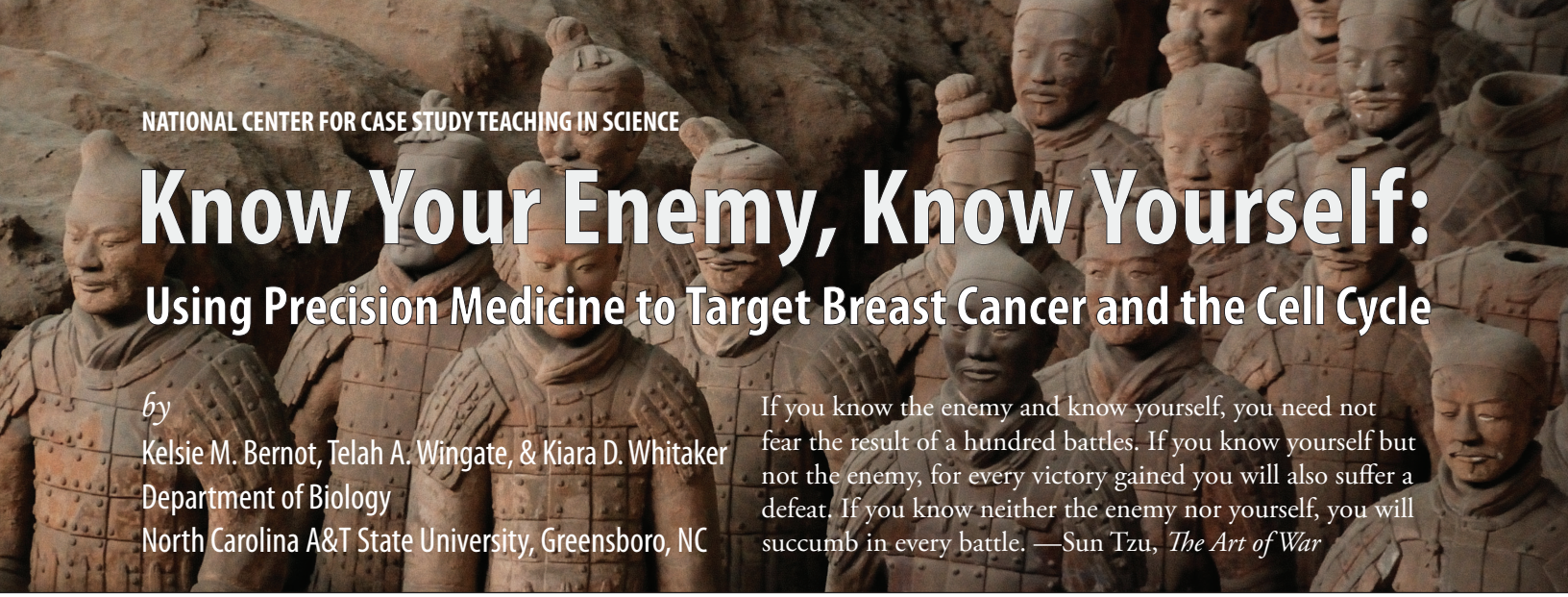

NATIONAL CENTER FOR CASE STUDY TEACHING IN SCIENCE

Know Your Enemy, Know Yourself:

Using Precision Medicine to Target Breast Cancer and the Cell Cycle

by
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If you know the enemy and know yourself, you need not fear the result of a hundred battles. If you know yourself but not the enemy, for every victory gained you will also suffer a defeat. If you know neither the enemy nor yourself, you will succumb in every battle. —Sun Tzu, *The Art of War*

Introduction

Learning Objectives

After completing this case study, you should be able to:

- Read and interpret breast cancer pathology results.
- Draw the phases of mitosis.
- Connect cell signaling to cell cycle regulation, and explain how dysregulation of those events can lead to cancer.
- Discuss how physicians use precision medicine to obtain genetic information about the individual's tumor tissue to target treatment specific to that individual's cancer.
- Discuss how physicians use precision medicine to obtain genetic information about the individual's somatic, non-cancerous cells to make decisions about treatment.
- Define *incidence* and *mortality*.
- Compare how health disparities impact patients' and physicians' abilities to know the enemy and know themselves in the United States and the world.
- Discuss the impact of ethnicity, age, geographic location and healthcare infrastructure on health disparities.

Videos

Please preapre for our class meeting by watching the following videos and taking notes on the phases of mitosis.

Video 1: Hasudungan, A. 2013. Cell cycle (mitosis). Running time: 9:52 min. <https://youtu.be/DePnK9TwU6w>.

Video 2: BiologySpotTutors. 2012. Cell cycle checkpoints. Running time: 3:44 min. <https://youtu.be/eljbYQihxXw>.

After watching these two videos you should be able to:

- Describe the cell cycle and what happens at each phase: G_0, G_1, S, G_2, M, and cytokinesis.
- Describe the three cell cycle checkpoints, what is being detected at each checkpoint, and what happens to the cell if it does not meet the requirements of the checkpoint.

Next, watch the following:

Video 3: Bozeman Science. 2014. Phases of mitosis. Running time: 10:41 min. <https://youtu.be/mXVoTj06zwg>.

You should now also be able to:

- List the phases of mitosis in the order in which they occur and describe what happens in each phase.

Part I – Uh-oh

Kelly and her husband Jack were fooling around in bed one evening, when Jack felt a lump on Kelly's breast. "What's this?" he asked.

Kelly replied, "Oh, I don't know, it's probably a cyst or something to do with my menstrual cycle."

At 33 years of age, Kelly wasn't concerned about the lump and was certain it would go away; however, her gynocologist wasn't so sure. After a mammogram showed a suspicious mass, the radiologist ordered a **biopsy**, telling Kelly "It's possible that this is a **benign** tumor; however we need to make certain that it is not **malignant**." During the next two days while Kelly was waiting to hear the results of the biopsy, she felt anxious. She periodically felt adrenaline coursing through her body as she finally considered the possibility that she might have **cancer**.

Questions

1. Explain the relationship between the following words: *benign, malignant, biopsy,* and *cancer.*

2. If you have studied epinephrine (adrenalin) signaling previously, consider why Kelly felt the effects of the hormone, adrenaline. What signals was this molecule sending to various parts of her body? (You may need to consult a reliable online source to fully understand the concept of how the hormone adrenalin acts on different tissues in the body.)

Part II – Results

Getting off the phone with the radiologist is kind of strange because I'm about to say to my coworkers for the first time, "I have cancer." I test it out in my head, and it sounds really weird, but I go ahead and say it anyway. We spend a little time talking (me laughing about things, which seems kind of inappropriate, but that's sometimes how I react to bad or sad news—I can't seem to help it). A few coworkers offer to drive me home, but I want to finish my work. What am I going to do at home—sit and cry? That's totally not my style, even if it would be understandable. Life hands you lemons, you gotta make lemonade—or at least put it in a bottle of Corona! — Kelly's blog

This news initiated a whirlwind of doctor's visits: eight doctors in seven days—radiologist, two breast surgeons, an oncologist, three plastic surgeons, and a radiation oncologist. Kelly was a scientist, yet she felt as though she was studying for the most important exam in her life—deciding how to treat her breast cancer. Kelly underwent a mastectomy, during which the breast surgeon removed her cancerous breast and sent the tissue to pathology. The plastic surgeon then inserted a tissue expander, which would gradually stretch her skin over several months so that she could receive a breast implant to normalize her physical appearance.

I really don't care about losing a breast. I don't need it for anything. It's not like it's my brain or even a kidney. Not only do I have two breasts, but they don't do anything essential anyway, so what's it matter if I have to get rid of one? I'm definitely in mental and physical preparation for this journey. I feel like I do just before a race—when I'm warning myself that's it's gonna hurt, and I'm gonna be exhausted, but I have to keep pushing my hardest. — Kelly's blog

Questions

1. The pathology report to the right is the exact information that Kelly received from the pathologist. Precision medicine requires the knowledge of factors that may differ between different cancers, even within the same type of cancer (e.g., breast cancer). This is a critical piece to "know your enemy." Number 1 on the pathology report shows Kelly's scores for various markers that determine the type and aggressiveness of Kelly's cancer, and may provide targets for treatment with precision medicine. Numbers 2–4 on the report tell the reader how to interpret the results. Use this information to interpret Kelly's results, scoring each of the following markers as low, high, positive, or negative. Note that this aspect of precision medicine is focused only on Kelly's cancer cells and what differences they have from normal cells that can be targeted by drugs.

   a. Ki-67 Proliferation Index _____

   b. Estrogen Receptor _____

   c. Progesterone Receptor _____

   d. Her2/Neu _____

2. Read this description of Ki-67 from Wikipedia: "The Ki-67 protein (also known as MKI67) is a cellular marker for proliferation. It is strictly associated with cell proliferation. Ki-67 protein is

Pathology Results

Infiltrating duct carcinoma
Ductal carcinoma in situ
Prominent lymphoid infiltrates

1. **Characterization:**
   a. **Ki-67 Proliferation Index – 40%**
   b. **Estrogen Receptor - >90% with strong intensity**
   c. **Progesterone Receptor – 5% with strong intensity**
   d. **Her2/Neu – 3+**

2. Interpretation of Results – Ki-67
   a. <10% = LOW
   b. >30% = HIGH

3. Interpretation of Results – Estrogen and Progesterone Receptors
   a. >10%, any intensity- POSITIVE
   b. <10%, strong or moderate – FOCALLY POSITIVE OR RARE POSITIVE
   c. 1-10%, weak-FOCALLY AND WEAKLY POSITIVE
   d. <1%, weak-NEGATIVE
   e. 0% - NEGATIVE

4. Interpretation of Results – Her2/Neu
   a. 0 = NEGATIVE (no staining)
   b. 1+ = NEGATIVE (weak, incomplete membrane staining or weak complete membrane staining in less than 10% of tumor cells)
   c. 2+ = EQUIVOCAL (complete membrane staining that is weak or non-uniform in more than 10% of tumor cells and/o4 intense complete membrane staining in less than 30% of tumor cells
   d. 3+ = POSITIVE (intense complete membrane staining in more than 30% of tumor cells)

present during all active phases of the cell cycle (G_1, S, G_2, and mitosis/cytokinesis), but is absent from resting cells (G_0). Ki-67 is an excellent marker to determine the growth fraction of a given cell population. The fraction of Ki-67-positive tumor cells (the Ki-67 labeling index) is often correlated with the clinical course of cancer." <http://en.wikipedia.org/wiki/Ki-67_(protein)>. Based on the Wikipedia description, does a high Ki-67 score mean that the cancer is more aggressive (growing faster) or less aggressive (growing slower)?

3. Estrogen receptor, progesterone receptor, and Her2/Neu (also known as epidermal growth factor receptor) are all signal transduction receptor proteins that transmit signals to the cell to divide. Predict the name of the ligand for each receptor. How could having too many growth factor receptors on the surface of the cell make a more aggressive cancer?

4. Some cells divide constantly, whereas other cells rarely divide if at all. Rank the following cell types by their frequency of cell division from never to constantly.
   a. Hair cells _____
   b. Liver cells _____
   c. Muscle cells _____
   d. Sensory Neurons _____

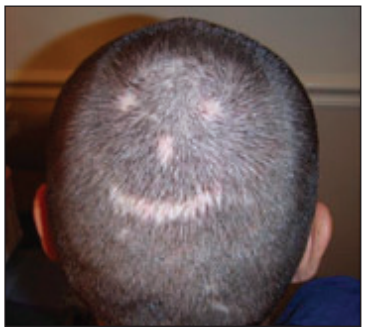
5. Review the phases of the cell cycle by placing the abbreviated phase name (G_1, S, G_2, or M/C) next to the event(s) that occur during that phase of the cell cycle.
   a. _____ Errors in DNA replication are repaired.
   b. _____ DNA is replicated.
   c. _____ The cell grows by producing more proteins and organelles.
   d. _____ The cell physically divides into two cells, with identical copies of DNA going into each new cell.

6. Review the three regulatory checkpoints that determine whether a cell progresses through the cell cycle, rests in G_0, or undergoes programmed cell death (apoptosis).

Checkpoint Name:	*What is the cell checking for at each checkpoint?*	*What happens to cell if it can't pass checkpoint?*
G_1		
G_2/M		
Metaphase (part of mitosis)		

Part III – Treatment

Kelly's breast cancer was determined to be aggressive, which meant that she needed to undergo chemotherapy. Her doctors recommended a combination of docetaxel (Taxotere®), carboplatin, and trastuzumab (Herceptin®). The following is her blog entry after her second chemo treatment:



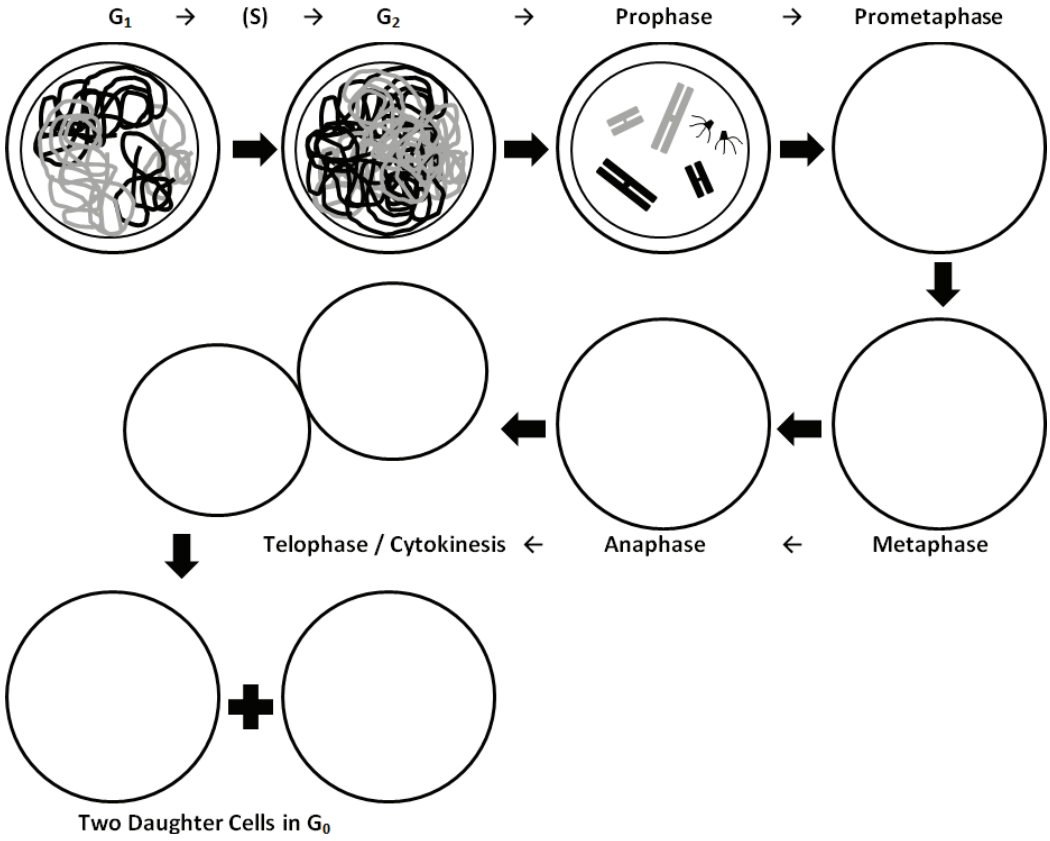
As far as chemo symptoms go, the biggest has been fatigue. I was just tired, grumpy, and not my usual, peppy self most of the week. Other very minor things—tiny bit of stomach upset at the beginning of the week, but just enough to make me not feel like eating a big steak, not enough to keep me from eating soup or sandwiches. My scalp felt a little irritated—for the girls out there, it was the feeling of having my hair in a tight ponytail all day and then letting it down, and the follicles just feel prickly—this is normal for several days before the hair falls out. Also, I have a weird dry mouth feeling, so some things taste a little different. But seriously, these are really minor issues. Jack has decided my new nickname is "Splotch.'" I shaved my head in preparation for my hair falling out. Now I have splotchy patches where my hair has fallen out and lots of zits…fun stuff! See the "design" Jack made in my hair when it first got super loose and was easily to accidentally pull out—pain free, I promise!

Questions

1. Use the diagrams provided below to review the phases of the cell cycle.
   a. Why is mitosis important? What is the end result of mitosis?

   b. Explain what has happened between G_1 and Prophase.

   c. Draw a representative picture to explain what happens in the remaining phases. Assume that the starting cell has two sets of homologous chromosomes—one set that is long, and one set that is short. Paternal chromosomes are black; maternal chromosomes are grey.



2. Kelly took the chemotherapy drug docetaxel, which interferes with the disassembly of microtubules. Microtubules make up spindle fibers, which are required for the movement of chromosomes to the center of the cell and then to opposite poles of the cell. Why is docetaxel an effective anti-cancer drug? At which stage of the cell cycle does docetaxel work?

3. Kelly also took the chemotherapy drug carboplatin, which directly damages DNA, preventing DNA replication and transcription. Why is carboplatin an effective anti-cancer drug? At which stage of the cell cycle does it work most prominently?

4. Prior to the year ~2000, women who were diagnosed with Her2+ breast cancer had a strong likelihood that the cancer would metastasize. Her2+ breast cancer is considered aggressive because women with Her2+ breast cancer had very poor survival rates. However, with the discovery of an antibody (trastuzumab) that specifically interferes with the ability of Her2 to transmit the growth signal to the cancer cells, now women with Her2+ breast cancer have a better chance of survival than women with none of the growth factor receptors (e.g., triple negative breast cancer). Why is it useful to block transmission of a growth signal to the cancer cells? At which stage of the cell cycle do growth factors work?

5. Would you predict that trastuzumab is effective on all breast cancer patients? Why or why not?

6. Why did Kelly's hair start to fall out? Why did she feel a bit of nausea? Why are these side effects of chemotherapy (docetaxel and carboplatin)? Go back to Question 6 and discuss which of the cells are most likely to be involved in chemotherapy side effects and why.

7. Precision medicine refers to a disease treatment approach that takes into account individual variability in genes, environment, and lifestyle. Precision medicine can target variability in the individual's cancer cells ("know your enemy") compared to another person's cancer cells or it can target variability within one person's somatic, *non*-cancerous cells ("know yourself"). Non-precision medicine is based on properties of the cancer cells that don't necessarily distinguish genetically between cancer cells and normal cells. Rank Kelly's three drug treatments (docetaxol, carboplatin, trastuzumab) as to whether you would consider them part of precision medicine or not.

Part IV – Survival

Kelly received chemotherapy every three weeks for a total of six cycles (four months). She then continued to receive trastuzumab every three weeks for another eight months. Her hair started growing back during this time. Kelly also underwent genetic testing of three genes that made up her normal somatic cells (not her cancer cells, but the cells that have made up her normal tissue since birth—"know yourself").

First, Kelly was tested for known cancer-promoting mutations in the BRCA1 and BRCA2 genes. This testing was necessary due to her family history of breast cancer (her paternal aunt had breast cancer at age 47; her cousin from the same family had breast cancer at 38; her father had prostate cancer; her mom had several aunts with breast cancer). Kelly tested negative for known BRCA1 and BRCA2 mutations, meaning she did not have these mutations that increase the risk of cancer. Second, Kelly was tested for her normal somatic genotype of the CYP2D6 gene. The enzyme produced from this gene is a strong metabolizer of drugs in the liver. For some drugs, it breaks them down and eliminates them. For other drugs, it activates them, by converting them from inactive to active structures. To limit recurrence, Kelly's doctors wanted to prescribe tamoxifen—a drug that interferes with the ability of the estrogen receptor (ER) in breast tissue to respond to estrogen. This is important because Kelly's breast cancer cells overexpressed estrogen receptor ("know your enemy"), meaning that naturally-produced estrogen could stimulate any remaining cancer cells to undergo mitosis. However, tamoxifen is administered as a prodrug. It must be converted to its active form by the CYP2D6 in the liver in order to be effective. Some people produce weak enzymes that are not very good at converting tamoxifen to its active form; thus tamoxifen would not be effective for those individuals. Kelly's somatic cell testing revealed normal CYP2D6 activity in her normal cells ("know yourself"), so tamoxifen was appropriate for her. She had to continue this medicine for ten years. During that time, she could not get pregnant because tamoxifen can cause birth defects if taken during the early stages of embryonic development. In addition, and, as of this publication, she has reached the nine year cancer-free mark. Although she is considered to be "cured," she still takes tamoxifen daily and has yearly mammograms and physical exams by a breast oncologist.

Kelly's thoughts nine years post-cancer:

> *It sounds trite, but having cancer was a life-changing experience for me. By the time I finish tamoxifen, I will likely be past child-bearing age, so no kids for me unless I adopt. I still look in the mirror every day and see the visible reminder of my mastectomy / reconstruction scars. I rarely forget for even a day that I'm a cancer survivor. If I have headaches a few days in a row, I fleetingly wonder if I might have metastatic cancer. Despite these stressors, facing cancer has had countless positive impacts on my life. For one, I learned to live in the moment. Two, I learned how resilient and tough I can be in the face of adversity—this has given me confidence to pursue challenges I never would have considered previously. Three, I've been able to help other people through their own experiences with adversity, and I learned how to be a better listener and supporter. I transformed my career and started speaking about "my story" to raise money for cancer research. Finally, I made my own impact on cancer research by performing research as a scientist, but more importantly by participating in numerous clinical trials at all stages of my treatment. As one of my mentors said, "When we treat a cancer patient with the standard care, we have the potential to help that individual patient. However when we enter a patient into a clinical trial, we have the chance to help the hundreds of thousands of patients who come later, through the knowledge and information that we gain from the trial."*
>
> *Now that I am a biology professor, I am also training the next generation of cancer researchers. I am extraordinarily lucky to have had such an "easy" time with cancer. But not everyone has the same story. One of my students (let's call her Cashonda) came up to me after class one year in tears because her mother had breast cancer. Cashonda's mom was a black woman originally from Nigeria, but living in the US. She was diagnosed with triple-negative breast cancer. Triple negative breast cancer means that her cancer genotype was different than mine—she didn't overexpress Her2, ER, or PR ("know your enemy"). Despite treatment, she passed away before the end of the semester. This experience had a profound impact on me, as I watched Cashonda struggle to graduate while taking her mom to doctor appointments, supporting her as she got sicker and sicker, and finally travelling across the Atlantic Ocean to bury her mother in her home country. It really made me think "I'm so lucky." And it made me ask why. Why did I have such a different experience than my*

student's mom? Did her normal somatic genotype ("know yourself") make her more prone to TNBC, which has fewer treatment options? Did her Nigerian environment or traditional diet make her pre-disposed to TNBC? Is TNBC more prevalent in Africa? How likely are African women in Africa, black women in the United States and white women in the United States to die from breast cancer?

Questions

1. Many medicines have a "standard" dose for adults. Others are given based on an individual's weight. Consider how one might use precision medicine to consider the impact of treatment decisions on the genetic variability of CYP2D6 activity from one person to the next. CYP2D6 activity is categorized as poor, intermediate, normal, or ultrarapid metabolism. Codeine is a narcotic pain reliever that is given in its prodrug form and is converted into the active form (morphine) by CYP2D6. Compare the impact of giving a "standard" dose to a poor metabolizer versus an ultrarapid metabolizer.

2. Unlike Kelly, who tested positive for all three receptors (Her2, PR, ER), patients with triple negative breast cancer (TNBC) do not overexpress any of these three receptors. Studies have shown that triple-negative breast cancer is more likely to metastasize and more likely to recur after treatment. African American women are more likely to develop TNBC than Caucasians. Kelly received several treatments for breast cancer (surgery, chemotherapy (docetaxel, carboplatin), trastuzumab, and tamoxifen.) Predict whether each of these treatments would be effective for TNBC patients, and explain why.

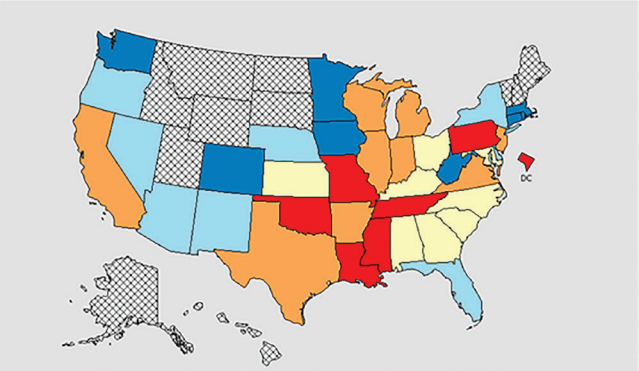
3. Is it possible to use precision medicine to target TNBC? What other information might be necessary?

4. The term "health disparities" refers to differences in the health status of different groups of people. These groups can be defined by gender, ethnicity, socioeconomic status, or their geographic location, to name a few. Some health disparities are directly related to *cancer incidence* and *cancer mortality.* Look up and write down the definitions of these terms. What is the relationship between them and how would you expect health disparities to influence them?

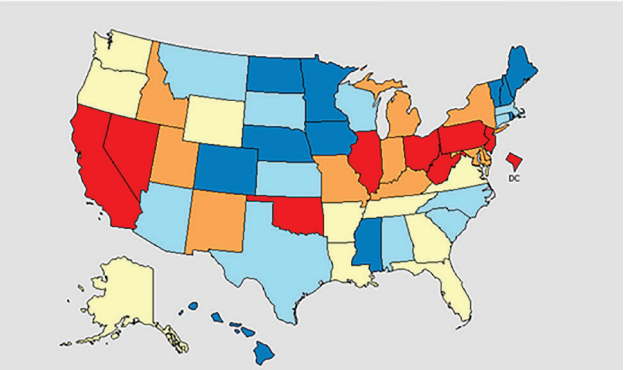
5. Consider the two maps below that show age-adjusted death rates due to breast cancer in the United States for black versus white females. What conclusions can you draw from these two maps? Write at least three separate conclusions. Discuss the implications of these findings. An updated map can be generated from <http://statecancerprofiles.cancer.gov/> using any number of characteristics such as cancer type, age, race, etc. Consider the following questions. Are there consistent differences between the two groups? Are there particular states or regions of the country that have better or worse records?

Death Rates for United States, Breast Cancer, 2009–2013

Black (includes Hispanic), Female, All Ages



White (non-Hispanic), Female, All Ages



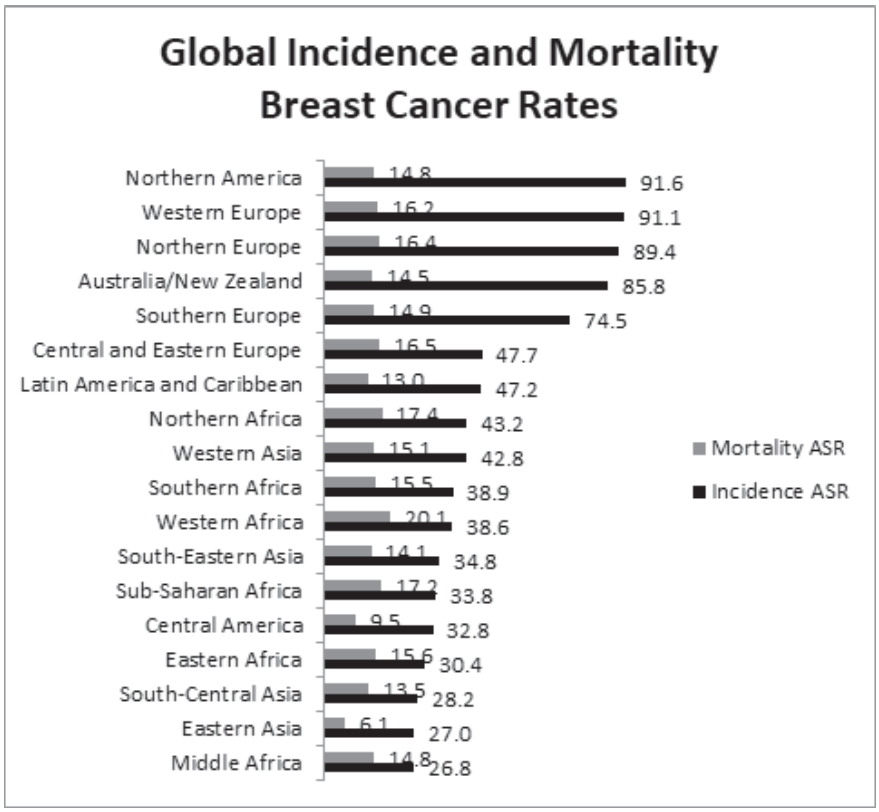
Quartile	*Age Adjusted Annual Death Rate (Deaths / 100,000) for Blacks*	*Age Adjusted Annual Death Rate (Deaths / 100,000) for Whites*
	19.8–24.4	18.3–20.0
	24.4–27.6	20.0–20.9
	27.6–30.3	20.9–21.4
	30.3–32.0	21.4–22.0
	32.0–34.8	22.0–25.4
	Suppressed due to lack of data*	

Notes:
Created by statecancerprofiles.cancer.gov on 12/21/2016 8:30pm. State Cancer Registries may provide more current or more local data. Data presented on the State Cancer Profiles Web Site may differ from statistics reported by the State Cancer Registries. *Source:* Death data provided by the National Vital Statistics System public use data file. Death rates calculated by the National Cancer Institute using SEER*Stat. Death rates (deaths per 100,000 population per year) are age-adjusted to the 2000 US standard population (19 age groups: <1, 1–4, 5–9,…,80–84, 85+). The Healthy People 2020 goals are based on rates adjusted using different methods, but the differences should be minimal. Population counts for denominators are based on the Census 1969–2014 US Population Data file as modified by NCI.

*Data have been suppressed to ensure confidentiality and stability of rate estimates. Data is currently being suppressed if there are fewer than 16 counts for the time period.

Healthy People Goal C-3: Reduce the female breast cancer death rate to 20.7.
Healthy People 2020 Objectives provided by the Centers for Disease Control and Prevention.

6. Work with your group to write at least three possible conclusions that you can draw from the following graph "Global Incidence and Mortality Breast Cancer Rates." Note that you can look online to get updated information from the current year (ASR= Age Standardized Rate). (Data from World Health Organization, <http://globocan.iarc.fr/Pages/summary_table_site_sel.aspx>.)



7. List and discuss factors that could explain why incidence and mortality rates vary so much between different regions of the world.