore videos obtained from a real-time experiment under different conditions.
- You will be able to relate the effect of each molecule

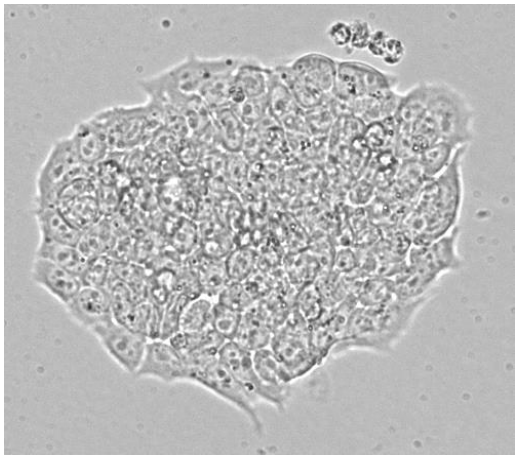
Now that we have reviewed the changes that occur during the differentiation process from a stem cell to a neural progenitor, let's go to analyze a real experiment. For this purpose, we have used a microscope designed by one of our collaborators. This equipment is designed to use 6-well plates and allows the capture of images at regular intervals, so the final output consists of 6 different videos of all the collected images. Moreover, the person conducting the experiment can access the program and view the collected images in real-time, thanks to our remote experimentation technologies.

Setting Our Experiment

The experiment that we are going to analyze during this class is a 2D differentiation from stem cells to neural progenitor. Remember that for cells to survive, they need to be submerged in a medium that allows it. Essentially, a culture medium consists of water and other components that provide nourishment to our cells. And depending on what we feed them, we can define their fate. Additionally, they need to be at an ideal temperature (T= 37°C) and a CO2 concentration of 5%, with the latter two parameters controlled by the incubator.

The experiment starts with the seeding of mESC in each of the wells of a 6-well plate. Approximately 125,000 cells/well were seeded, with each well receiving a different treatment. In the following image, we can see the experimental setup, as well as a photograph of one mESC colony used in our experiment.





At this point, you may be wondering what each of the words enclosed in the wells of the plate means. First, we have the "control" wells. In these wells, the mESC will remain in what we call "mESC media," a medium formulated to maintain the pluripotency of the stem cells. It is important that in every experiment you conduct in the future, you have a control group with which you can compare your results.

The next wells correspond to the "experimental" wells, which means they are the ones where we aim for differentiation towards a specific fate. These wells have in common the use of a neuronal induction medium or Sasai 1 (named after the author who defined this medium), to which different molecules are added (indicated in each well) that will help determine the fate of the stem cells.

Before delving into each of the molecules used, why do you think it is necessary to use different conditions to achieve a single objective, in this case, the generation of neural progenitors?

Activity 1: Make your own hypothesis

In the following table, you can review each of the molecules used in a bit more detail.

Compound	Full Name	Description
SB	SB431542	SB431542 is a small molecule inhibitor that targets the transforming growth factor-beta (TGF-β) signaling pathway. It specifically inhibits the activity of the TGF-β type I receptor, also known as activin receptor-like kinase 5 (ALK5) by binding to its ATP-binding pocket, which is essential for the receptor's kinase activity.
IWR	IWR-1-endo	IWR-1-endo is a potent inhibitor of WNT signaling through the stabilization of a destruction complex member called AXIN2. The destruction complex is responsible for the phosphorylation and subsequent degradation of β-catenin in the absence of WNT signaling. As a result, the nuclear translocation of β-catenin and the activation of WNT target genes are effectively inhibited.
NZ	Neurodazine	Neurodazine is a small molecule which can act as a Stem Cell neuronal differentiation inducer.

Before we start analyzing the data, let's pay attention to the following image, which is an illustration of the changes that mESC go through on their path.

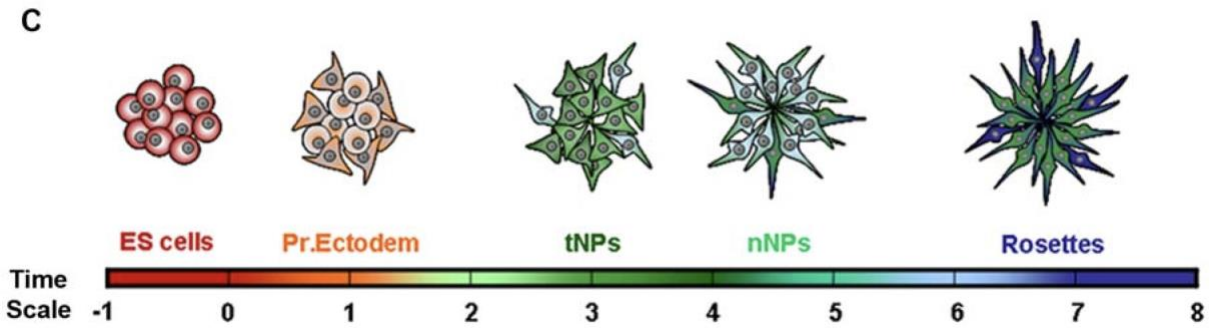


Figure 1. Schematic representation of the successive cellular states that occur along the path to neural differentiation. ES: Embryonic Stem Cell, Pr.Ectoderm: Primitive ectoderm, tNPs: transient NP, nNPs: neurogenic progenitors. doi:10.1371/journal.pone.0006286.g006

During the process of neural induction, stem cells initiate the neural differentiation process under specific conditions that exclude inhibitory signals like BMP. Bone morphogenetic proteins (BMPs) belong to the transforming growth factor beta (TGFβ) superfamily and have diverse functions in the development of the central and peripheral nervous systems. In vertebrates, the absence of BMP signaling is associated with neural induction from the ectoderm, which occurs during or before gastrulation. As they commit to the neural progenitor cell lineage, stem cells express pan-neural genes, indicating their specification towards the neural lineage. Subsequently, these neural progenitor cells organize themselves into three-dimensional structures known as rosettes, which closely resemble embryonic neural tube primordia and signify an advanced stage of in vitro neuronal differentiation. Within the rosettes, the cells exhibit a distinct apico-basal polarity as they acquire competence for neuronal production. Cells in neural rosettes are capable of multiplying by symmetrical division over an extended period in culture.

However, the process of becoming mature neurons extends beyond the previous time-scale. We can use the following image to have a more comprehensive overview of the entire process.

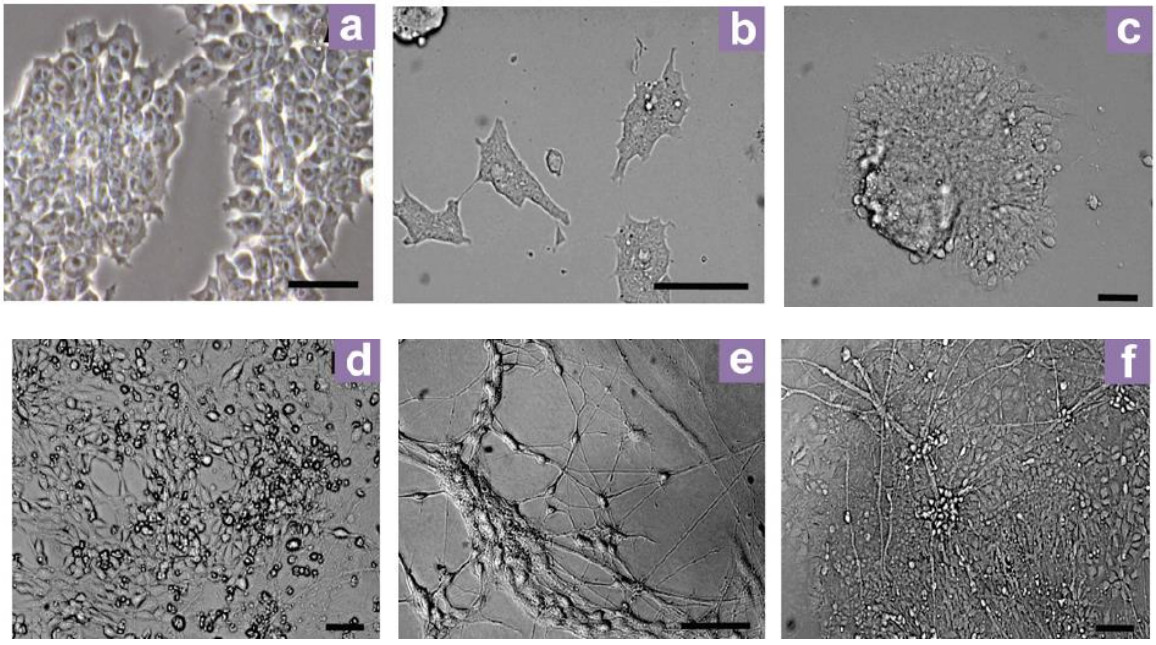


Figure 2. The progression of mouse embryonic stem cell differentiation after neural induction can be observed in the following stages: (a) Day 0: Mouse embryonic stem cells, (b) Day 3: Neural progenitor cells, (c) Day 7: Precursor cells, (d) Day 11: Immature neural cells, (e) Day 17: Immature/differentiating neural cells and (f) Day 22: Differentiating/mature neural cells. PMID: 25941460

Considering the information provided in this section and the one reviewed in the previous section, could you predict what you would find after 5 days of starting the experiment?

Control: (mESC media)

Sasai 1 (SB)

Sasai 1 (SB + IWR)

Sasai 1 (Neurodazine)

Activity 2: Checking the differentiation process

Great! Now it's time for you to compare the hypothesis you have proposed for each condition with the results of our experiment. You will now review 4 different videos corresponding to days 5 - 6 (24 hours) of cultivation, all compressed into just 10 seconds. Please access the following link which will allow you to download the videos, each corresponding to one of the described conditions.

After reviewing the content of each video, please identify any differences from the provided hypothesis or if it is validated. Please describe your findings in the following table for each condition.

Conditions	Description
Control	

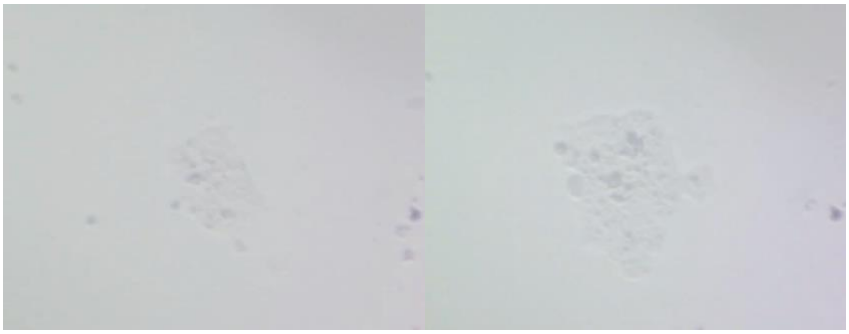
Sasai 1 (SB)	
Sasai 1 (SB + IWR)	
Sasai 1 (Neurodazine)	

It is very likely that during the content review, you may have noticed details in the cells, such as 'movements' or changes in their morphology. Let's take some time to analyze each of them in detail in the following minutes.

Activity 3: Analyzing each of the conditions:

Condition 'Control' (Click to watch)

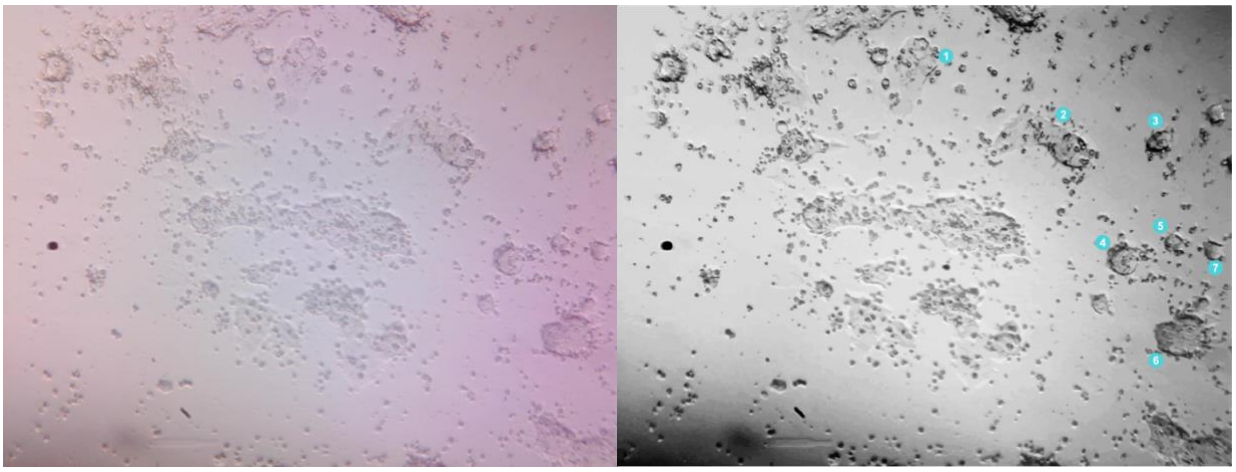
First, let's analyze the control of our experiment. Remember that the control well contains mESCs in a specific medium to maintain their pluripotent state. The following two images represent the starting and ending point of the video:



What is the biggest change that you can observe between the beginning and the end of the video? How would you describe the behavior of the cells during the video? Does it align with what was expected?

Condition 'SB' (Click to watch)

Now let's analyze the condition in which our cells have been in our base medium supplemented with the SB molecule. Feel free to track each of the initial colonies, but for analysis purposes, let's focus on some of them, which have been labeled as can be distinguished in the following graph.

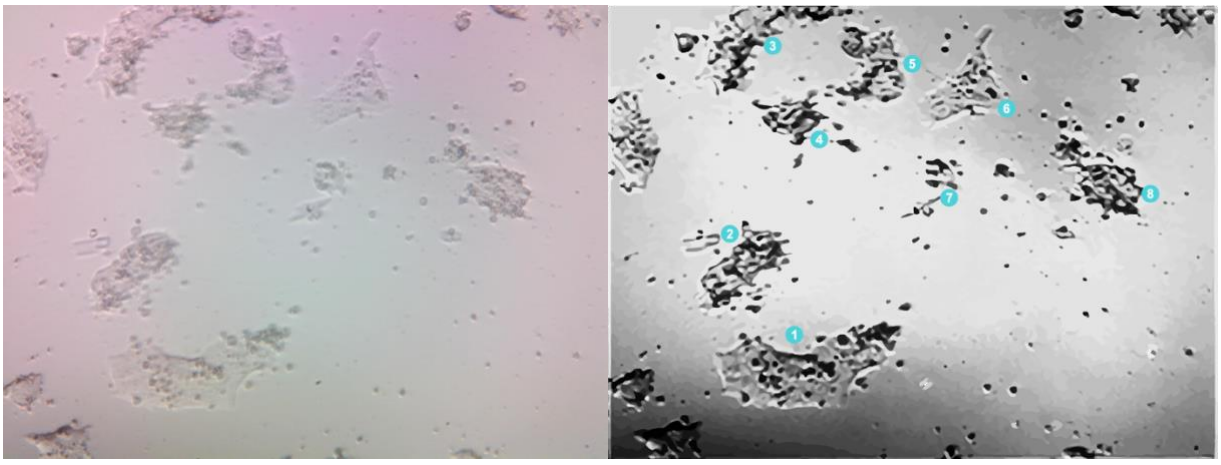


Describe the behavior of colonies 1 and 2; 4 and 5; 6 and 7. What are they doing? What's difference with the stem cells?

If we focus on each of the selected colonies (1-7), we can distinguish certain changes in the colony's morphology. Describe it. Additionally, explain if the observed morphology corresponds to what was expected for the time of the experiment (5 days after the initiation of differentiation).

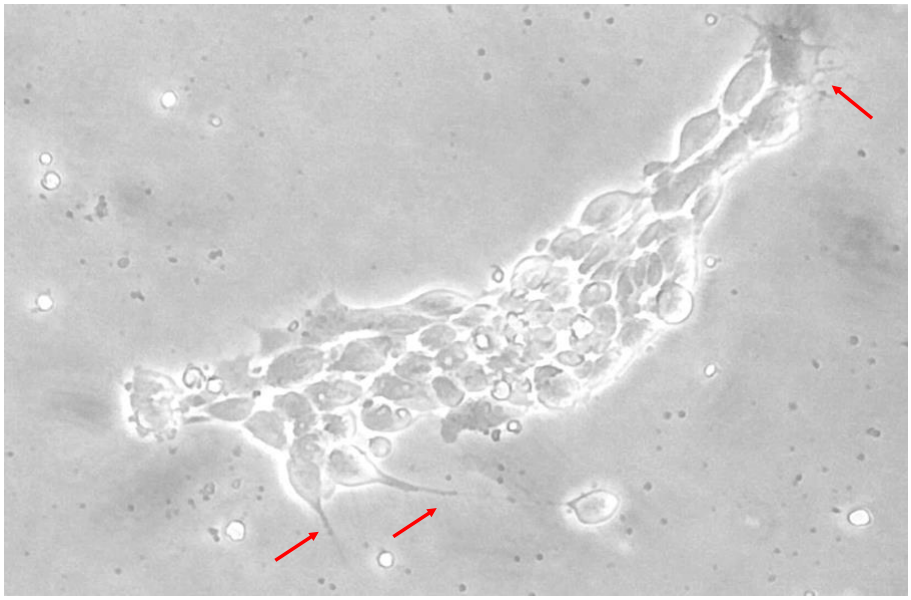
Condition 'SB + IWR' (Click to watch)

This condition is similar to the previous one, with the addition of a second molecule: IWR. Some colonies have been selected to track each one of them in the video.



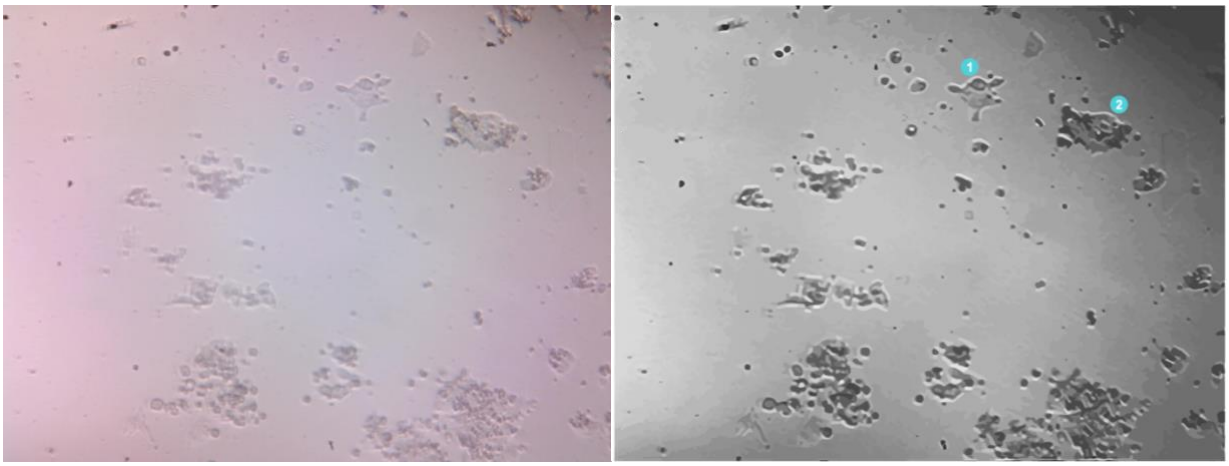
Considering the enumerated colonies, what changes can you notice happening to them during the video? Can you find any differences compared to the previous condition?

At the end of day 6, we managed to capture an image of certain cells using a higher magnification of 20X. What are the most notable characteristics that you can observe?



Condition 'Neurodazine' (Click to watch)

Our latest condition involves the use of Neurodazine, a molecule that has not been fully characterized yet, although it is proposed to act as an inducer of neuronal differentiation. We have selected 2 colonies to perform tracking in the video.



Can you find any differences in terms of cell morphology compared to the previous conditions?

Activity 4: Final Considerations

After analyzing each of the conditions, which molecules would you use in a subsequent experiment to induce stem cell differentiation?