

Teacher's Guide: Mowing Down Cancer Cells

Class time: 30-50 minutes.

Purpose: To analyze a graph of data from an experiment testing two different cancer treatments against laboratory-cultured cancer cells.

Notes to the teacher: After students have had a chance to review the article "[Cancer's sweet cloak](#)," pass out the accompanying Student Guide ([Blackline Master 3](#)) and rulers.

If your students are unfamiliar with the concept of a log scale, explain what it is and how to make approximate measurements on it before students start working. Students may also view this [Khan Academy video](#) on logarithmic scales. The main idea to emphasize is that the major tick marks on a log axis represent factors of 10 (1, 10, 100, 1,000 and so on). In this way, it's possible to show a wider range of values on one graph. That means the halfway point between the values of 1 and 10 is not 5.5 as students might expect, but roughly 3. In order to find the halfway point, one must take the square root of the product of the left tick mark's value (1) and the right tick mark's value (10). For example, the square root of 10 (because $1 \times 10 = 10$) is about equal to 3.16. [See visualization here](#). The value 2 is somewhat to the left of that midpoint, and all the other values (4 through 9) are scrunched up closer and closer between the midpoint and the next tick mark. For another example, if two adjacent tick marks indicate 100 and 1,000, the midpoint between them is roughly 316. Take the square root of the product of the left tick mark's value (100) and the right tick mark's value (1,000). The square root of 100,000 ($100 \times 1,000 = 100,000$) is about equal to 316. Two hundred is somewhat left of the midpoint and 400 through 900 get closer and closer together between the midpoint and 1,000.

Introduction:

The graph titled "Effect of HER2 antibody plus sialidase on immune activity" is from [an experiment](#) with laboratory-cultured populations of ZR-75-1 human breast cancer cells, which compared with normal breast cells have elevated (but not astronomical) levels of the HER2 protein on their surfaces. HER2 protein is a growth factor receptor, which helps to sense signals from the environment telling cells to grow. If cells have too much HER2, they can grow and divide too much, a hallmark of cancer cells.

The graph shows the effect of two different treatments on these cells:

- Herceptin, or trastuzumab, an antibody that specifically binds to the HER2 protein.
- Herceptin-sialidase, the same antibody connected to a bacteria-produced sialidase enzyme that trims sialic acid sugars.

For each of these two treatments, the graph plots the “Percent cytotoxicity” (the percent of cancer cells killed by natural killer cells) versus the treatment concentration in picomolar ($\text{pM} = 10^{-12} \text{ M}$). Circles show measured data points, and curves extrapolate the apparent trend for the data points.

Questions:

1. Using your ruler and a calculator, add and label the pM concentration for at least five additional tick marks on the x-axis.
2. If you were repeating this experiment, what additional concentration might you want to test? What result would you expect to see? *[Based on the trend lines drawn, students should pick at least one or two concentrations around 100–200 pM. They should expect to see an intermediate cytotoxicity.]*
3. Using a ruler, approximately what is the lowest percent cytotoxicity? *[Approximately 2 to 3 percent cytotoxicity.]*
4. Why might there be a nonzero amount of cytotoxicity even with no treatment? *[Cell death occurs. Even individual cancer cells that are kept well-fed in a laboratory experiment can die.]*
5. Using a ruler and estimating from the log scale on the x-axis, approximately what is the highest treatment concentration tested? *[Approximately 20,000 pM.]*
6. At the highest concentration of the Herceptin only treatment, approximately what is the percent cytotoxicity? How much larger is that value (in percentage points) than the lowest measured cytotoxicity? *[Approximately 12–13 percent cytotoxicity total, or approximately 10 percent more cytotoxicity than with no treatment.]*
7. At the highest concentration of Herceptin-sialidase treatment, approximately what is the percent cytotoxicity? How much larger is that value than the lowest measured cytotoxicity (in percentage points)? *[Approximately 32–33 percent cytotoxicity total, or approximately 30 percent more toxicity than with no treatment.]*
8. At the highest concentration, approximately how much more cytotoxicity does Herceptin-sialidase cause than Herceptin (approximately what multiplicative factor)? *[Roughly 2.5–3 times more cytotoxicity, depending on whether you are talking total cytotoxicity or cytotoxicity above the lowest level recorded.]*

9. Using a ruler and estimating from the log scale on the horizontal axis, at approximately what concentration does Herceptin have half of its maximum possible effect? [*Approximately 1,000 pM.*]
10. Using a ruler and estimating from the log scale on the horizontal axis, at approximately what concentration does Herceptin-sialidase have half of its maximum possible effect? [*Approximately 100 pM or a smidgen over that.*]
11. Herceptin and Herceptin-sialidase treatments require different concentrations to achieve half of their maximum effects. What's the fraction of Herceptin-sialidase required relative to Herceptin alone? [*To achieve a half-maximal effect, roughly 1/10 as much Herceptin-sialidase is required as Herceptin. The concentration required to achieve half the maximum effect is called the effective concentration or inhibitory concentration, for 50 percent of the effect, EC₅₀ or IC₅₀. The lower it is, the less of a treatment you have to take.*]
12. Based on what you know, by what mechanism(s) does Herceptin kill ZR-75-1 breast cancer cells in this experiment? [*Herceptin blocks HER2 growth factor receptors, so cells get fewer signals telling them to grow. Some cells will likely die.*]
13. Based on what you know, by what mechanism(s) does the Herceptin-sialidase treatment kill ZR-75-1 breast cancer cells in this experiment? [*Herceptin-sialidase contains Herceptin that blocks HER2 growth factor receptors, but it also contains sialidase that clips sialic acid sugars on the cell surface. With their sialic acid sugar disguise shaved off, the cancer cells are recognized and killed by natural killer cells.*]
14. Which treatment does a better job of killing ZR-75-1 breast cancer cells? How is that shown in this experiment, and what difference in mechanism explains that difference in results? [*Based on this study, the Herceptin-sialidase combination is much more effective at killing ZR-75-1 cells than Herceptin alone is killing approximately 2.5–3 times more cells at roughly 1/10 the required concentration. Because it has the sialidase enzyme, it clips sialic acid sugars on the surface of targeted cells and triggers an immune response.*]
15. Why is it important to test both Herceptin and Herceptin-sialidase in the same experiment? What else might you want to see tested at the same time and why? [*Herceptin is a control to see if the effects of the Herceptin-sialidase treatment are due only to the benefits of Herceptin binding to HER2, or whether the sialidase enzyme is also playing a role. It might also make sense to test sialidase by itself, just to make sure it does not cause the same levels of cytotoxicity at the concentrations observed for the coupled Herceptin-sialidase.*]

16. What experiments could you do with cultured cells to test how safe Herceptin and Herceptin-sialidase treatments might be? *[It is great to show that these treatments kill breast cancer cells, but it is also very important to show that they do not kill normal breast cells, or other types of normal cells. Tests could look at normal cells and even a breast cancer cell line that does not have elevated levels of HER2.]*
17. Would you expect Herceptin and Herceptin-sialidase to be effective against all types of breast cancer cells? Why or why not? *[Herceptin and Herceptin-sialidase both target cells with elevated levels of HER2, but some breast cancer cell types have normal amounts of HER2. The sialidase portion works by stripping off sugars, but it is possible that cancer cells exist that don't rely on sugars as a cloak but have found some other way to keep the immune system from attacking.]*
18. Name an additional factor that might affect the success of Herceptin-sialidase treatment in a whole animal or human? *[A human or animal immune system might recognize the bacteria-produced sialidase as foreign and attack the molecule or its carriers.]*
19. How could you change the design of Herceptin-sialidase to help minimize problems in a whole animal or human? *[Ideally the sialidase (and the rest of the treatment) should come from the species or individual in which it is being used, so that it will not be recognized as foreign by the immune system. Even that is no guarantee because the immune system might react to how the molecules are connected.]*
20. How could you change the design of Herceptin-sialidase to target other types of cancer cells? *[One end of Herceptin-sialidase is the Herceptin antibody that binds to HER2 that is found in excess on the surface of some breast cancer cell types. That end could be replaced with an antibody that binds to some other protein that is found in excess on the surface of some other cancer cell types. But tests need to show the success of these approaches because some cancer cell types do not have excess surface proteins, and some proteins abundant on cancer cells are also abundant on normal cells.]*
21. If you changed the design of Herceptin-sialidase to target some other type of cancer cells, what sort of simple experiments could you do with cultured laboratory cells? *[Link sialidase to another antibody that binds to some other protein found in excess on the surface of some other cancer cell type. Test that antibody, antibody-sialidase and sialidase alone against cultured cancer cells to measure the percent cytotoxicity at a wide range of concentrations. Also, do the same experiment with cells that have a normal amount of that target protein on their surfaces, in order to test for undesirable toxicity in normal cells.]*

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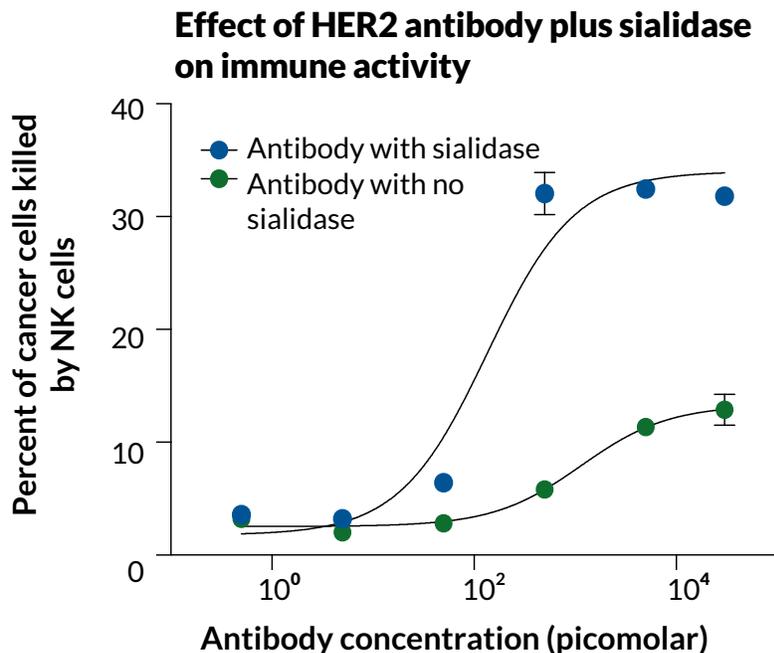
Purpose: To analyze a data graph from an experiment testing two different cancer treatments against laboratory-cultured breast cancer cells.

Introduction: The graph titled “Effect of HER2 antibody plus sialidase on immune activity” is from [an experiment](#) with laboratory-cultured populations of ZR-75-1 human breast cancer cells, which compared with normal breast cells have elevated (but not astronomical) levels of the HER2 protein on their surfaces. HER2 protein is a growth factor receptor, which helps to sense signals from the environment telling cells to grow. If cells have too much HER2, they can grow and divide too much, a hallmark of cancer cells.

The graph shows the effect of two different treatments on these cells:

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For each of these two treatments, the graph plots the “Percent cytotoxicity” (the percent of cancer cells killed by natural killer cells) versus the treatment concentration in picomolar ($pM = 10^{-12} M$). Circles show measured data points, and curves extrapolate the apparent trend for the data points.



Directions: Analyze the graph and explore cancer immune therapy treatments by answering these related questions:

1. Using your ruler and a calculator, add and label the pM concentration for at least five additional tick marks on the x-axis.
2. If you were repeating this experiment, what additional concentration might you want to test? What result would you expect to see?
3. Using a ruler, approximately what is the lowest percent cytotoxicity?
4. Why might there be a nonzero amount of cytotoxicity even with no treatment?
5. Using a ruler and estimating from the log scale on the x-axis, approximately what is the highest treatment concentration tested?
6. At the highest concentration of the Herceptin only treatment, approximately what is the percent cytotoxicity? How much larger is that value (in percentage points) than the lowest measured cytotoxicity?
7. At the highest concentration of Herceptin-sialidase treatment, approximately what is the percent cytotoxicity? How much larger is that value than the lowest measured cytotoxicity (in percentage points)?
8. At the highest concentration, approximately how much more cytotoxicity does Herceptin-sialidase cause than Herceptin (approximately what multiplicative factor)?
9. Using a ruler and estimating from the log scale on the horizontal axis, at approximately what concentration does Herceptin have half of its maximum possible effect?
10. Using a ruler and estimating from the log scale on the horizontal axis, at approximately what concentration does Herceptin-sialidase have half of its maximum possible effect?

11. Herceptin and Herceptin-sialidase treatments require different concentrations to achieve half of their maximum effects. What's the fraction of Herceptin-sialidase required relative to Herceptin alone?
12. Based on what you know, by what mechanism(s) does Herceptin kill ZR-75-1 breast cancer cells in this experiment?
13. Based on what you know, by what mechanism(s) does the Herceptin-sialidase treatment kill ZR-75-1 breast cancer cells in this experiment?
14. Which treatment does a better job of killing ZR-75-1 breast cancer cells? How is that shown in this experiment, and what difference in mechanism explains that difference in results?
15. Why is it important to test both Herceptin and Herceptin-sialidase in the same experiment? What else might you want to see tested at the same time and why?
16. What experiments could you do with cultured cells to test how safe Herceptin and Herceptin-sialidase treatments might be?
17. Would you expect Herceptin and Herceptin-sialidase to be effective against all types of breast cancer cells? Why or why not?
18. Name an additional factor that might affect the success of Herceptin-sialidase treatment in a whole animal or human?
19. How could you change the design of Herceptin-sialidase to help minimize problems in a whole animal or human?
20. How could you change the design of Herceptin-sialidase to target other types of cancer cells?
21. If you changed the design of Herceptin-sialidase to target some other type of cancer cells, what sort of simple experiments could you do with cultured laboratory cells?