

Ghost in Your Genes

PROGRAM OVERVIEW

NOVA explores how the epigenome—the body's complex chemical network that controls gene expression—plays a role in human biological destiny.

The program:

- notes that, after mapping the human genome, researchers wondered how so few genes could account for so much diversity among the species.
- recounts how one scientist determined how the deletion of a key sequence of DNA on human chromosome 15 could lead to two different syndromes depending on whether the deletion originated from the mother or the father.
- explains that this was the first human evidence that something other than genes themselves could determine how genes are expressed.
- provides an animation of two ways that scientists think the epigenome works to turn genes on and off.
- cites differentiation as an example of the epigenome at work—during development, cells switch on or off to differentiate cell function; as the cells divide they retain a memory of their cell type.
- notes that while the epigenome is normally incredibly stable, epigenetic switches sometimes can be thrown and later lead to disease.
- looks at how the epigenetic profiles of two 66-year-old twins are very different from that of two 6-year-old twins, suggesting that epigenetic changes accumulate over time.
- presents several experiments with rats that reveal how the epigenome works in an animal model.
- reports how epigenetic changes may promote disease by silencing tumor suppressor genes or activating oncogenes.
- reviews a clinical trial in which half the patients recovered after being treated with a drug designed to remove chemical tags silencing tumor suppressor genes.
- presents the work of two scientists looking for an epigenetic influence on autism.
- reports on a review of generations of historical records from a remote Swedish village that suggests epigenetic changes may be passed down through the generations.



BEFORE WATCHING

- 1 Epigenetic effects employ the chemical mechanisms involved with DNA. As a class, review the following definitions: *DNA*, *chromosome*, and *genome* (see *Key Terms* on page 3). Use a model of DNA to have students identify the two strands that make up DNA, the sugar-phosphate backbone, the individual nucleotide bases, and the nucleotide base pairs.
- 2 Review the concept of gene expression with students. What is it? (*Gene expression is the ability of a gene to produce a biologically active protein.*) To help students understand this process, use the graphic on page 2 to guide them through the process of transcription and translation. Once you have reviewed the process with students, have them work in pairs to transcribe and translate a gene online at: learn.genetics.utah.edu/units/basics/transcribe

AFTER WATCHING

- 1 Discuss the concept of epigenetics. What is it? (*Epigenetics is the study of the modifications to genes, such as by methylation, which do not involve changing the underlying DNA.*) How does normal gene expression differ from epigenetic effects on the genome?
- 2 The program raises some social and ethical implications regarding epigenetic effects. While scientists still don't know exactly what affects the epigenome or how it may be passed down to future generations, if lifestyle choices or environmental effects are passed down, what would students consider changing about their current lifestyle for any potential offspring they may have? How certain would they need to be that their epigenome was being affected before they made the change?

Taping rights: Can be used up to one year after program is recorded off the air.

CLASSROOM ACTIVITY

Activity Summary

Students model how scientists use DNA microarrays to determine levels of gene expression in breast cancer patients, and then choose treatments based on what they learn.

Materials for Teacher

- 1 white plastic ice cube tray per team, with at least 12 wells
- 500 mL vinegar
- 600 mL salt water (600mL water mixed with 6 Tbsp. salt)
- 500 mL of water
- pipettes
- small self-stick notes

Materials for Each Team

- copy of “Checking Up on Genes” student handout
- copy of “How DNA Microarrays Work” student handout
- copy of “Gene Locations on Array” student handout
- copy of “Cancer Therapies” student handout
- 15 mL of phenolphthalein
- pipettes

Background

Normal-functioning DNA codes for proteins through the processes of transcription and translation. During transcription, one strand of DNA in a cell’s nucleus is used to synthesize a strand of mRNA. After the mRNA is produced, it moves into the cell’s cytoplasm. During translation, transfer RNA (tRNA) and the cell’s ribosome work together to create a protein by building a series of amino acid sequences specified by the nucleotides in the mRNA. (The tRNA transports the amino acids while the ribosome synthesizes them into proteins.) Proteins are involved in nearly every aspect of the physiology and biochemistry of living organisms.

LEARNING OBJECTIVES

Students will be able to:

- define DNA microarray and gene expression.
- describe how microarrays are used to determine gene expression.
- explain how understanding gene expression can lead to improved treatments for disease.

STANDARDS CONNECTION

The “Checking Up on Genes” activity aligns with the following National Science Education Standards (see books.nap.edu/html/nses).

GRADES 9–12

Life Science

- The cell
- The molecular basis of heredity

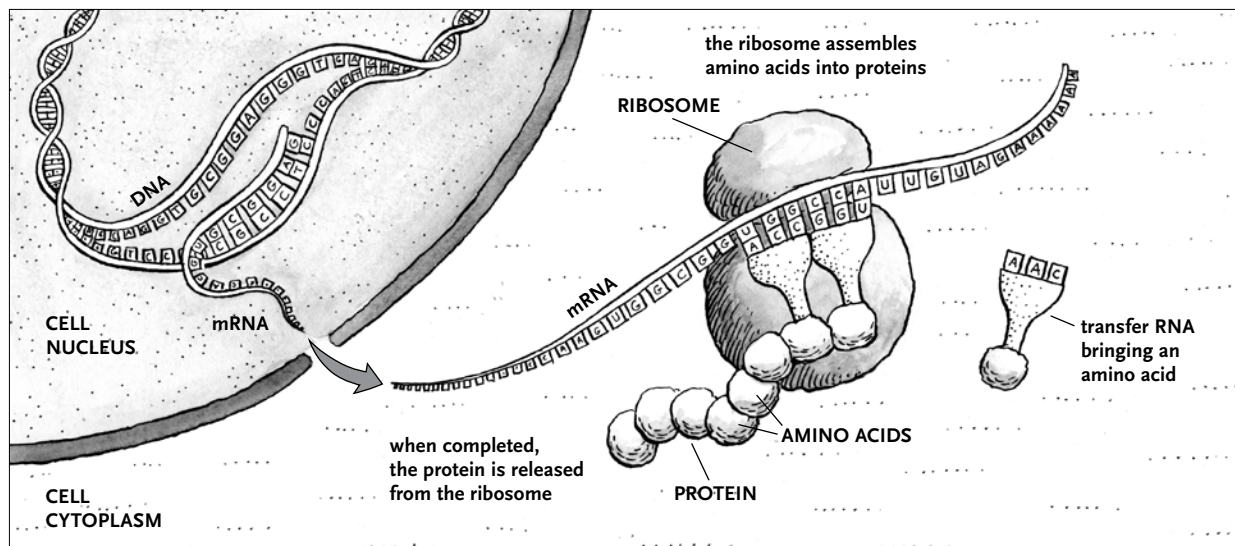
Science and Technology

- Understandings about science and technology

Science in Personal and Social Perspectives

- Personal and community health

Video is not required for this activity.



CLASSROOM ACTIVITY (CONT.)

If a DNA molecule mutates, it may produce faulty proteins. If these proteins are involved in controlling the processes of cell growth and division, the mutation could trigger a cell to become abnormal and divide uncontrollably. For many years, this was the only mechanism known to cause cancer. Treatment of this type of cancer mainly relied on trying to destroy the mutated cells.

But researchers have now discovered that cancer can be triggered by epigenetic changes—modifications to mechanisms associated with DNA that alter gene expression without mutating the original DNA. These changes are like switches turning genes on and off. Some epigenetic effects turn on, or activate, genes that stimulate tumor growth; other effects turn off, or silence, genes that would normally suppress tumor growth. Since epigenetic changes do not alter the DNA sequence itself, they hold the promise of being chemically reversed with drug (and potentially nutritional) therapies.

Cancer may be caused by several different mutations or epigenetic changes that cause genes to be expressed (turned on) and/or silenced (turned off) when they should not be. By identifying which genes in the cancer cells are working abnormally, doctors can better diagnose and treat cancer.

One way scientists try to determine which genes are working abnormally is to use a DNA microarray (see “How DNA Microarrays Work” student handout for a complete description of how these arrays function). These gene-expression “fingerprints” allow a doctor to determine both the genes involved in a patient’s cancer and the possible reaction of each patient to different drug treatments. This activity models how doctors use microarrays to determine levels of gene expression in breast cancer patients and then choose treatments based on what they learn.

Procedure

- 1 Before class, prepare enough microarrays for the number of teams you will be organizing. The activity is designed for a tray with 16 wells. If needed, you can delete columns 7 and 8 for trays with fewer wells. Columns 1–6 are needed to complete the activity. The microarray models you will be creating work on the basis of an acid, base, and neutral. The solutions you prepare will be simulating the genes that are already on a microarray before a patient’s cDNA is added. To prepare the trays:
 - Use a self-stick note to mark “TL” on the top left and “BR” on the bottom right of each ice cube tray.

KEY TERMS

chromosome: A tightly coiled macromolecule of DNA and its associated proteins. Chromosomes contain many genes. Sexually reproducing organisms have two of each chromosome, one from each parent. Organisms vary in the number of chromosomes they have.

complementary DNA (cDNA): A single strand of DNA synthesized in the lab to complement the bases in a given strand of messenger RNA. Complementary DNA represents the parts of a gene that are expressed in a cell to produce a protein.

deoxyribonucleic acid (DNA): A double-stranded chain of nucleotides. It carries a cell’s genetic information and is found in the cells of all living organisms. It is capable of self-replication and the synthesis of RNA.

DNA microarray: A collection of microscopic DNA spots attached to a solid surface, such as glass, plastic, or silicon chip, forming an array. Scientists use DNA microarrays to measure gene expression levels.

gene: The basic unit of inheritance. A gene is made up of a sequence of four different bases: A (adenine), T (thymine), G (guanine), and C (cytosine). The way that these bases are combined determines the gene’s function. Genes control the production of proteins.

gene expression: The process by which the information encoded in DNA is converted into a final gene product (i.e., a protein or any of several types of RNA).

genome: An organism’s basic complement of DNA.

messenger RNA (mRNA): Serves as a template for protein synthesis.

transfer RNA (tRNA): A set of RNA molecules that transfer amino acids to the ribosomes, where proteins are assembled according to the genetic code carried by mRNA. (Each type of tRNA molecule is linked to a particular amino acid.)

CLASSROOM ACTIVITY (CONT.)

- Put 15 mL of the pure vinegar, salt water solution, or water in the wells according to the following key for each patient.
 - A = acid—vinegar (will stay clear)
 - B = base—salt water (will turn light pink)
 - N = neutral—water (will turn dark pink)
- Set up an equal number of Patient 1 and Patient 2 microarrays. (The materials list specifies enough materials for up to eight arrays, four of Patient 1 and four of Patient 2.)

Patient 1 Profile

	1	2	3	4	5	6	7	8
A	A	A	B	B	N	B	N	A
B	N	A	N	A	B	N	B	N

Patient 2 Profile

	1	2	3	4	5	6	7	8
A	N	B	N	B	A	B	A	N
B	A	B	A	B	B	N	B	A

- Tell students they will be playing the role of oncologists specializing in breast cancer and will be conducting microarray analyses on two newly diagnosed breast cancer patients, Mrs. Jones and Mrs. Brown. Inform students that Mrs. Jones is a 46-year-old African-American woman with no family history of breast cancer and that Mrs. Brown is a 63-year-old Caucasian woman who has had breast cancer on her mother's side of the family.
- Organize students into teams. Assign half the students Patient 1 and the other half Patient 2. Distribute copies of the student handouts to each team. Review the activity with students.
- Make sure students understand the flow of DNA to mRNA to protein (see *Background* and *Key Terms* on page 3 for more information).
- Have students read the "Checking Up on Genes" handout that explains how they will do the activity. Then have them read the "How DNA Microarrays Work" handout. Clarify any misunderstandings about any terminology (see *Key Terms* on page 3) or the technology before students work with their own microarray. (Note: For this activity, the technique has been simplified.)
- Distribute the trays you have prepared to each team, the phenolphthalein, and the pipettes. Have students use the phenolphthalein solution to add three drops to each of the spots on their array. After all the spots have been treated, have students interpret the results using the key on their "Gene Locations on Array" handout. Then have each team record the result for its patient under each gene name on the same handout.

SAFETY NOTE

If students spill any of the phenolphthalein on their skin, have them immediately rinse it off thoroughly with water. After completion of the activity, rinse the tubes and droppers with a weak acid, such as vinegar.

CLASSROOM ACTIVITY (CONT.)

- 7 After teams have interpreted their results, have them use their “Cancer Therapy Options” handout to determine which therapies might be indicated for their patients. Point out that if the genes listed in the “Do not use if” category for each therapy are expressed in the manner indicated, then the patient would react badly or not respond to the treatment. Ask students to use this information to determine which treatments are safe to use for each patient. Have them record their answers on the handout.
- 8 Discuss students’ results and answers to the questions on the “Checking Up on Genes” student handout. If student DNA microarray results differ, ask students why that might be. *(Some reasons include that the substances in the prepared microarray were not distributed evenly or that students may have added different amounts of the substance representing the cDNA.)* Ask a representative from a Patient 1 team and a representative from a Patient 2 team to report the treatment choices for each patient. Are they the same? *(No.)* Ask students why, if both women have breast cancer, the treatments are different. *(The two recommendations are different because even though both patients have breast cancer, their gene expression profiles are different, and call for different treatment regimens.)*
- 9 To illustrate how chemicals in the body can control gene expression, show students the portion of the program (2:32) that describes and animates this process.
www.pbs.org/nova/teachers/activities/3413_genes.html#video
(QuickTime or Windows Media plug-in required.)
After students have viewed the video, ask them to describe two ways researchers know how genes can be turned on and off. *(Chemical tags, such as methyl groups, can attach directly to DNA and switch genes on or off, or tags can grab onto proteins called histones around which genes are wrapped. Tightening or loosening the histones effectively hides [turns off] or exposes [turns on] the genes.)*
- 10 As an extension, have students choose two of the genes in the microarray profile and research what cell regulation processes the genes control. Have students report to the class what they learned. Students can find the genes in the National Center for Biotechnology Information Gene database at
www.ncbi.nlm.nih.gov/sites/entrez?db=gene

Classroom Activity Author

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ACTIVITY ANSWER

Gene Locations on Array

Patient 1 Profile

	1	2	3	4	5	6	7	8
A	ESR1 ○	ABC-C6 ○	BCL2 –	DPYD –	TOP2A +	GSTP1 –	CDC2 +	GATA3 ○
B	DHFR +	EGFR ○	ERB-B2 +	ABC-B2 ○	MT1 –	TGFB3 +	ANXA –	GRB7 +

Patient 2 Profile

	1	2	3	4	5	6	7	8
A	ESR1 +	ABC-C6 –	BCL2 +	DPYD –	TOP2A ○	GSTP1 –	CDC2 ○	GATA3 +
B	DHFR ○	EGFR –	ERB-B2 ○	ABC-B2 –	MT1 –	TGFB3 +	ANXA –	GRB7 ○

Cancer Therapy Worksheet

Cyclophosphamide: Patient 1: yes; Patient 2: yes

Doxorubicin: Patient 1: yes; Patient 2: yes

Fluorouracil (5-FU): Patient 1: yes; Patient 2: no

Methotrexate: Patient 1: no; Patient 2: no

Paclitaxel: Patient 1: no; Patient 2: no

Tamoxifen: Patient 1: no; Patient 2: yes

Trastuzumab: Patient 1: yes; Patient 2: no

Key

+ = overexpressed
(dark pink)

– = underexpressed
(light pink)

○ = normal
(clear)

Student Handout Questions

- Which treatment or treatments would you recommend for your patient?

Patient 1: a combination of cyclophosphamide, doxorubicin, fluorouracil, and trastuzumab. Patient 2: a combination of cyclophosphamide, doxorubicin, and tamoxifen.

- Some genes, such as ERB-B2 and ESR1, have been found to be associated with particular diseases or conditions such as cancer. Other genes, such as the ABC-B2 gene, are not associated with a disease but are involved in resistance to certain drugs or treatments. Why would it be useful to test for the expression of genes like the ABC-B2 gene on a microarray? *If the gene is strongly expressed, it would mean that a particular treatment might not work, or might even be harmful to the person taking that drug.*

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LINKS AND BOOKS

Links

NOVA—Ghost in Your Genes

www.pbs.org/nova/genes

Contains articles and multimedia features to accompany the NOVA program.

Backgrounder: Epigenetics and Imprinted Genes

www.hopkinsmedicine.org/press/2002/November/epigenetics.htm

Provides a basic introduction to epigenetics.

DNA Is Not Destiny

discovermagazine.com/2006/nov/cover

Explains some ways that epigenetic changes unfold at the biochemical level and details recent research in epigenetics.

DNA Microarray

learn.genetics.utah.edu/units/biotech/microarray

Offers a step-through interactive that explains how microarrays work.

Epigenetics: The Science of Change

www.ehponline.org/members/2006/114-3/focus.html

Provides an overview, with a diagram, of the connection between epigenetic factors and disease in humans.

What Is Epigenetics?

epigenome-noe.net/aboutus/epigenetics.php

Offers a brief yet informative overview of the field of epigenetics.

Book

The Epigenome: Molecular Hide and Seek

by Stephan Beck and Alexander Olek (editors).

Wiley, 2003.

Presents nine essays that cover the historical origins of epigenetics and its role in development, gene regulation, disease, diet, and aging.

Checking Up on Genes

You are oncologists specializing in breast cancer and will be conducting a microarray analysis on one of two newly diagnosed breast cancer patients, Mrs. Jones and Mrs. Brown. You will be adding a solution to each spot on the array that represents the complementary DNA (cDNA) of your patient to determine her gene expression profile. After you complete your microanalysis, you will decide on her course of treatment.

Procedure

- 1 Read your "How DNA Microarrays Work" student handout to learn what microarrays are used for and how they work.
- 2 The plastic grid your teacher will give you represents the microarray for your patient. Each spot represents one gene. The solution represents the cDNA of a cancer patient. Using the solution your teacher has given you, use a pipette to add three drops in each spot on the microarray for your patient.
- 3 Once all the spots have been treated, use the key on your "Gene Locations on Array" handout to interpret your results. Then record the result for your patient under each gene name on the same handout.
- 4 After you have interpreted the results, use your "Cancer Therapy Options" handout, which describes several treatments for breast cancer. Use the results of your microarray analysis to determine which therapies might be indicated for your patient. Then answer the questions on this page.



Questions

Write your answers on a separate piece of paper.

- 1 Which treatment or treatments would you recommend for your patient?
- 2 Some genes, such as ERB-B2 and ESR1, have been found to be associated with particular diseases or conditions such as cancer. Other genes, such as the ABC-B2 gene, are not associated with a disease but are involved in resistance to certain drugs or treatments. Why would it be useful to test for the expressions of genes like the ABC-B2 gene on a microarray?

How DNA Microarrays Work

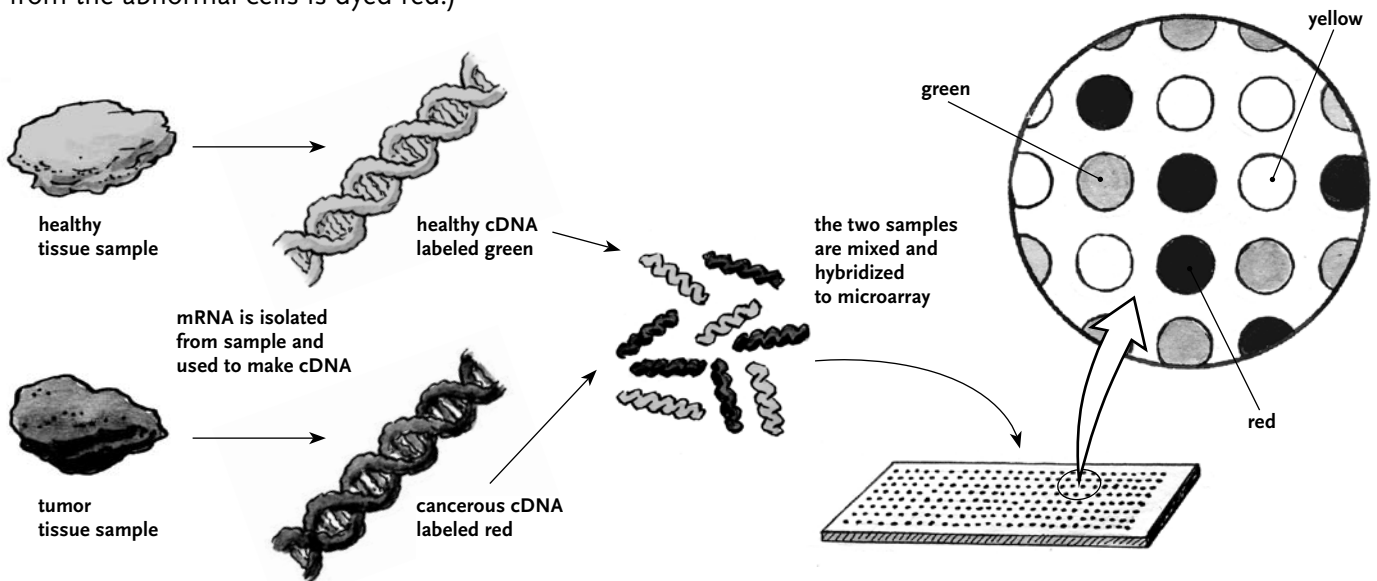
In each type of cell, like a muscle cell or a skin cell, different genes are expressed (turned on) or silenced (turned off). If the cells that are turned on mutate, they could—depending on what role they play in the cell—trigger the cell to become abnormal and divide uncontrollably, causing cancer.

By identifying which genes in the cancer cells are working abnormally, doctors can better diagnose and treat cancer. One way they do this is to use a DNA microarray to determine the expression levels of genes. When a gene is expressed in a cell, it generates messenger RNA (mRNA). Overexpressed genes generate more mRNA than underexpressed genes. This can be detected on the microarray.

The first step in using a microarray is to collect healthy and cancerous tissue samples from the patient. This way, doctors can look at what genes are turned on and off in the healthy cells compared to the cancerous cells. Once the tissues samples are obtained, the messenger RNA (mRNA) is isolated from the samples. The mRNA is color-coded with fluorescent tags and used to make a DNA copy (the mRNA from the healthy cells is dyed green; the mRNA from the abnormal cells is dyed red.)

The DNA copy that is made, called complementary DNA (cDNA), is then applied to the microarray. The cDNA binds to complementary base pairs in each of the spots on the array, a process known as hybridization. Based on how the DNA binds together, each spot will appear red, green, or yellow (a combination of red and green) when scanned with a laser.

- A red spot indicates that that gene was strongly expressed in cancer cells. (In your experiment these spots will be dark pink.)
- A green spot indicates that that gene was strongly repressed in cancer cells. (In your experiment these spots will be light pink.)
- If a spot turns yellow, it means that that gene was neither strongly expressed nor strongly repressed in cancer cells. (In your experiment these spots will be clear.)
- A black spot indicates that none of the patient's cDNA has bonded to the DNA in the gene located in that spot. This indicates that the gene is inactive. (All of the genes in your experiment are active.)



A microarray is an orderly arrangement of rows and columns on a surface like a glass slide. Each of the spots on an array contains single-stranded DNA molecules that correspond to a single gene. An array can contain a few, or thousands, of genes.

Gene Locations on Array

The following charts show which genes are represented by each spot on your array. Use the key on this page to determine the expression level of each gene for your patient. Then record whether each gene was strongly expressed (+), strongly repressed (-), or neither strongly expressed nor repressed (o) underneath the name of each gene.

Patient 1 Profile

	1	2	3	4	5	6	7	8
A	ESR1	ABC-C6	BCL2	DPYD	TOP2A	GSTP1	CDC2	GATA3
B	DHFR	EGFR	ERB-B2	ABC-B2	MT1	TGFB3	ANXA	GRB7

Patient 2 Profile

	1	2	3	4	5	6	7	8
A	ESR1	ABC-C6	BCL2	DPYD	TOP2A	GSTP1	CDC2	GATA3
B	DHFR	EGFR	ERB-B2	ABC-B2	MT1	TGFB3	ANXA	GRB7

Key

- + = strongly expressed (dark pink)
- = strongly repressed (light pink)
- o = neither strongly expressed nor repressed (clear)

Cancer Therapies

Cyclophosphamide

Brand Names: Cytoxan, Neosar

What it is: chemotherapy drug

How it works: Cyclophosphamide acts by transferring one or more saturated carbon atoms to cellular macromolecules. This damages the cancer cell DNA, and slows or stops the growth of the cancer cells.

Do not use if one or more is true: ABC-B2 = +
GSTP1 = +
MT1 = +

Safe to use for: Patient 1 yes no Patient 2 yes no

Doxorubicin

Brand Names: Adriamycin, Rubex

What it is: chemotherapy drug

How it works: Doxorubicin inhibits RNA synthesis and causes DNA strand breakage. This slows or stops the growth of the cancer cells.

Do not use if one or more is true: EGFR = +
ABC-C6 = +

Safe to use for: Patient 1 yes no Patient 2 yes no

Fluorouracil (5-FU)

Brand Name: Adrucil

What it is: chemotherapy drug

How it works: Fluorouracil binds with and deactivates a key enzyme (thymidylate synthetase) in thymidine biosynthesis. This slows or stops the growth of the cancer cells.

Do not use if one or more is true: EGFR = +
BCL2 = +
DPYD = +

Safe to use for: Patient 1 yes no Patient 2 yes no

Methotrexate

Brand Names: Mexate, Folex

What it is: chemotherapy drug

How it works: Methotrexate binds to and inactivates the enzyme dihydrofolate reductase (DHFR), and inhibits the synthesis of purine and pyrimidine. This prevents the growth of cancer cells.

Do not use if one or more is true: BCL2 = +
DHFR = +

Safe to use for: Patient 1 yes no Patient 2 yes no

Paclitaxel

Brand Name: Taxol

What it is: chemotherapy drug

How it works: Paclitaxel binds to tubulin and blocks cell division. This slows or stops the growth of cancer cells.

Do not use if one or more is true: BCL2 = +
ERB-B2 = +

Safe to use for: Patient 1 yes no Patient 2 yes no

Tamoxifen

Brand Name: Nolvadex

What it is: hormone (antiestrogen)

How it works: Tamoxifen binds to the estrogen receptor, preventing cell growth. It also affects the cycling of the cell in the natural cell cycle.

Do not use if one or more is true: ESR1 = 0 or -
ERB-B2 = +

Safe to use for: Patient 1 yes no Patient 2 yes no

Trastuzumab

Brand Name: Herceptin

What it is: monoclonal antibody

How it works: Herceptin binds to the ERB-B2 growth factor receptor and prevents the cell from dividing.

Do not use if one or more is true: ERB-B2 = 0 or -

Safe to use for: Patient 1 yes no Patient 2 yes no