# Biomarkers in Cancer

## An Introductory Guide for Advocates

Research Advocacy Network

*Advancing Patient-Focused Research*

www.researchadvocacy.org

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# CHAPTER 1. INTRODUCTION TO BIOMARKERS

When we go into our physician's office for an annual check-up, we are likely to have our cholesterol levels and blood pressure checked. These procedures are deemed important because high cholesterol is a biomarker for cardiovascular disease and high blood pressure is a biomarker for stroke. In bygone days, physicians used to look at the color of their patients' urine to determine whether they were healthy. As can be seen from these examples, biomarkers have been with us a long time and have become a routine part of medical care.

**What Are Proteins?**
When we hear the word protein, the first thing many of us think of is the protein in the foods we eat. Protein-rich foods include eggs, meat, cheese, beans, and nuts. Technically, proteins are large, complex 3-dimensional molecules made up of hundreds or thousands of smaller components called amino acids. Our bodies take proteins from the foods we eat and break them down into individual amino acids. These amino acids are then re-assembled in a different order to form specific proteins that our cells need to maintain their structures and carry out their functions.

## What is a Biomarker?

Ideally, different organizations and publications would agree on the definition of a biomarker. However, defining biomarkers is not straightforward because the term is used in a number of different disciplines and the types of biological measures that are considered biomarkers have expanded over time.

For instance, our examples of blood pressure and cholesterol demonstrate the use of biomarkers in medicine. However, biomarkers are also used in ecology to indicate the health of ecosystems or the effects of human intervention on other animal species. For the purposes of this guide, we will limit our discussion of biomarkers to those used in human medicine and biomedical research.

Even in these disciplines, what is considered a biomarker has changed over time as new technologies have been developed. In many areas of medicine, biomarkers used to be limited to proteins that were identifiable or measurable in the blood or urine. Today, imaging techniques allow us to view aspects of the body that we could not "see" before and have resulted in the discovery of many new biomarkers. For instance, imaging techniques permit the detection of structural changes in the human brain that can be used as indicators of certain diseases or conditions. As a result of these changes, defining the term biomarker requires a bit more exploration.

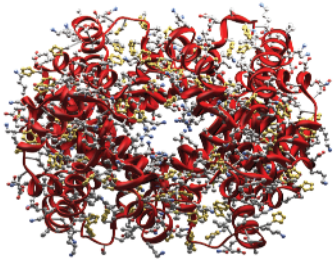
The following table lists definitions of biomarkers provided by various organizations and publications. As can be seen in this table, most definitions of biomarkers consist of two parts.

1. What kinds of things can be biomarkers?
2. What is the purpose of a biomarker? That is, what does it indicate or tell us?

Let's consider each of these in turn.

**Definitions of Biomarkers**

| Source | Definition |
|---|---|
| National Cancer Institute | A biological molecule found in blood, other body fluids, or tissues that is a sign of a normal or abnormal process, or of a condition or disease. A biomarker may be used to see how well the body responds to a treatment for a disease or condition. Also called molecular marker and signature molecule |
| MedicineNet dictionary | A biochemical feature or facet that can be used to measure the progress of disease or the effects of treatment |
| Center for Biomarkers in Imaging (Massachusetts General Hospital) | Anatomic, physiologic, biochemical, or molecular parameters associated with the presence and severity of specific disease states |
| Biomarkers Consortium (Foundation of National Institutes of Health) | Characteristics that are objectively measured and evaluated as indicators of normal biological processes, pathogenic processes, or pharmacologic responses to therapeutic intervention |



*Protein Structure*

***What kinds of things can be considered biomarkers?***

The first part of most definitions specifies the kinds of things that qualify as biomarkers. As shown in the table, some definitions limit the scope of biological markers to certain types of biological entities. For instance, the National Cancer Institute's definition states that biomarkers are "biological molecules." Similarly, the definition provided by the dictionary at medicine.net limits a biomarker to a "biochemical feature or facet." Because these definitions severely limit the types of biological characteristics that can qualify as biomarkers, they are probably too narrow. According to these definitions, high blood pressure, anatomical structures, and blood flow would not qualify as biomarkers.

In contrast, the definition provided by the Center for Biomarkers in Imaging includes a wider variety of biological measures: "anatomic, physiologic, biochemical, or molecular parameters." However, other organizations have opted to use even broader definitions that do not specify the type of parameter. An example is the definition provided by the Biomarkers Consortium. This definition states that biomarkers can include characteristics that are objectively measured and evaluated, without specifying the type of characteristic. According to this definition, high blood pressure qualifies as a biomarker, as do anatomical structures and physiological measures. This broader definition also leaves open the possibility that other types of biomarkers could be discovered in the future. The broader definitions are probably more useful in today's ever-changing medical and research environments.

| Role of Biomarker | Description of Use |
|---|---|
| Diagnostic | To help diagnose a cancer, perhaps before it is detectable by conventional methods |
| Prognostic | To forecast how aggressive the disease process is and/or how a patient can expect to fare in the absence of therapy |
| Predictive | To help identify which patients will respond to which drugs |

***What is the purpose of biomarkers?***

The second component of the definition refers to the uses of biomarkers or the purpose for identifying and measuring them. Most of the definitions note that biomarkers may have at least one of several purposes: (i) to help diagnose a condition, perhaps before the cancer is detectable by conventional methods; this is known as a diagnostic biomarker, (ii) to forecast how aggressive the disease process is and/or how a patient can expect to fare in the absence of therapy; this is known as a prognostic biomarker, and (iii) to help identify which patient will respond to which drug; this is known as a predictive biomarker. Several of the definitions also specify that biomarkers may be used to indicate normal biological processes. There is much more agreement across definitions on the purpose of biomarkers (part 2 of the definition) than on the form of biomarkers (part 1 of the definition).

A final note about the definition of biomarkers is that they may be referred to by several different names, especially in cancer medicine and research. The National Cancer Institute notes that biomarkers in cancer may also be called molecular markers and signature molecules, although, as we have seen, not all biomarkers fit into these categories. Tumor marker is another common name for biomarkers, as explained in the callout box.

**Tumor Markers**

The National Cancer Institute defines a tumor marker as "a substance that may be found in tumor tissue or released from a tumor into the blood or other body fluids." The phrase *tumor marker* is often used interchangeably with biomarker. However, the definition of biomarker is broader. Biomarkers include not only substances associated with or released from tumor tissue, but also physiological markers or markers visualized using imaging technology. Biomarkers may also be substances released by the body in response to the tumor but not by the tumor per se. For instance, the immune system may react to the tumor by producing substances that can be detected in the blood. These substances may indicate the presence of a tumor, but are not actually produced by the tumor cells. Additionally, the term biomarkers can apply to blood cancers, which do not form solid tumors.

## Types of Biomarkers

The biomarkers used today in medicine and research generally fall into several categories. Molecular biomarkers, also called molecular markers or biochemical markers, are one of the most common types. These are often genes or proteins, such as HER-2/neu in breast cancer. However, as we've seen, physiologic processes such as blood pressure and blood flow are also used as biomarkers, as are some anatomic structures such as the size of a brain area. In the following text, we describe these three categories of biomarkers, along with some examples.

***Molecular or biochemical biomarkers***

Molecular or biochemical markers are biological molecules found in body fluids or tissues. In cancer, molecular biomarkers are often genes or gene products such as proteins. An example is prostate specific antigen. Prostate specific antigen is a protein produced by prostate cells that is normally found in low levels in the blood of men. Increased levels of prostate specific antigen are used as a diagnostic biomarker for prostate cancer, although high levels can also indicate inflammation of the prostate or other conditions. As we will see in later chapters, molecular biomarkers are no longer confined to a single molecule. Instead, they may consist of a panel of different biochemical entities that together serve as a biomarker signature.

Image courtesy of National Human Genome Research Institute

### *Physiologic biomarkers*

Physiologic biomarkers are those that have to do with the functional processes in the body. For instance, blood flow in brain areas affected by stroke is being investigated as a potential indicator of treatment success. As imaging techniques become more advanced, we are likely to see an increase in the investigation and use of physiologic biomarkers.

### *Anatomic biomarkers*

Anatomic biomarkers are those that have to do with the structure of an organism and the relation of its parts. Anatomic biomarkers include the structure of various organs such as the brain or liver. For instance, the size of certain brain structures in relation to one another is a biomarker for a movement disorder known as Huntington disease. The discovery of anatomic biomarkers is also being facilitated by the development of imaging techniques.

**Examples of Some Biomarkers**

| Biomarker | Type | Condition |
|---|---|---|
| C reactive protein | Molecular/biochemical | Inflammation |
| High cholesterol | Molecular/biochemical | Cardiovascular disease |
| S100 protein | Molecular/biochemical | Melanoma |
| HER-2/neu gene | Molecular/biochemical | Breast cancer |
| BRCA genes | Molecular/biochemical | Breast and ovarian cancers |
| Prostate Specific Antigen (PSA) | Molecular/biochemical | Prostate cancer |
| CA-125 | Molecular/biochemical | Ovarian cancer |
| Cerebral blood flow | Physiologic | Alzheimer disease, stroke, schizophrenia |
| High body temperature | Physiologic | Infection |
| Size of brain structures | Anatomic | Huntington disease |

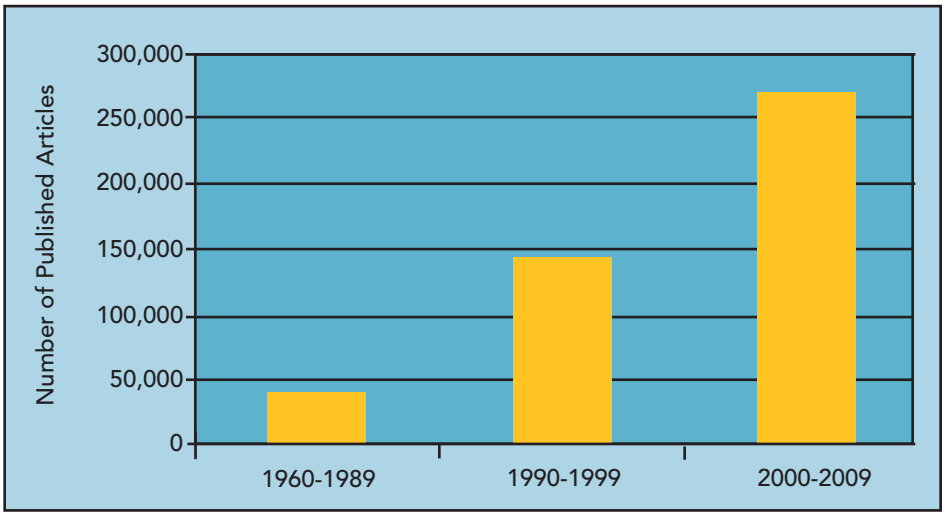
## Are Biomarkers Perfect Predictors or Prognosticators?

The answer to this question is an unequivocal "no": Biomarkers are not perfect predictors of health or disease, or response to treatment. The accuracy of biomarkers varies greatly depending on a variety of factors such as how specific they are for the disease and how accurately they can be measured. We will discuss biomarker testing in greater detail in Chapter 4. For now, however, we will simply state that the accuracy of prediction varies for different biomarkers and different conditions, and no biomarker is perfect. The ideal diagnostic biomarker would detect 100% of the people who have prostate cancer and 0% of those who do not. In reality, very few (if any) biomarkers ever achieve this level of prediction.

## Expanding Interest in Biomarkers

As you may have guessed, biomarkers are an active area of research. One way to examine the interest in biomarkers is to count the number of scientific or medical articles published on the topic over the past several decades. Between the years 1960 and 1989, approximately 42,000 such articles were published in peer-reviewed journals indexed on the PubMed database – the predominant biomedical publication database in the United States. This number more than doubled in the 1990s and nearly doubled again between 2000 and 2009. In the year 2009 alone, more than 24,000 articles related to biomarkers were published in the scientific and medical literature.

**Number of Published Scientific or Medical Articles Related to Biomarkers**



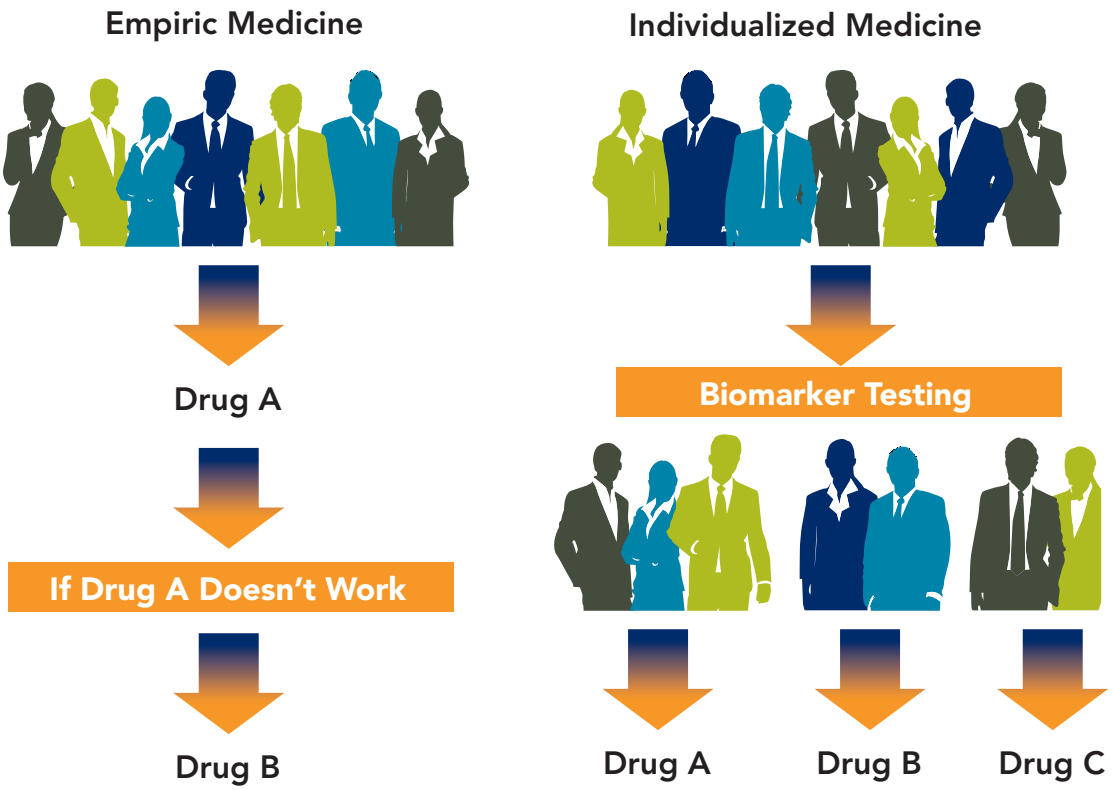

Source: National Library of Medicine, Pub Med database, keyword "biomarker" limited to the years stated

Another indicator of the interest in biomarkers is the existence of biomedical journals devoted entirely to the topic. For instance, a journal called *Biomarkers: Biological Markers of Disease and of Response, Exposure and Susceptibility to Drugs and Other Chemicals* is published 8 times per year. Other journals devoted to biomarkers include *Journal of Molecular Biomarkers & Diagnosis and Genetic Testing* and *Molecular Biomarkers.*

## Biomarkers and Individualized Medicine

A major reason for the increasing interest in biomarkers is the potential they hold for individualized or personalized medicine, also referred to as targeted therapy. One thing that is certain about cancers is that they are not all alike. As we learn more about cancer cells and their surrounding environment, the number of subtypes of each cancer increases. The subtypes are often based on biomarkers that distinguish the cancer based on some important feature such as the aggressiveness of the disease (prognostic biomarkers) or response to treatment (predictive biomarkers).

Individualized medicine is a field that focuses on differences between people and the potential for these differences to influence medical outcomes. With individualized medicine, a person's cancer may be subtyped according to some biomarker that is present or absent, increased or decreased. This may result in a greater likelihood of receiving treatment that is appropriate and effective for our particular cancer. Individualized medicine contrasts with the trial-and-error method used in the past, and still used frequently today, to determine treatment. This trial and error strategy is commonly referred to as the empiric method.




As we will see, individualized medicine is a recurring theme in the context of biomarkers. In the next chapter, we will discuss genes and gene products such as proteins, which form the basis of individualized medicine. It is the differences in these biomolecules that distinguish one cancer from another and serve as targets for many of the new cancer treatments.

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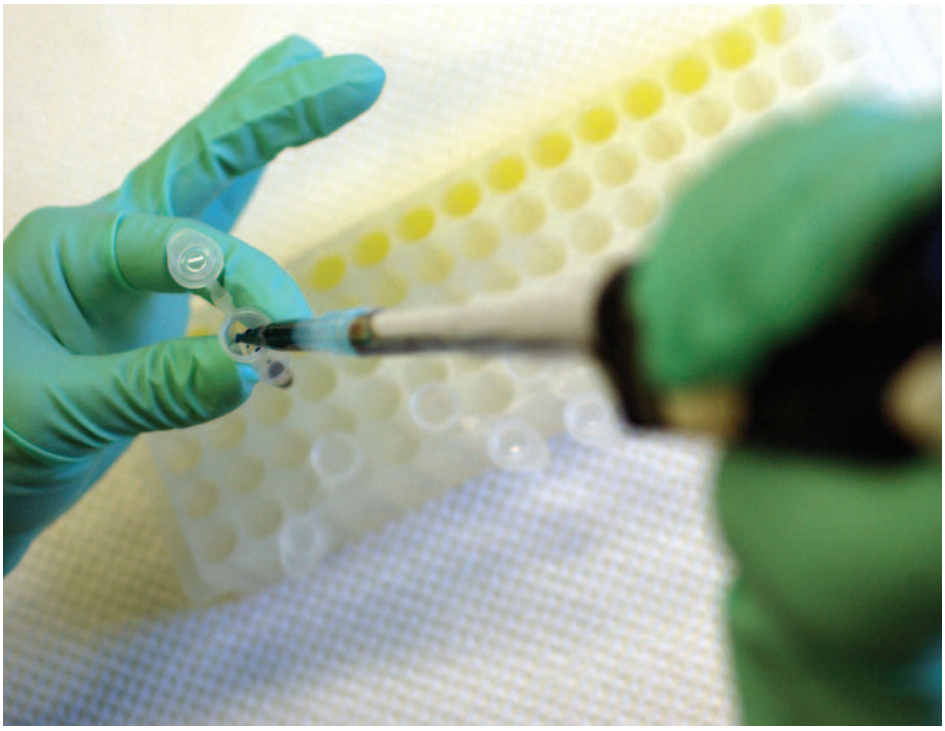


# CHAPTER 2. EXPLANATION OF GENES AND PROTEINS: COMMON BIOMARKERS IN CANCER

Humans have about 20,000 to 25,000 genes – approximately the same number as mice and plants and just a few thousand more than roundworms. This finding was surprising to some people who thought that complex animals such as humans would have many more genes than mice or rats. The fact that number of genes is not related to whether an animal builds airplanes or burrows under the ground for the winter is only one of many unexpected discoveries that scientists have made about our genes.

In this chapter we discuss genes and the proteins that result when they are turned on or activated. Genes are made up of DNA, the substance that ensures that hens have baby chicks and lionesses have baby cubs, and not vice versa. DNA is found in nearly every cell in our bodies. It provides the recipes for proteins – the biomolecules that go on to perform all cellular functions. We will also consider what can happen when our genes contain errors or alterations. Finally, we will discuss the major international undertaking known as the Human Genome Project that resulted in discoveries about the number of human genes and their chemical sequences. Let's begin by discussing the basics of DNA.

## DNA

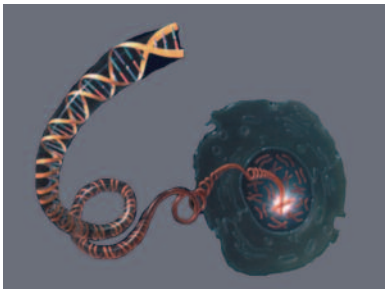
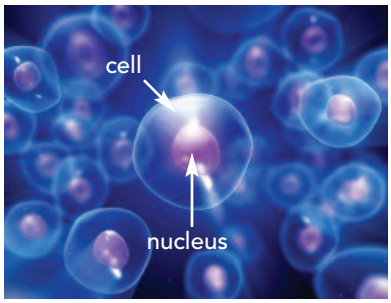


Image courtesy National Human Genome Research Institute

DNA, short for deoxyribonucleic acid, has been the focus of much attention since its double-helix structure or twisted ladder shape was first discovered by James Watson and Francis Crick in 1953. The discovery revealed what many researchers had long believed, which is that DNA actually carries the genetic information for the development and functioning of living organisms.

DNA holds within it the information that instructs cells to develop specific features that enable them to perform specific roles in the body. For instance, muscle cells are designed to contract, nerve cells are designed to communicate information, and cancer cells are designed to grow and replicate. Also, DNA carries the genes that make up the hereditary information that is passed from generation to generation. No two people have exactly the same DNA, except for identical twins. As is often seen in the news today, DNA is the genetic fingerprint used to help solve crimes when bodily fluids such as blood, saliva, or semen are recovered from a crime scene. These analyses are possible because no two people's DNA (except for that of identical twins) is exactly the same.

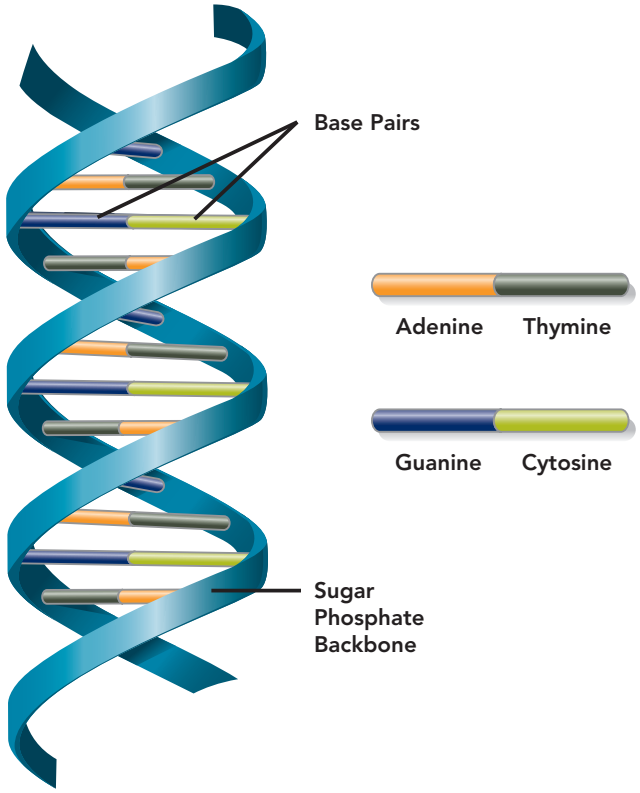



*This graphic shows a cluster of normal cells. The large round structures inside of each cell are the nuclei.*

DNA is found within the nucleus of nearly every cell in our bodies. The nucleus is a round or oval-shaped structure within the cell known mainly for its role as the home of DNA. In the cell nucleus, DNA is found tightly wound with proteins in structures called chromosomes, which we will discuss in more detail later in this chapter.

As noted previously, DNA is made up of chemical building blocks that form a double helix, a complex structure that could be compared to a twisted ladder. The steps of the twisted ladder are pairs of chemicals. It is the order of these chemicals that makes humans different from cats and makes one person susceptible to cancer and another to Alzheimer disease.

The four chemicals that pair up in DNA are known as nucleotides or nucleotide bases. These four bases are adenine, cytosine, guanine, and thymine, usually known as A, C, G, and T for the first letters of their names. The rule of base pairing is that A must pair with T, and C must pair with G. Note that either letter of the pair can be "first" in the pairing, such that A pairs with T and T pairs with A; C pairs with G and G pairs with C.

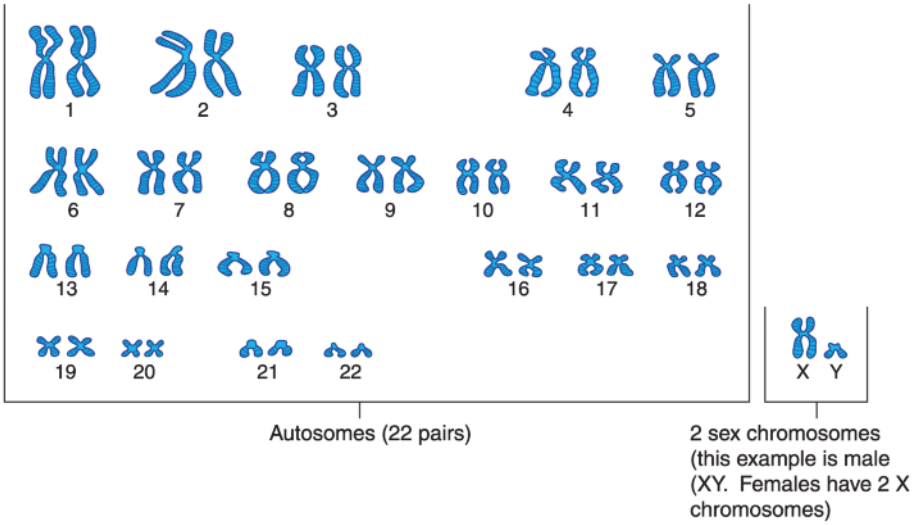



**Gene:** Pieces of DNA that contains the information for making a particular biochemical, usually a protein

A strand or sequence of DNA in humans may consist of up to 2 million A, C, G, and T bases. Located within these long strands are shorter sequences that contain instructions to make a protein. These sequences are called genes. Genes may contain hundreds or thousands of nucleotide bases. We have two copies of each gene, one from each parent.

## Chromosomes

Chromosomes are made up of tightly packed DNA supported by proteins called histones. Each chromosome has two sections, or "arms." A chromosome is an organized package of DNA found in the nucleus of the cell. Different organisms have different numbers of chromosomes. Human cells normally have 23 pairs of chromosomes: 22 pairs of numbered chromosomes called autosomes that look the same in both males and females and a pair of sex chromosomes, which differ between males and females. Females receive two X chromosomes, and males have one X and one Y chromosome.



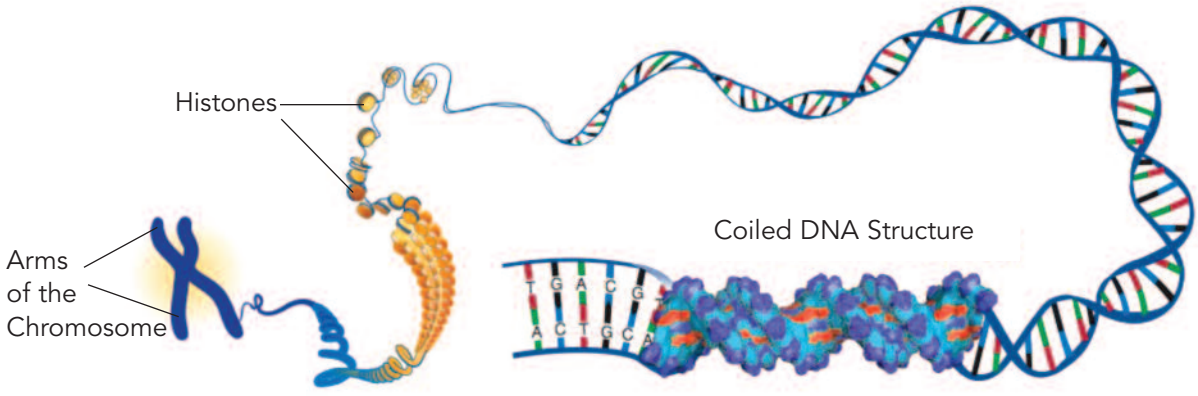
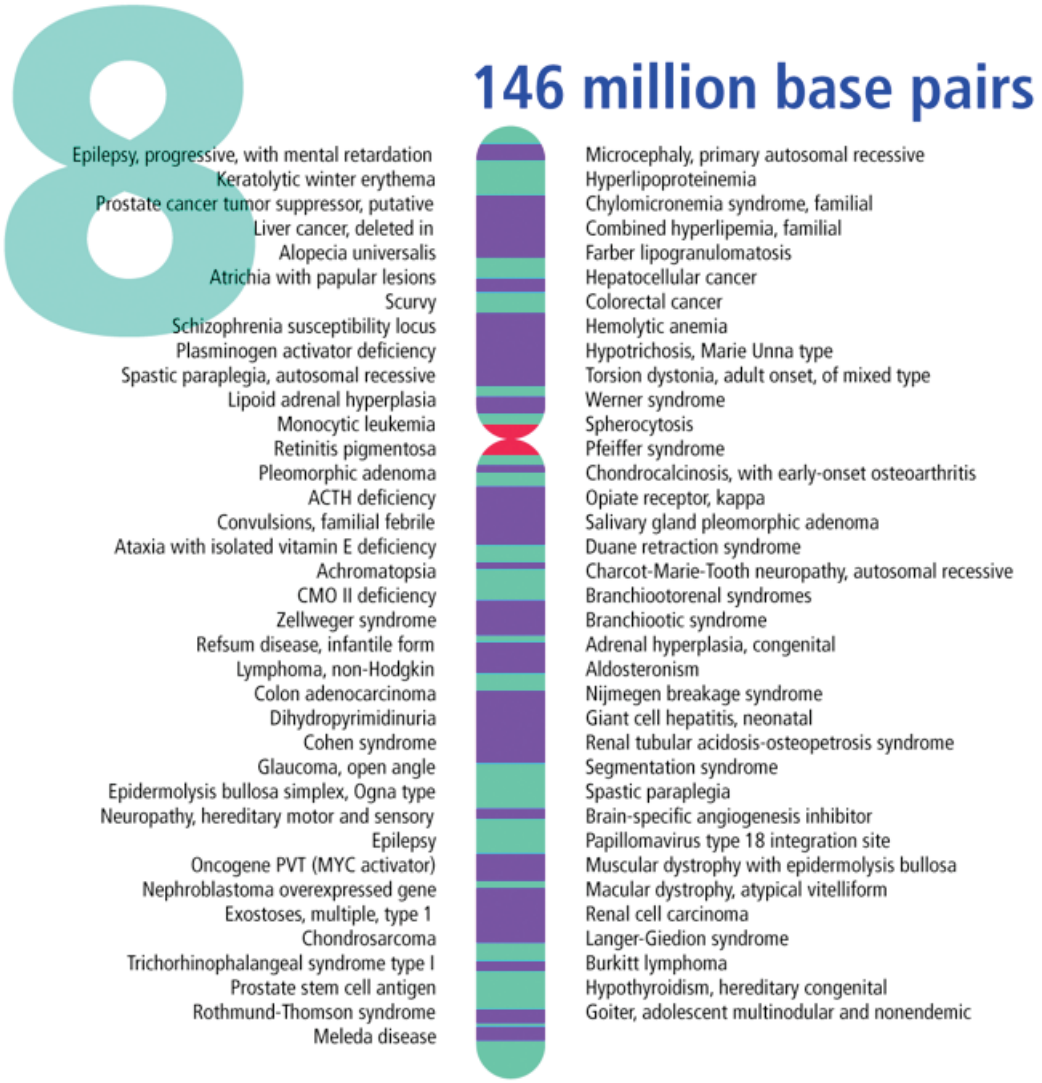




Image credit: Darryl Leja, National Human Genome Research Institute

A change in the number of chromosomes from the normal 23 pair can cause a variety of problems. Some individuals are born with conditions that are the result of having too many or too few chromosomes, such as Down syndrome, in which the person typically has three copies of chromosome 21 in each cell, totaling 47 chromosomes per cell instead of the normal 46.

Cancerous cells can also have chromosomal abnormalities, although these abnormalities may not be inherited. Such abnormalities can occur in cells other than the egg or sperm as a cancerous tumor forms or progresses.

## Medical Conditions Localized to Chromosome #8




*Researchers have mapped or localized many conditions to different human chromosomes. This graphic shows some of the conditions that are due to alterations in chromosome #8. Some chromosomes have more diseases associated with them, and some have fewer. To view the list of diseases associated with each chromosome, please visit the Department of Energy's website: http://genomics.energy.gov/gallery/chromosomes/gallery-01.html.*

**Gene Expression:** The process by which a gene gets turned on in a cell to make RNA (ribonucleic acid) and proteins.

## DNA and Gene Expression: How Are Proteins Made from DNA?

DNA does not spend all of its time wound up in chromosome form. The unique double-helix structure allows it to unwind during cell division in order to be copied and have the copies transferred to new cells. It also unwinds in order for its instructions to be used to make proteins in the process known as gene expression. Gene expression is the process by which a gene gets turned on in a cell to make a copy chemical known as RNA (ribonucleic acid), that then may be translated into a protein. The process of gene expression is comprised of two major steps known as transcription and translation.

Major Steps in Making a Protein From DNA

1. **Transcription:** copying the DNA sequence.
2. **Translation:** changing the DNA sequence into a protein.

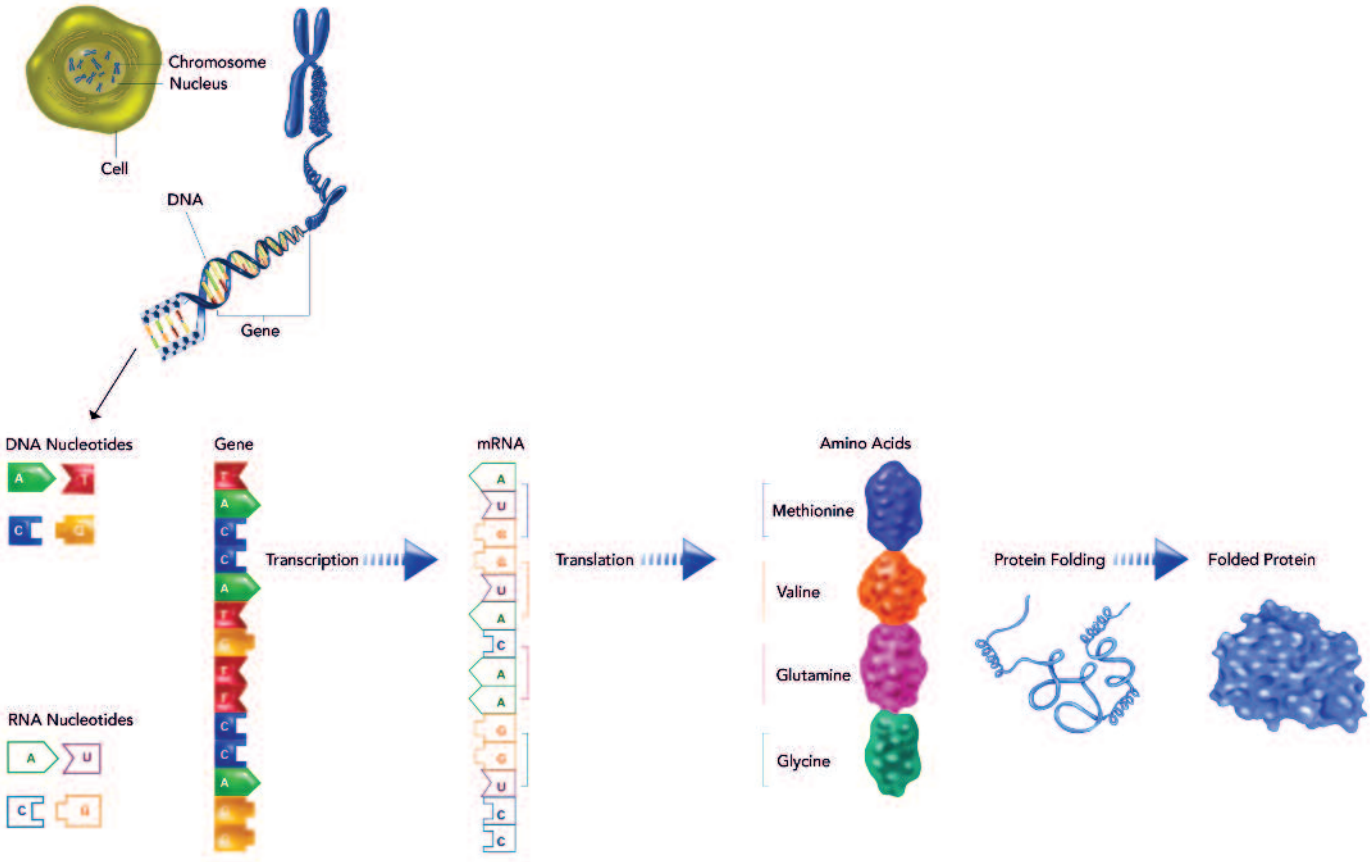



Image credit: National Institute of General Medical Sciences: http://images.nigms.nih.gov/

*This graphic shows the overall processes of transcription and translation that occur in cells. Each of these steps is explained in the text on the following pages.*

### *Step One: Transcription*

The first step in gene expression is known as transcription. During transcription, the information contained in the gene's DNA is transferred to a similar molecule called RNA. The particular RNA that receives the information is called messenger RNA (mRNA) because it carries the information out of the nucleus of the cell and into the cell's cytoplasm for the second step of the process. The transcription step is essentially a "copying" step where the DNA is copied to an RNA. It can be likened to putting your hand into a substance such as wet concrete that hardens into a mold. This mold can then be used to create a model of your hand.

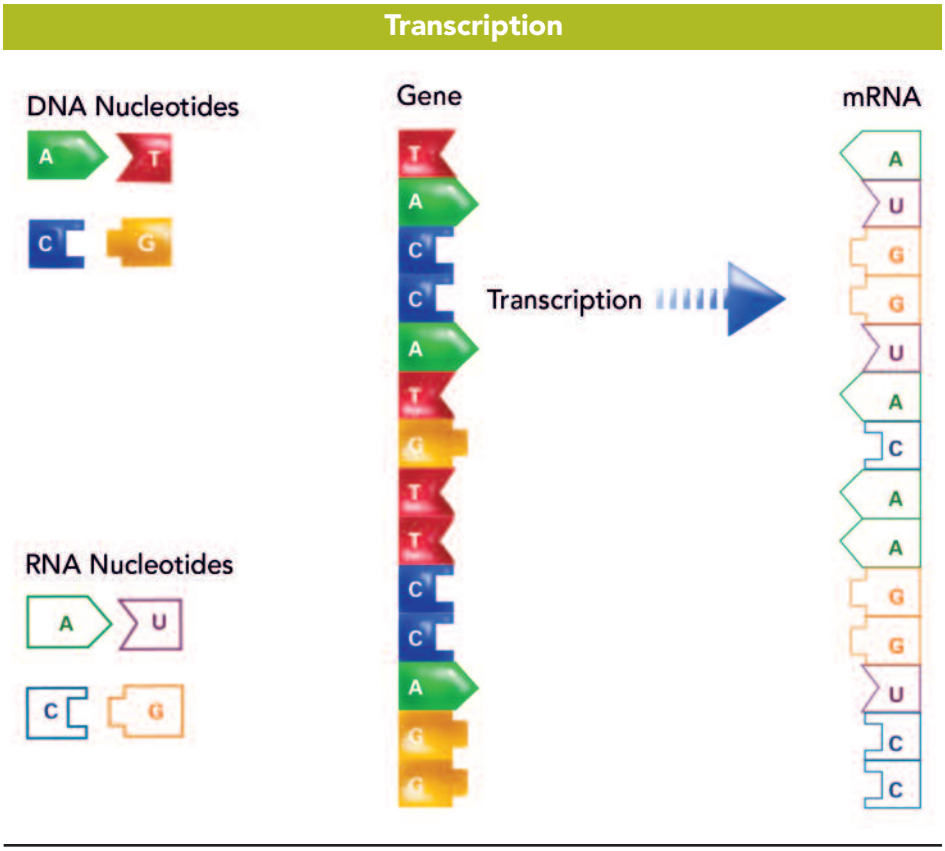



Image credit: National Institute of General Medical Sciences: http://images.nigms.nih.gov/

*This graphic shows the basic process of transcription. The DNA molecule unzips and the gene on one strand is copied to mRNA. Copying occurs by generating a strand of mRNA whose nucleotide bases pair with those of the DNA. The only exception is that RNA uses a nucleotide base called uracil instead of thymine (U instead of A) to pair with T. This pairing is shown in the lower left corner: U with A and G with C. The DNA strand to be copied is shown in the middle (TACCAT . . .). The mRNA produced by transcription is shown in the right column. As you can see, the mRNA produced contains the sequence of nucleotides that pairs with those in the DNA sequence: T pairs with A, A pairs with U, C pairs with G, etc.*

### *Step Two: Translation*

During the second step of gene expression, known as translation, the information that is contained in the mRNA is translated into another language by a structure within the cytoplasm called a ribosome. The ribosome reads the sequence of nucleotide bases, with three nucleotides coding for a particular amino acid. This sequence of three nucleotides is called a codon. Amino acids are the building blocks of proteins. A type of RNA called transfer RNA (tRNA) then assembles the amino acids in the order read off by the ribosome. Proteins are simply long chains of amino acids that take on different folding or coiling patterns depending on their length and sequence of amino acids.

**Translation**

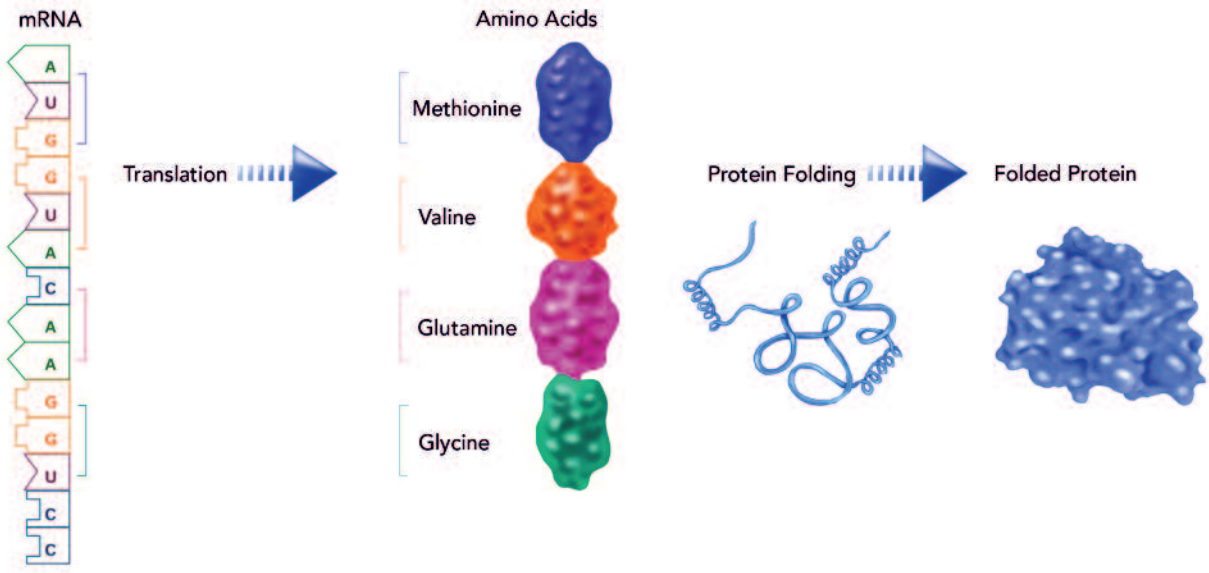



Image credit: National Institute of General Medical Sciences: http://images.nigms.nih.gov/

*This graphic shows the basic process of translation. The mRNA strand shown on the left moves out of the cell nucleus onto a ribosome. Here each set of three nucleotide bases is translated into a single amino acid as shown in the center. The spelling of the nucleotide bases tells the cell which amino acid to add. As shown in this example, AUG codes for methionine; GUA codes for valine; CAA codes for glutamine; and GGU codes for glycine. This graphic shows four amino acids: methionine, valine, glutamine, and glycine, but there are more than 20 different amino acids. As amino acids are added in the correct order, the structures become proteins. Depending on their size and the sequence of amino acids, proteins can fold or coil into certain shapes. These proteins then go on to perform nearly all cellular functions.*

## DNA to Protein

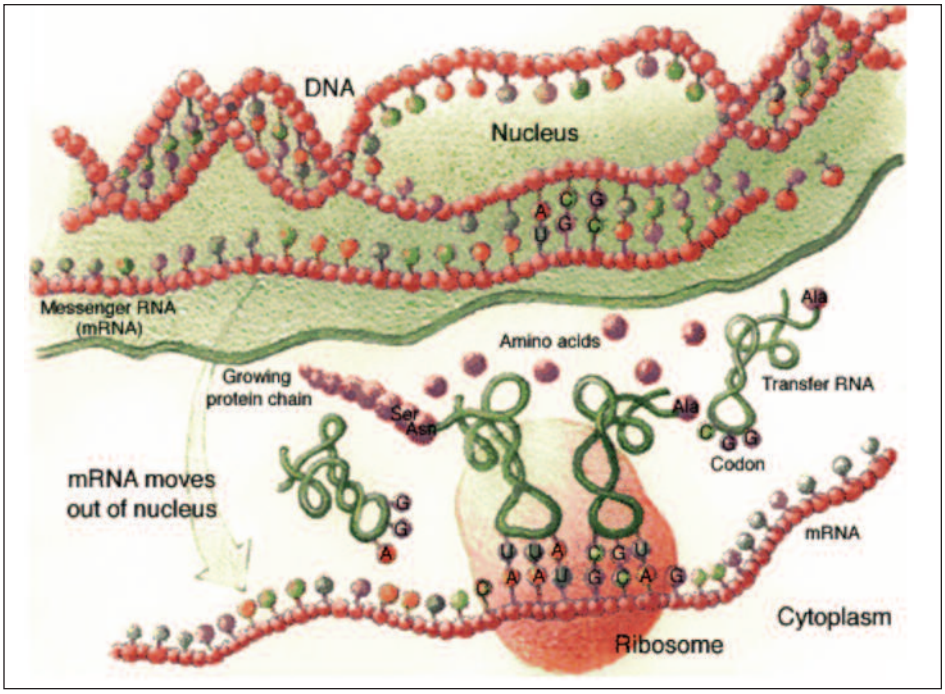



Image Credit: Genome Management Information System, Oak Ridge National Laboratory; http://genomics.energy.gov.

*This graphic shows another depiction of the process by which DNA is transcribed into mRNA and then translated into protein. The nucleus is shown in green at the top of the graphic. The coiled DNA helix is shown unraveling and being copied to mRNA inside the nucleus. The mRNA chain then moves out of the nucleus to the ribosome (lower middle part o the graphic), as indicated by the arrow. At the ribosome, a type of RNA called transfer RNA (tRNA; represented as the green squiggly lines) binds to the mRNA. Each tRNA carries three nucleotides that pair with three mRNA nucleotides. A sequence of three nucleotide bases that encodes a certain amino acid is called a codon. The tRNA adds a specific amino acid to the growing protein chain based on the sequence of nucleotides in the codon*

## Proteins

As we discussed earlier, our bodies break down protein from the foods we eat into individual amino acids. These amino acids are then re-assembled into specific proteins that our bodies require, including cell structure and function, as well as regulation of the body's tissues and organs. A list of some of essential functions of proteins is shown in the following table.

| Some Protein Functions | |
|---|---|
| **Protein Type** | **Function** |
| Antibody | Bind to specific foreign particles to protect the body |
| Enzyme | Carry out nearly all chemical reactions within a cell. Assist in formation of new molecules by reading genetic information stored in DNA |
| Messenger | Transmit signals to coordinate processes between cells, tissues, and organs |
| Structural component | Provide cellular and bodily structure and support |
| Transport/storage | Bind and transport atoms and molecules within cells and the body |

## Genetic Variation

Earlier in this chapter, we noted that no two humans have exactly the same DNA sequence unless they are identical twins. Differences in our DNA are referred to as variation. Variations can be those we are born with or those we acquire over the course of our lives.

All of us undergo changes in our DNA during our lifetimes, most of which are simple copying errors that occur during replication. Other changes in our DNA occur due to environmental damage such as sun exposure or cigarette smoke. These generally are limited to our body's DNA and not passed on to the next generation because our cells have built-in mechanisms to repair such damage. This ability to repair slows as we age, resulting in accumulating DNA damage over time. However, changes can occur in the DNA of cells that make eggs and sperm, resulting in mutations that are, indeed, passed on to the next generation. These mutations are responsible for hereditary diseases.

There are a number of different types of variations that can occur. For the purposes of this chapter, we will consider two of them: single nucleotide polymorphisms and mutations.

### *Single Nucleotide Polymorphisms*

Single nucleotide polymorphisms (SNPs; pronounced "snips") refer to a difference in only one nucleotide base pair in our DNA sequence that occurs in at least 1% of the population. These are specific, identifiable differences in DNA that account for 90% of all variation in human DNA. SNPs are not exclusively good or bad for us as organisms: some may benefit and some may harm, whereas others may have no detectable effect.

SNPs are differences in one nucleotide base pair that affect 1% or more of the population, whereas mutations are changes in the DNA sequence that affect less than 1% of the population. Mutations are not necessarily limited to changes in one single nucleotide base pair.

***Mutations***

Mutations are changes in the DNA sequence that affect less than 1% of the population. Unlike SNPs, mutations usually refer to changes that have negative consequences. DNA mutations can result in the mutated gene creating too much or too little of a given protein, the creation of an abnormal protein, or a protein in the wrong cell at the wrong time.

Scientists are continually searching for the mutations that cause specific disorders and diseases so that we can identify these mutations through genetic testing and in order to find cures or ways to prevent such conditions altogether. Most inherited genetic disorders and diseases have already been mapped by researchers.

One of the first genetic variations identified in cancer families was the BRCA1 gene, officially called the "breast cancer 1, early onset gene." Individuals who possess mutations in this gene are at higher risk of developing early onset breast cancer as well as fallopian tube cancer, male breast cancer, and pancreatic cancer. Researchers believe that mutations in the BRCA1 gene result in an abnormal protein that cannot perform its job, which is, in part, to help repair damaged DNA or fix mutations that occur in other genes.

## BRCA1 Genetic Mutation Location on Chromosome 17

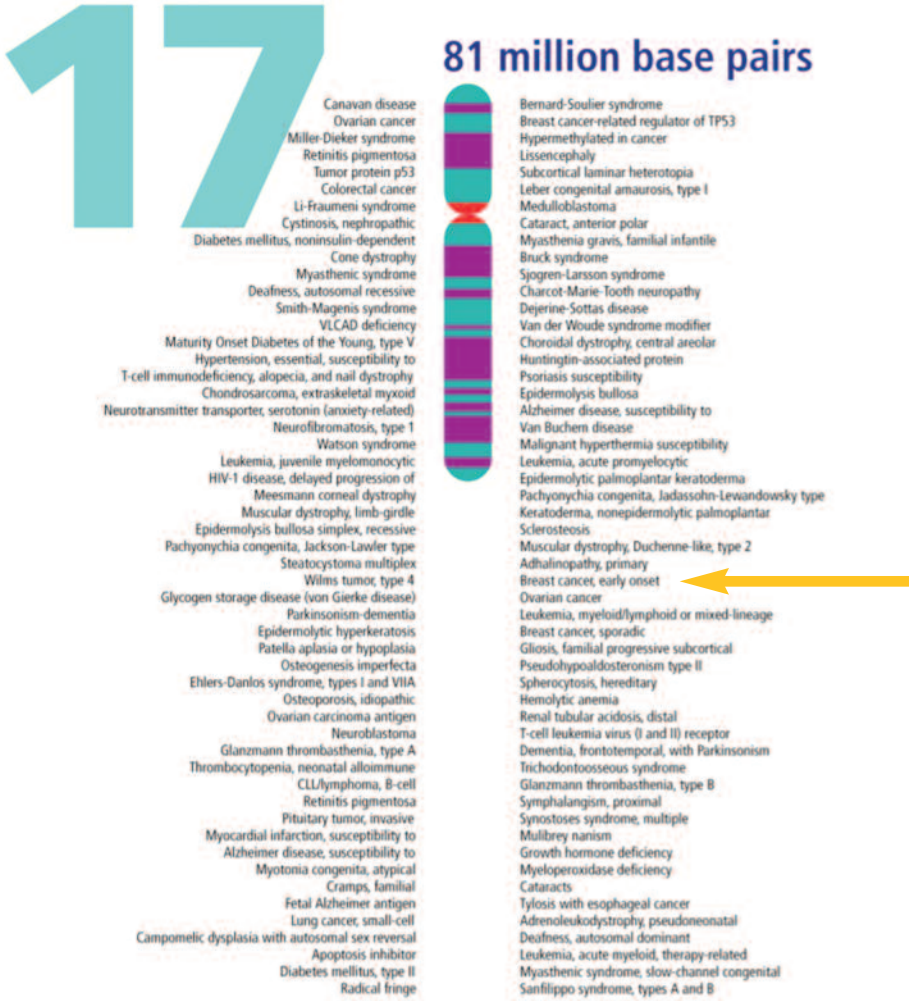



Image credit: U.S. Department of Energy Genome Program's Genome Management Information System (GMIS); http://genomics.energy.gov

Another genetic mutation that has been mapped is that of Lynch syndrome, or hereditary nonpolyposis colorectal cancer (HNPCC). This cancer is related to variations in the MLH1, MSH2, MSH6, and PMS2 genes. These genes develop proteins that repair mistakes made when DNA is copied in preparation for cell division. Abnormal cells are copied and can lead to uncontrolled cell growth and cancer. These genetic variations put individuals at a higher risk of developing HNPCC.

## The Human Genome Project

The Human Genome Project was a 13-year, international project designed to map and identify all of the approximately 20,000 to 25,000 genes in the human genome. Although the Project was completed in 2003, it continues to be a work in progress, and updates are continually posted at the Project's website (www.genome.gov). The undertaking was a coordinated effort by the US Department of Energy and the National Institutes of Health, as well as the Wellcome Trust of the United Kingdom and 18 countries around the world.

The goals of the Project were as follows:
- To identify all of the approximately 20,000-25,000 genes in human DNA;
- To determine the sequences of the 3 billion chemical base pairs that make up human DNA;
- To store this information in databases;
- To improve tools for data analysis;
- To transfer related technologies to the private sector; and
- To address the ethical, legal, and social issues that may arise from the project.

In 2006, the Project announced the completion of the DNA sequence for the last of the human chromosomes. This landmark project has provided a wealth of information for researchers worldwide and has even led to the development of new fields of science designed to understand and integrate all of the knowledge gained.

### National Human Genome Project Timeline

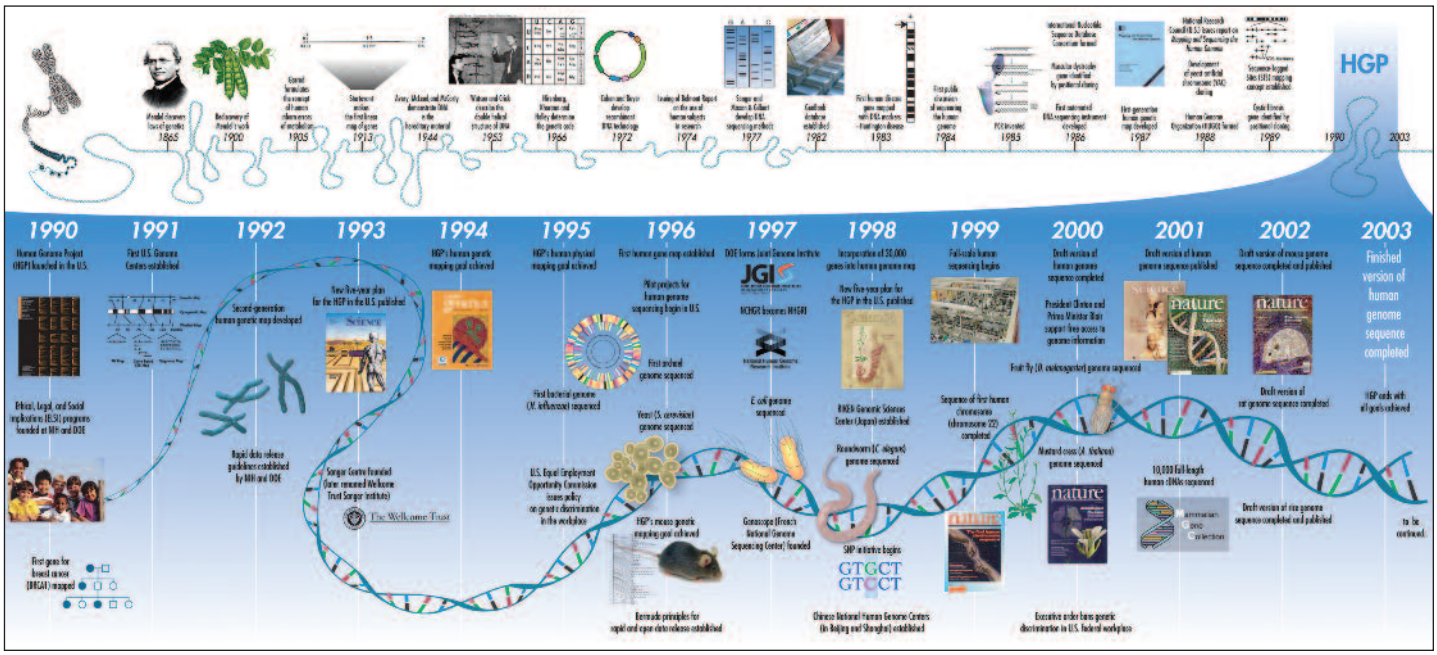



Image Credit: Darryl Leja, National Human Genome Research Institute; Available at: www.genome.gov.

As a result of the vast amounts of information provided by the Human Genome Project, more genes and proteins are being explored as potential biomarkers. As we will see in subsequent chapters, biomarkers for cancer are increasingly multiple genes and proteins instead of single genes and proteins, as in the past.

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# CHAPTER 3. USES OF BIOMARKERS IN CANCER

Bill and John are 200-pound men in their late 60s. They have both been diagnosed with colon cancer and have elected to undergo treatment with a medicine called irinotecan. However, it has been decided that Bill will receive a normal dose of irinotecan, and John will receive a lower dose? Why?

It turns out that John has tested positive for a biomarker known as UGT1A1*28 that can be detected by analyzing samples of blood or cells from a cheek swab. John is one of approximately 10% of individuals who have a genetic variation that leads them to metabolize irinotecan more slowly. Reducing John's dose may prevent the accumulation of high drug levels in his body and may help reduce toxic side effects.

This example illustrates the use of biomarkers in determining drug dose. Biomarkers have many other uses in cancer – not only in the treatment of patients, but also in the development of new drugs. In this chapter, we first consider the uses of biomarkers in cancer medicine and then turn to the uses of biomarkers in cancer drug discovery. As we will see, a given biomarker may have more than one use and some biomarkers are used in both cancer medicine and drug discovery.

## Uses of Biomarkers in Cancer Medicine

***Risk assessment***

The use of biomarkers in cancer medicine potentially begins even before we ever develop any detectable disease. That is, some genetic mutations increase the risk of eventually developing cancer. These biomarkers are said to predispose us to cancer. Examples of biomarkers associated with an increased risk of cancer are the BRCA1 and BRCA2 genes. Harmful mutations in these genes can increase the chance of developing breast and other cancers in both men and women. Individuals with these mutations can obtain more frequent screenings that may detect cancer in its early stages when it is more readily treated. In the future, drugs that prevent the mutations from causing cancer may become available, potentially increasing the utility of risk assessment biomarkers.

***Diagnosis***

Biomarkers can also aid in the diagnosis of cancer. Although many cancers are diagnosed by looking at cells under a microscope, it can sometimes be difficult to determine the primary or main type of tumor in cases where cancer has spread to more than one location. Biomarkers may help determine this. One example comes from a study conducted at Johns Hopkins in the late 1990s. Researchers wanted to determine whether tumors in the lung were primary disease or metastases (tumors that had spread from their original location). To determine this, they compared the chromosome structure from cells in the lung tumor to those in the primary tumor. They found similar chromosomal alterations in the different tumors when the lung tumor represented a metastasis. In contrast, the chromosomal alterations differed when the lung tumor was not a metastasis. On this basis, the researchers were able to use the chromosomal information to help determine the diagnosis or primary tumor type.

***Prognosis***

Another use of biomarkers in cancer medicine is for disease prognosis, which may take place after an individual has been diagnosed with cancer. Prognosis refers to the natural course of the disease in the absence of treatment. Some cancers are more aggressive than others and knowing this can help guide treatment. If a biomarker can help distinguish a cancer that is likely to grow rapidly from one that is likely to grow slowly, then patients with these two types of cancers might receive different treatments. Additionally, patients with slowly-growing tumors may be spared aggressive treatment.

An example of a potential prognostic biomarker is a protein called tissue inhibitor of metalloprotease-1 or TIMP-1. In a recent study conducted at the University of Athens in Greece, TIMP-1 levels in the blood were tested in 55 patients who had just been diagnosed with multiple myeloma, a type of blood cancer. In these newly-diagnosed and untreated patients, lower levels of TIMP-1 in the blood were associated with a better prognosis. On the other hand, high levels of TIMP-1 in the blood were associated with a worse prognosis. Further research will be necessary before TIMP-1 can routinely be used as a prognostic biomarker in multiple myeloma. However, results of this small study provide an example of how researchers are investigating various biomarkers for use in cancer prognosis. If biomarkers can be identified that reliably differentiate patients with more aggressive cancers from those with less aggressive cancers, treatment can be planned accordingly. That is, patients with more aggressive cancers may need more aggressive treatments.

***Prediction of treatment response***

Biomarkers may also be used to predict response to treatment. Even cancers that affect the same body part may exhibit differences from person to person that can influence how they respond to a given treatment.

An example of a biomarker used to predict response to treatment is the HER2/neu gene. HER2 stands for human epidermal growth factor receptor 2. Approximately one fourth of all breast cancers have too many copies of the HER2 gene, which go on to produce too much HER2 protein. Breast cancers that have this characteristic may respond to a drug called trastuzumab, which inhibits the activity of the HER2 protein. In contrast, trastuzumab is not recommended for the treatment of breast cancers that lack extra copies of HER2/neu.

Another aspect of HER2/neu overexpression is that it causes breast cancers to grow and divide more quickly. For this reason, over-expression of this gene is also used as a prognostic biomarker whose presence indicates a more aggressive cancer. Thus, HER-2/neu is an example of a biomarker with more than one use.

***Pharmacokinetics or predicting drug doses***

As we discussed in our initial example of Bill and John and their different doses of irinotecan for colorectal cancer, biomarkers can sometimes be used to determine drug doses. This use is often referred to as pharmacokinetics, which is the study of the how a drug is absorbed, distributed, metabolized, and eliminated by the body. In cancer research, this typically means studying how levels of the drug vary based on variations in metabolism. Because of differences in our genes, some people metabolize or change the chemical structure of drugs differently. In some

cases, decreased metabolism of a certain drug causes high levels of the drug to accumulate in the body. This may cause the drug's effects to be more intense and prolonged than expected, and may lead to more toxic side effects. In other words, if we have mutations that affect drug metabolism, we may experience worse side effects than people without these mutations. If the genetic alterations that cause reduced metabolism of a drug are known in advance, we can be given a lower drug dose.

Another example of this is a gene that codes for an enzyme called thiopurine methyl-transferase (TPMT). Some individuals have mutations in this gene that prevent them from metabolizing a drug called mercaptopurine. Mercaptopurine is often used to treat a type of childhood leukemia. Patients with certain mutations in the TPMT gene who are given mercaptopurine cannot adequately metabolize the drug, leading to a sustained reduction in the number of white blood cells. White blood cells fight infections and their prolonged decrease can be life threatening. If it is determined that someone has a mutation in this gene, he or she can be given a lower dose of mercaptopurine that may be safer and more tolerable.

***Monitoring treatment response***

Biomarkers can also be used to monitor how well a treatment is working. An example of this is the use of a protein biomarker called S100-beta in monitoring the response of malignant melanoma. Melanoma is a type of skin cancer involving the melanocytes, the cells that produce the pigment that gives our skin its color. Melanocytes make a protein called S100-beta that is found in high levels in the blood of individuals with large numbers of cancer cells. Response to treatment is associated with reduced levels of S100-beta in the blood of individuals with melanoma.

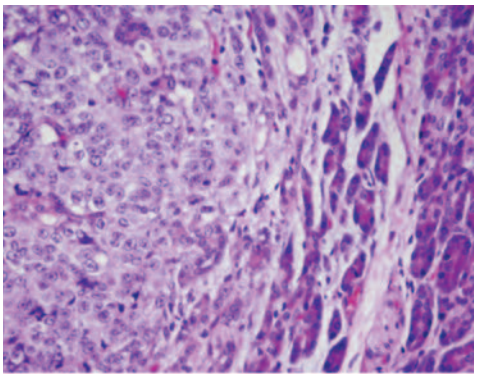


Photo courtesy Richard Lee, MD, PhD, National Cancer Institute (NCI) www.genome.gov

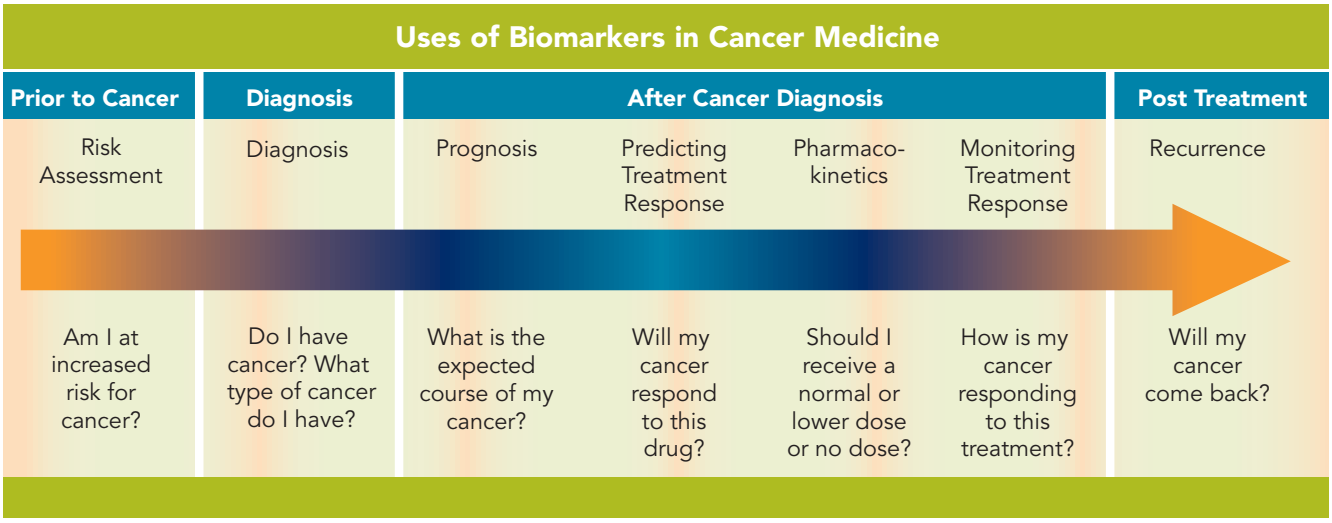
*Metastatic melanoma cells (left)*

***Recurrence***

Another use of biomarkers is in predicting or monitoring cancer recurrence. Onco*type* DX® is an example of a test used to predict the likelihood of breast cancer recurrence. This test is specified for use in women with early-stage (Stage I or II), node-negative, estrogen receptor-positive (ER+) invasive breast cancer who will be treated with hormone therapy. Onco*type* DX® evaluates a panel of 21 genes in cells taken from a tumor biopsy. The results of the test are given in the form of a recurrence score that indicates the likelihood of distant recurrence at 10 years: the higher the score, the more likely the tumor is to recur. This test can also be used to help predict who will benefit from chemotherapy. Onco*type* DX® differs from some other biomarkers in that the biomarker is actually a panel of 21 genes instead of just a single gene or protein.

However, not all biomarkers that predict recurrence serve a clinically useful purpose. An example of this is a protein biomarker in the blood known as CA-125 that has been associated with ovarian cancer recurrence. High levels of CA-125 often precede the recurrence of clinical symptoms or signs of ovarian cancer. It seems logical that when individuals whose ovarian cancer was previously in remission begin to show high levels of CA-125, they may benefit from early treatment. However, a study of more than 1000 patients with ovarian cancer in remission did not support this assumption. This study found that patients who received early treatment when they showed high levels of CA-125 did not live longer than patients who received treatment that began when they showed signs

and symptoms of recurrent ovarian cancer. These findings led the authors of the study to conclude that CA-125 is not useful as a routine marker of recurrence of ovarian cancer.




## Uses of Biomarkers in Cancer Drug Discovery

In addition to their uses in cancer medicine, biomarkers are also routinely used in cancer drug discovery. Two areas we consider here are the development of drug targets and surrogate endpoints in clinical trials.

***Developing drug targets***

In 1960, two researchers at the University of Pennsylvania School of Medicine reported the discovery of a strange chromosome in the white blood cells of patients with chronic myelogenous leukemia. This abnormality was dubbed the "Philadelphia chromosome" and was eventually found to be caused by a translocation of genetic material from chromosomes 9 and 22. When these two chromosomes are combined, they create a cancer-causing gene known as BCR-ABL. The DNA sequence of this gene is eventually transcribed and translated into a protein that causes leukemia.

For many years, the BCR-ABL gene was used as a biomarker for a certain subtype of leukemia. However, eventually its discovery led to the development of a drug designed to block the effects of the protein encoded by the BCR-ABL gene. This drug, named imatinib (Gleevec®), effectively inhibits this protein and decreases the production of cells containing the Philadelphia chromosome. This decreases progression of the leukemia.

***Surrogate endpoints***

In the development of a new cancer drug, the gold standard of proof that the drug is effective is whether it decreases cancer progression in humans, and ultimately, whether it prolongs survival. However, it would save great amounts of time, effort, and money if drugs that do not work could be eliminated from the development pipeline before they were tested in such clinical trials.

This is where surrogate endpoints come in. A surrogate is simply a stand-in for something else. In this case, a surrogate endpoint is a stand-in for the effects of a drug on cancer progression and survival. The use of validated surrogate endpoints would ideally prevent patients from having to undergo tumor biopsies and lengthy clinical trials to determine if a new drug works. Instead, patients may provide blood tests to determine whether the biomarker has increased or decreased in response to the drug. If the biomarker shows consistent changes, the drug manufacturer may then decide to conduct full efficacy clinical trials with a reasonable amount of assurance that patients will benefit and their drug will be a success. On the other hand, if the biomarker does not change, patients can be spared a treatment that will likely be ineffective and the drug company can focus their attention on drugs that are more likely to succeed. In this way, biomarkers may serve as surrogate endpoints.

Surrogate endpoint biomarkers, often referred to as SEBs, are the subject of much research. A potential surrogate endpoint biomarker that is currently receiving a lot of attention is the level of circulating tumor cells – that is, the number of tumor cells present in the blood. The number of circulating tumor cells is an established biomarker for tumor progression and metastasis (spread to distant areas). Some scientists and physicians are interested in using circulating tumor cells as a surrogate endpoint biomarker and consider it a way to speed up drug development. However, one challenge is that the level of these cells in the blood is typically very low and difficult to measure. New technologies and strategies may change that.

**Some Ideal Characteristics of Surrogate Endpoint Biomarkers**

- Biomarker should be involved in the process that causes the cancer
- Changes in biomarker should be highly related to changes in the disease
- Levels of biomarker should be high enough that they can be measured easily and reliably
- Levels or presence of biomarker should readily distinguish normal vs. cancerous or precancerous tissue
- Effective treatment of the cancer should change level of the biomarker
- Level of the biomarker should not change spontaneously or in response to other factors not related to successful treatment of the cancer

Adapted from Cohen V, Khuri FR. Progress in lung cancer chemoprevention. *Cancer Control: Journal of the Moffitt Cancer Center*. 2003;10:315-324.

## Multiple Uses of a Single Biomarker

It is worth reiterating that a single biomarker may have multiple uses. However, the characteristics needed to make a biomarker good for one purpose will not necessarily be the same ones needed to make it good for another purpose. An example of this is prostate specific antigen. As we discussed in chapter 1, levels of prostate specific antigen in the blood can be increased by conditions besides prostate cancer. However, because high levels of prostate specific antigen cannot distinguish men who have prostate cancer from those whose levels are increased for another reason, most men with high levels are likely to undergo cancer tests. These tests include a digital rectal exam, possibly followed by a biopsy. As a result, many men will undergo a biopsy for prostate cancer that they actually do not have. This can be alarming for patients and their families, and can also increase healthcare costs. When screening large numbers of men for prostate cancer, we would prefer to have a test that is better at separating those who have cancer from those who do not.

The use of PSA in cancer medicine is controversial, and advocates should be aware of the issues. PSA testing is discussed in *Chapter 4: Challenges With Biomarkers*.

On the other hand, levels of prostate specific antigen in the blood can be very useful in monitoring prostate cancer once it has been diagnosed. For instance reduced levels of this protein in the blood are a biomarker for effective treatment of prostate cancer. As explained by several investigators at the National Cancer Institute, "The arguments about use of PSA [prostate specific antigen] for screening continue, but its value in monitoring diagnosed prostate cancer or its treatment would be hard to dispute." (Ludwig, Weinstein, 2005)

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# CHAPTER 4. CHALLENGES WITH BIOMARKERS

Let's digress from our cancer discussion for a moment to consider a condition for which there is a highly reliable biomarker that is present in the urine and is easily detected with a test: pregnancy. Pregnancy tests are a good example of the desirable combination of factors we would ideally like to see in tests for cancer biomarkers:

1. The biomarker is reliably present in people with the condition but is very rarely present in people without the condition.
2. The biomarker is present in an easily accessible bodily fluid such as urine.
3. The biomarker is readily detected with a reliable and valid standardized test that is simple to perform correctly.

To understand the challenges with cancer biomarkers, it may be useful to consider each of these points.

## Biomarker Specificity and Sensitivity

During pregnancy, a woman's body produces a hormone called human chorionic gonadotropin or hCG for short. This hormone is very rarely detectable in the body if the woman is not pregnant. This is a nearly ideal situation in which the biomarker is present in all women who are pregnant but is rarely present in those who are not pregnant.

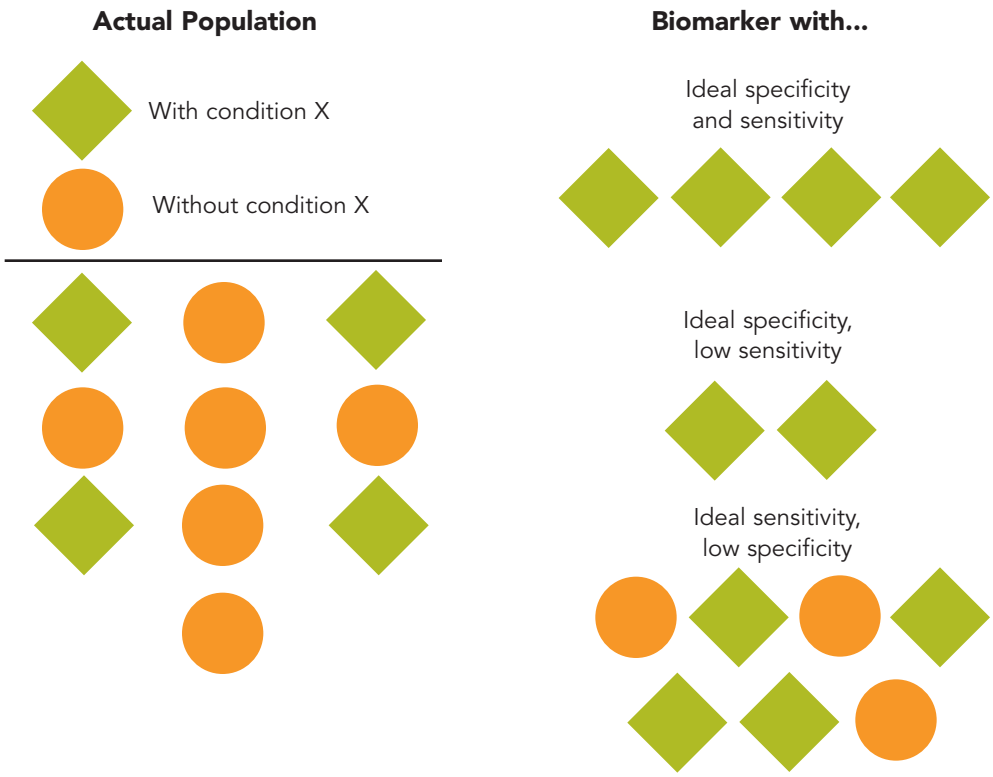
**Specificity:** Likelihood of obtaining a negative result when the target is not present. Said another way, a specific test only gives a positive result when the person has a given condition. For instance, a specific test for pregnancy would only identify people who are actually pregnant. In this case, if the test gives a negative result, you can be relatively certain that you are not pregnant. The likelihood that you do not have hCG in your system if you are not pregnant is very high; conversely, if you do have hCG in your system, it is likely that you are pregnant instead of having some other condition.

For diseases such as cancer, it is often difficult to identify a specific and sensitive biomarker. We may find that a candidate biomarker is associated not only with cancer, but also with other diseases or conditions. In this case, the presence of the biomarker would not necessarily tell you if a person has cancer; it may indicate some other condition. In this example, the biomarker does not have good specificity.

In contrast, people who do have cancer may not always have a candidate biomarker. This too is an undesirable situation because the absence of the biomarker is not reliably associated with an absence of cancer. In this case, the biomarker does not have good sensitivity. As we will see later in this chapter, biomarker tests are rated according to their specificity and sensitivity. Because these concepts are major determinants of how useful a biomarker or a biomarker test is, they are very important to consider.

**Sensitivity:** Likelihood of obtaining a positive result when the target is actually present. In our examples, this is the likelihood that the biomarker will be present when the person does have the given condition (pregnancy or cancer). The likelihood that you do have hCG in your system if you are a pregnant woman is very high.

## Specificity and Sensitivity




*In this imaginary population of 10 people (circles and diamonds), 4 have the condition (green diamonds) and 6 do not (orange circles). A biomarker with ideal specificity and sensitivity would be evident in all 4 green people but 0 orange people. A biomarker with ideal specificity but low sensitivity might be evident in 2 green people but 0 orange people. In other words, it would miss some of the people with the condition, but wouldn't falsely identify anyone with the condition. A biomarker with ideal sensitivity but low specificity would be evident in all green people but might also be evident in 3 orange people. In other words, it would correctly identify all people who have the condition but would falsely identify some people as having the condition when they actually do not.*

## Tissue Accessibility

What if a woman had to have surgery on her uterus in order to determine if she were pregnant? If this were the case, most women would probably opt for the "wait and see" method. In contrast, performing a urine test to detect a biomarker does not require a doctor to cut into one's body and thus is much more acceptable to most people.

In cancer, researchers must often take a sample of the cancerous tissue in order to determine whether a biomarker is present. For adults with blood cancers such as the leukemias, this is not typically a big issue, as most people accept having their blood drawn (although children may be an exception here). For solid tumors, sampling the tissue can be more of a challenge. Many people have tumor tissue samples removed for the purpose of diagnosis, but may be less willing to have a subsequent tissue sample taken if it requires an additional surgery. In some cases, a second tissue sample is necessary to identify relevant biomarkers. For instance, researchers may want to know how the tumor's genetic expression changes in response to treatment.

Sampling tumor tissue does not always require surgery. Some tissue samples can be obtained via needle biopsies or endoscopy. However, certain needle biopsies can be major procedures, and can cause patients pain and distress.

The need to obtain multiple tissue samples from tumors has become a challenge for researchers attempting to develop new biomarkers. In many cases, there is simply not enough tissue available to perform the biomarker studies they have designed. Even in today's clinical cancer trials, serial tissue samples are not typically included as part of the protocol. A related challenge is that the cancerous tissue must be collected in a specific way in order to be analyzed using certain genomic techniques. For instance, some genetic studies require that tissue be frozen immediately in liquid nitrogen. Because these conditions are not always met, some of the tissue collected cannot be used in genomic studies.

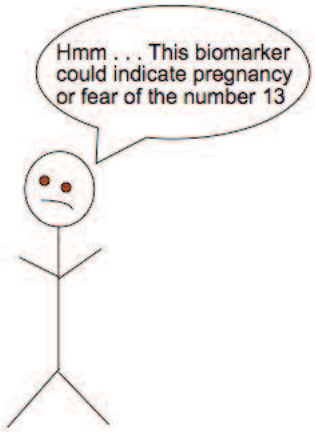
Tissue sampling is an important issue for advocates. This is discussed further in *Chapter 7: Ethical, Legal, and Social Issues With Cancer Biomarkers.* Additional information about tissue sampling for research can be found at the following

- Research Advocacy Network (www.researchadvocacy.org): *Understanding Pathology and Tissue Research.*
- Cancer Coalition of Cancer Cooperative Groups, Inc. *Cancer Research: A Guide to Clinical Trials. Tissue and Its Use.* Available at: http://www.cancertrialshelp.org/patient_content/pdMainContent.aspx?intAppMode=5. Accessed February 1, 2010.
- Prostate SPORE National Biospecimen Network Pilot. NBN blueprint. Available at: http://prostatenbnpilot.nci.nih.gov/blue_full1.asp. Accessed February 1, 2010.
- U.S. Department of Energy Office of Science, Office of Biological and Environmental Research, Human Genome Program. Ethical, Legal, and Social Issues. Available at: http://www.ornl.gov/sci/techresources/Human_Genome/elsi/elsi.shtml. Accessed February 1, 2010.:

One way to get around the problem of multiple tissue samples is to look for biomarkers in easily-accessible bodily fluids such as blood or urine. As we will see in the subsequent chapters, this is exactly what many researchers are trying to do.

## Tests for Detecting Biomarkers

In order to determine whether a biomarker is present or absent – or to determine the level of a biomarker – a test must be performed on the tissue in question. Naturally, we want biomarker tests to be as accurate as possible so that we can have confidence in the results. Similarly, the test should give reliable results. If we test positive for a biomarker today, we should also get a positive result if we take the test again tomorrow. If we provide a blood sample to two different laboratories for a biomarker test, both laboratories should get the same result. These features are important to any test and form the basis for test evaluation – essentially testing the test. It would also be ideal if the tests were easy to perform. Tests that are difficult to perform may introduce a greater potential for error.




### *Test Validity*

Test validity is the ability of a test to measure what it is supposed to measure. Validity has many different components, two of which we have already discussed: specificity and sensitivity. A test is specific if it gives a positive result only if the biomarker is present and gives a negative result when the biomarker is not present. A test is sensitive if it gives a positive result every time the biomarker is present.

Clinical validity is also an important aspect of biomarker tests. Clinical validity refers to the ability of the test to accurately predict a clinically important outcome. Often, a clinically valid test will correlate with improvement in patient care. A biomarker test that has high specificity and sensitivity is no good if the result doesn't tell us something important about our health status. For instance, clinically valid biomarker tests may tell us something about how likely we are to respond to a given treatment or how likely it is that our cancer will recur.

The reliability, validity, and clinical utility of biomarker tests is an important issue for many advocates. Government regulations differ depending on whether biomarker tests are sold as kits or services. The type of regulation the tests are subject to determines how stringent the requirements are for demonstrating test validity and reliability. Test regulation and the level of validity/ reliability that tests must show are controversial issues that we discuss more fullyin in *Chapter 8: What Can Advocates Do With This Information*?

***Test Reliability***

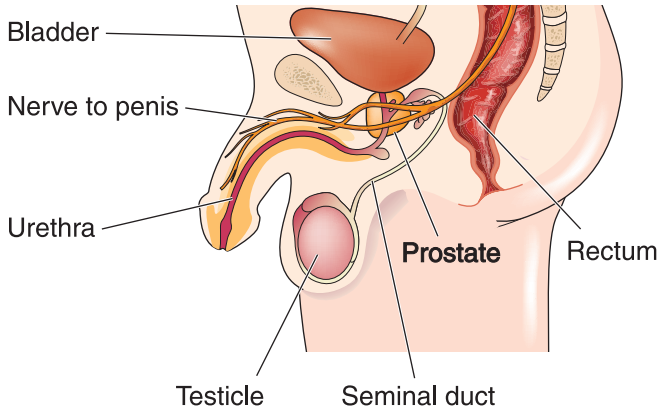
Test reliability means that the results of the test are repeatable. A tire pressure gauge that shows your tire pressure to be 32 pounds per square inch one minute and 14 pounds per square inch the next is not reliable. Because biomarker tests often require precise measurements, complicated equipment, and/or different mixtures of chemicals, reliability can be difficult to achieve. Ideally, tests would be standardized, meaning that they would be performed exactly the same way on the same equipment with the same chemicals each time. However, this is often not the case for biomarker tests. In order to get around this problem, some companies that have designed biomarker tests require that samples for testing be sent to the company's own laboratory. In this case, the biomarker testing can be standardized – performed the same way each time – and the company has control over the reliability of their test results.

## Selected Cancer Biomarkers Illustrate Challenges

The challenges with biomarkers we just discussed are not theoretical examples – they represent some of the real benefits and drawbacks of cancer biomarkers available today. In this section, we will consider three examples of cancer biomarkers in clinical use today: prostate specific antigen, HER2/Neu, and CA-125. These examples serve to illustrate the real-world challenges with developing a good cancer biomarker that meets all of the criteria we just discussed.

***Example #1: Prostate Specific Antigen***

The prostate is a gland that makes up part of the male reproductive system. Cells of the prostate produce prostate specific antigen (PSA), a protein that can be detected at a low level in the blood of all adult men.




Several medical conditions can increase the levels of PSA in the blood. These conditions include inflammation of the prostate, benign prostatic hyperplasia (enlargement of the prostate), and prostate cancer. The link between high levels of PSA in the blood and prostate cancer led to the use of this biomarker for prostate cancer screening and the monitoring of recurrence. The United States Food and Drug Administration has approved the PSA test to be used along with a digital rectal exam to help detect prostate cancer in men 50 years of age and older. The goal of these screening tests is to help identify prostate cancer before symptoms appear.

Although the PSA test has been used as a biomarker for prostate cancer since 1986, its value as a screening tool is controversial for several reasons. The first concern is that high levels of PSA are not specific to prostate cancer, but rather can be due to a number of different conditions. That is, the specificity of PSA as a biomarker is not very high. This was illustrated in a study conducted by researchers at Washington University in St. Louis. Researchers tested the PSA levels of 30,000 men in the community. Results showed that 25% to 33% of the men who had high PSA levels in their blood had prostate cancer. This means that 67% to 75% of the men in this study with high PSA levels did not have prostate cancer. When a positive result on the test falsely predicts that someone has a given condition, it is called a false positive. Thus, the PSA test has a high false positive rate. If we go back to our previous example of green vs. orange people in the test validity section, a high false positive rate means that many of the orange individuals (who do not have condition X) test positive for condition X. In this case, condition X is prostate cancer.

**False positive:** A positive test result is found when the condition being tested is actually absent.

The problem with a high false positive rate is that it can lead people to undergo additional medical procedures unnecessarily. For instance, men with high PSA levels and/or abnormal findings on a digital rectal exam may elect to undergo a needle biopsy. Such biopsies can cause stress and anxiety and are associated with financial costs. Although prostate needle biopsies are relatively safe, they can cause severe bleeding or infection of the prostate gland or urinary tract in 1% of patients. Thus, these tests are not without drawbacks and risks and, as with all tests, it is best to minimize the number of patients who undergo them unnecessarily.

## Evaluating Biomarker Tests

Ideally, the results of biomarker tests would give an accurate picture of the person's actual condition. The test would give a yes result (called a positive result) if the person has a given condition, and would give a no result (called a negative result) if the person does not have a given condition. In these cases, the test results are a "true" representation of whether a person has a given condition. Thus, we want tests with "true" results – true positives and true negatives. When used to describe test results, the words positive and negative are not used to mean good and bad but rather to mean yes (positive) or no (negative).

| Does the person actually have the condition being tested for? | Test Result | |
|---|---|---|
| | Positive (yes) | Negative (no) |
| Yes | True positive | False negative |
| No | False positive | True negative |

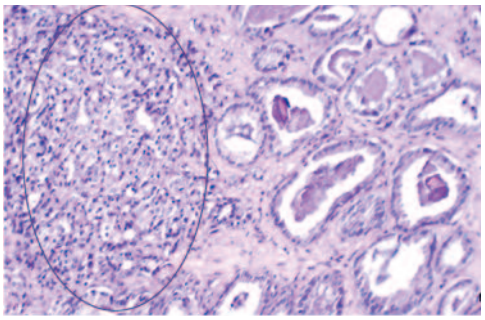
On the other hand, we don't want test results that begin with the word "false". Such results indicate that the test results do not match the person's condition. A false positive indicates that that the test result is positive but that the person does not actually have a given condition. A false negative means that the test result is negative when the person actually does have a given condition.

Tests are typically rated on their specificity and sensitivity, as we described earlier. A specific test is one that has a low false positive rate – if test says you have the condition, it is likely that you do. It rarely gives a positive result if a person doesn't actually have the condition. In contrast, a sensitive test is one that has a low false negative rate – it rarely misses anyone who does actually have the condition.

A simple example of the relationship between test results and health is given below:

- True positive: Sick people correctly diagnosed as sick
- False positive: Healthy people incorrectly identified as sick
- True negative: Healthy people correctly identified as healthy
- False negative: Sick people incorrectly identified as healthy

### Prostate Cancer Cells



*Cancer cells are located inside the oval; they appear to be in a jumbled state (undifferentiated) in contrast to the cells on the right.*

Image courtesy Otis Brawley and National Cancer Institute

Another continuing issue with the PSA test is whether it saves lives. Two large studies have attempted to answer this question, one in Europe and one in the United States. The American trial did not find any difference in deaths due to prostate cancer between the group that was required to undergo annual screening (PSA + digital rectal exams) and the group that was not required to undergo annual screening as part of the study. In the European trial, 0.29% of

men in the screening group died compared with 0.37% of the men in the no screening group. Another way to look at the results of this study is that, in order to prevent 1 death from prostate cancer, 1410 men need to be screened and 48 men treated for prostate cancer. Together, the results of these trials suggest that PSA screening does not have a large effect on saving lives.

It should be noted that there is less controversy in the use of PSA levels for monitoring cancer recurrence, with most experts agreeing that the test is useful for this purpose. However, the use of PSA testing as a screening tool illustrates several of the challenges with biomarkers.

***Example #2: HER2***

As we discussed briefly in Chapter 2, approximately one quarter of breast cancers are characterized by overexpression of a gene called HER2. This overexpression leads cells to produce too much HER2 protein. Breast cancers that overexpress HER2 often respond to trastuzumab, a drug that inhibits the activity of the HER2 protein. However, this drug is not used for breast cancers that do not overexpress HER2. As a result, HER2 may be used as a biomarker for response to a specific treatment – trastuzumab.

Because the HER2 protein is involved in cell growth and replication, cells that overexpress this protein receive too many signals telling them to grow and replicate. Levels of HER2 protein over time are not generally monitored as a response to treatment, although new findings are raising the possibility that the levels of part of the HER2 protein in the blood may have prognostic value as outlined in the following table.

**Additional Information About HER2 Levels Over Time**

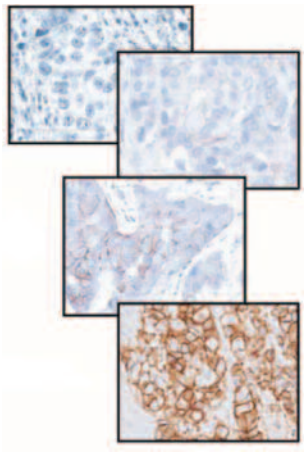
| Study Citation | Findings |
|---|---|
| Finn RS, Gagnon R, Di Leo A, et al. Prognostic and predictive value of HER2 extracellular domain in metastatic breast cancer treated with lapatinib and paclitaxel in a randomized phase III study. *J Clin Oncol*. 2009;27(33):5552-8. | • Decreased blood levels of a certain part of the HER2 protein over time were associated with longer survival in a group of 579 patients with newly-diagnosed metastatic breast cancer that was HER2 negative.<br>• However, these decreases occurred regardless of the treatment individuals received and did not predict benefit to the drug lapatinib (an inhibitor of HER2 and epidermal growth factor receptor kinases – proteins that help mediate the effects of the receptor). |
| Bramwell VH, Doig GS, Tuck AB, et al. Changes over time of extracellular domain of HER2 (ECD/HER2) serum levels have prognostic value in metastatic breast cancer. *Breast Cancer Res Treat*. 2009;114(3):503-11. | • Increases over time in blood levels of a certain part of the HER2 protein were associated with shorter survival in 1282 women with metastatic breast cancer. |

*Breast Cancer Cell*

Two different types of tests may be used to detect HER2 overexpression. One test uses a method called immunohistochemistry (IHC), which measures the level of HER2 protein on the outside of tumor cells. IHC tests are scored as a 0, 1+, 2+, or 3+, with 3+ indicating that the cells overexpress HER2. The score is based on how completely the cell membranes show a stain that marks HER2 and how intense that stain is. The intensity of the stain is based on the interpretation of the test results. Professional guidelines specify that a sample should be considered HER2 positive if >30% of invasive tumor cells show uniform, intense membrane staining.

## IHC (Herceptest®) Scoring

| Staining pattern | Score | Interpretation |
|---|---|---|
| No staining | 0 | Negative |
| Faint incomplete staining of cell membrane in >10% of tumor cells | 1+ | Trace Negative |
| Weak to moderate complete staining of cell membrane in >10% of tumor cells | 2+ | Weak Positive |
| Strong complete staining of cell membrane in >10% of tumor cells | 3+ | Strong Positive |



*Scoring method used in the HercepTest® IHC assay (DAKO HercepTest® [Package insert]. Carpinteria, Calif:DAKO Corp., 2004). Figure courtesy of Kenneth Bloom, MD. Reprinted with permission from the National Comprehensive Cancer Network; originally published in Carlson RW, Moench SJ, Hammond EH, et al. HER2 testing in breast cancer: NCCN Task Force Report and Recommendations. J Nat Comprehen Can Network. 2006;4(Suppl 3):S1-S22.*

*For another view of IHC results of breast cancer cells rated as 0, 1+, 2+, and 3+, visit the following website: http://www.herceptin.com/hcp/HER2-testing/interpreted-results.jsp.*

The other method used to detect HER2 is called FISH (fluorescence in situ hybridization). This method measures the underlying gene alteration in the tumor cells instead of the protein. FISH tests actually measure the number of copies of the HER2 gene. A result of more than 6 copies of the HER2 gene is considered positive. FISH scores may also be provided as a ratio of the number of HER2 signals to the number of signals from normal parts of the chromosome containing the HER2 gene. Normal cells show ratios of less than 1.8, whereas cells that overexpress HER have ratios of 2.2 or higher. Those with a ratio between 1.8 and 2.2 are considered to be indeterminate and require additional testing.

Whereas the problem with PSA was that the actual biomarker was not specific for the prostate cancer, the problems with HER2 appear to be related to the tests for the biomarker rather than the biomarker itself. One problem with the HER2 tests is that they do not always give the same result for the same specimen. Guidelines from the American Society for Clinical Oncology (ASCO) and College of

**False negative:** A negative test result is found when the condition being tested is actually present.

American Pathologists (CAP) state that 20% of current HER2 testing may be inaccurate. As we saw in the first part of this chapter, this is a problem with test reliability. This means that some women who initially test negative for HER2 overexpression may actually overexpress HER2. This is referred to as a false negative. Ideally, a test would minimize the potential for misinterpretation.

False negatives have the potential to be extremely detrimental to patients. If a test result for HER2 overexpression is negative, the woman will probably not receive trastuzumab. In this case, if the breast tumor actually overexpresses HER2, the woman could fail to receive a treatment that could prolong her life.

The tests for HER2 overexpression may also return false positives. Several studies have found that approximately 6% to 9% of patients who were initially deemed to have HER2 overexpressing tumors were found to have HER2 negative tumors when the tissue was re-tested. However, these women had been treated with trastuzumab in the study because of their initial HER2 positive results. When the researchers looked at whether trastuzumab was of benefit to these patients, they found that it was. Among women who were HER2 negative upon retesting, the relapse rate for those treated with trastuzumab was less than half that for those not treated with trastuzumab. A subsequent study also found a trend toward improvement in women with HER2 negative cancers treated with trastuzumab.

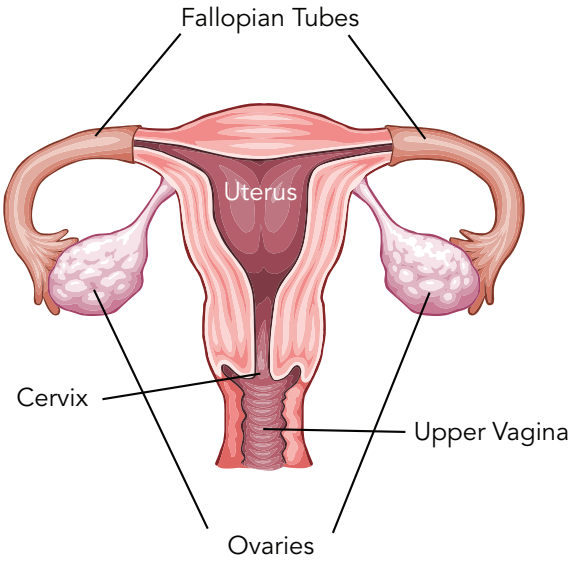
Many variables can affect the outcomes of HER2 tests. One variable is the collection of the pathology sample – it is important that the sample contain only cancerous cells and not normal cells that may surround the borders of the tumor. Additionally, many laboratory- and technique-related variables can affect the two different methods. The National Comprehensive Cancer Network (NCCN) indicates that both HER2 tests can be performed successfully if adequate controls and verifications are in place. They indicate that strict quality control and assurance measures must be conducted by each laboratory performing these tests for clinical purposes, including formal test validation and concordance studies. The American Society for Clinical Oncology (ASCO) and College of American Pathologists (CAP) also recommend formal validation of laboratory assays for HER2 testing, in addition to the use of standardized operating procedures and compliance with defined testing criteria. According to published guidelines, compliance with these procedures should be monitored via the implementation of strict laboratory accreditation standards and ongoing proficiency testing.

Like PSA, the example of HER2 overexpression illustrates the challenges with biomarkers: namely, reliability and validity. Tests may be difficult to conduct and/or interpret, leading to lack of reliability. Tests may lead to numerous false positives or false negatives, leading to lack of validity. Tests may not predict anything that is clinically important, leading to lack of clinical validity and utility.

***Example #3: CA-125***

A third example of a biomarker in clinical use today is CA-125 or cancer antigen-125. This biomarker is a protein that may be found in high amounts in the blood of patients with certain types of cancer, including ovarian cancer.

**Anatomy of the Ovaries and Nearby Structures**




Unfortunately, the use of CA-125 as a biomarker for ovarian cancer is not specific – the same problem we saw with the use of PSA. Elevated levels of CA-125 can be associated with many other conditions, including diverticulitis, endometriosis, liver cirrhosis, normal menstruation, pregnancy, uterine fibroids, and non-ovarian cancers. In fact, an expert panel concluded that 98% of women in the general population who show abnormal CA-125 levels in their blood do not have ovarian cancer. Because of this extremely high false positive rate, CA-125 is not currently recommended as a general screening test for individuals without a history of ovarian cancer.

Another problem with the use of CA-125 is that there is very little evidence to suggest that earlier detection of ovarian cancer will delay death.

The current recommendations for CA-125 are that the test should not be used to screen for ovarian cancer because of the low prevalence of this cancer and the invasive nature of diagnostic testing that would likely follow a positive test. The government's expert panel concluded that the potential harms of CA-125 testing for ovarian cancer screening outweigh its benefits.

Similarly, study results that became available in 2009 called into question the clinical validity of using CA-125 levels to monitor recurrence. In this study, women were treated for recurrent ovarian cancer either when their CA-125 levels became high or when they exhibited clinical symptoms or signs of ovarian cancer. Results showed no difference between groups in the duration of survival. That is, the earlier treatment given to women when their CA-125 levels increased did not increase the length of life compared with women who were given treatment later when they began to show symptoms. Thus, knowing one's CA-125 levels may not be clinically useful.

The examples of these three biomarkers illustrate the variety of challenges associated with identification of a good biomarker and the development of an accurate, reliable test. As we will see in the next chapter, new technologies are moving us toward the identification of groups of relevant biomarkers or biomarker signatures that together predict something important about cancer. However, the challenges we have seen with the older group of biomarkers described in this chapter are unlikely to go away any time soon. This may be particularly true in the development of biomarker tests, although researchers are approaching this problem from a variety of different angles that, as we will see in the next chapter, do not always involve conducting a laboratory test.

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# CHAPTER 5. THE PROMISE OF BIOMARKERS: HOW DO WE GET FROM HERE TO THERE?

Imagine visiting your physician's office and undergoing a full body scan designed to detect cancer in its very early stages. This scan would be painless and accurate and would be done as part of your routine medical care, just like having your blood pressure taken. Although scans such as these are not currently possible, scientists are working on technologies that provide an image or "picture" of certain types of cancerous cells in their very early stages.

In this chapter, we explore the various methods that are being used to discover new biomarkers, including the "omic" sciences, imaging techniques, and computer technology. We also consider the question of why more biomarkers aren't routinely used in the clinic and potential strategies to increase their use in cancer medicine.

## Methods of Biomarker Discovery

A handful of cancer biomarkers are in clinical use today. The promise of personalized medicine, however, anticipates that many more will be discovered as the result of advances in the study of our genes and also in technologies designed to help us view the body at work. In this section, we discuss several methods of biomarker discovery that investigators are using today to identify new biomarkers.

### *Methods of Biomarker Discovery: The "Omic" Sciences*

The completion of the Human Genome Project in 2003 stimulated a whole new group of sciences affectionately termed the "omics". Omics is simply a suffix that is attached to the biochemical unit under study, such as gene or genome + omics (genomics) or protein or proteome + omics (proteomics). The common thread running through all of the omic sciences is that they focus on multiple parts as opposed to individual parts. For example, instead of studying one gene, genomics looks at many genes and how they may work together. Instead of studying one protein, proteomics looks at multiple proteins that are expressed at a given time.

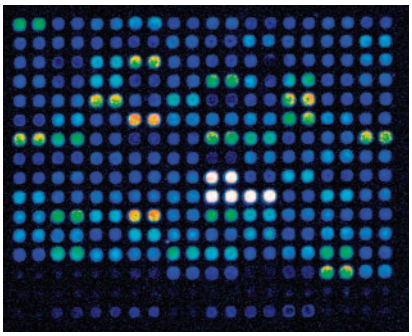
By looking at multiple genes or proteins, the omics sciences allow scientists to study the integration of information as it occurs in the body. This is important because many diseases are not determined by a single gene or protein. We know that, sometimes, people with the same genes develop different diseases. By studying the overall response of cells to a mutation or change in their environment, scientists can learn much more about complex diseases like cancer.

**Some of the "Omic" Sciences**

| "Omic" Science | Description |
|---|---|
| Genomics | Study of the genome – all of an organism's DNA – and its function |
| Transcriptomics | Study of the transcriptome – all of the genes that are transcribed into RNA in a cell or organism at a given time |
| Proteomics | Study of the proteome – all of the proteins expressed in a cell or organism at a given time point |
| Metabolomics | Study of the metabolome – all of the metabolites in a cell, tissue, or organism under given conditions |

## Genomics

Genomics, or the study of multiple genes and how they work together, is being used to figure out which genes differ between cancer cells and normal cells. Genes that distinguish the cancer based on some important feature may be useful as biomarkers. Some types of genomic studies evaluate the amino acid sequences of genes that a person possesses or that are present in cancer cells. However, this information does not tell us whether the genes are activated. For this reason, scientists are increasingly using genomic techniques that tell us whether the genes are actively being transcribed into RNA. The study of all of the genes that are transcribed in a cell at a given time is called transcriptomics. Using techniques to study the genes that are actively transcribed, researchers can develop a so-called gene expression signature or profile. Expression signatures are often better than a single gene in predicting important features of the cancer such as whether it is aggressive or whether it is likely to recur following treatment.



*DNA Microarray – A Common Genomics Tool*

An example of the importance of genomic biomarkers is in the prediction of breast cancer recurrence. It has been estimated that 55-75% of women in the United States with early-stage breast cancer receive adjuvant chemotherapy (chemotherapy after surgical tumor removal), which provides no benefit but causes side effects. Biomarkers may help identify women who are unlikely to benefit from this post-surgical treatment. Several different gene expression profiles make up tests that are now used as biomarkers for breast cancer. These tests examine the expression of 21 genes (Onco*type* DX®) or 70 genes (MammaPrint®) that provide information about the likelihood of breast cancer recurrence.

**Genome-wide association:** approach that involves rapidly scanning markers across the complete sets of DNA, or genomes, of many people to find genetic variations associated with a particular disease.

When attempting to discover new biomarkers, one of the important questions that researchers must ask is where in the genome to look. Humans have many thousands of genes and it can be somewhat overwhelming trying to figure out which ones are most likely to be biomarkers. There are at least two approaches to this problem. One strategy is to take a comprehensive approach, without the bias of prior scientific assumptions. In this approach, researchers analyze the complete set of DNA and try to relate a pattern of gene expression to some feature of the cancer. This type of analysis is also referred to as genome-wide association study (GWAS). An example of the comprehensive approach is to scan all of the genes expressed in a group of individuals with pancreatic cancer and to compare those to the genes express in a group of individuals without pancreatic cancer.

## Steps in Conducting a Genome-Wide Association Study

1. Identify two groups of participants: one with the disease under study and one "control" group that does not have the disease.
2. Obtain a sample of DNA from each person in the study.
3. Analyze these samples to determine the sequence of each person's DNA and identify markers of genetic variation known as single nucleotide polymorphisms or SNPs.
4. Apply bioinformatics and statistics to the results to determine which genetic variations (such as SNPs) are more frequent in people with the disease than those without the disease.
5. These variations are said to be "associated" with the disease. They can often provide information about where in the genome the disease originates. However, these variations do not necessarily cause disease but rather may "tag along" with the gene or genes that do cause the disease.

Information from the Human Genome Research Institute (www. genome.gov).

An alternate strategy is called the candidate-driven or hypothesis-driven approach. In this approach, researchers determine which genes to examine based on the pre-existing scientific literature. An example of the hypothesis-driven approach is to compare the expression of selected genes involved in cell growth in a group of individuals with pancreatic cancer to those in a group of individuals without pancreatic cancer. Each of these methods has its benefits and drawbacks, as illustrated in the following graphic.

## Comprehensive Vs. Hypothesis-Driven Approach to Biomarker Discovery

| Comprehensive Approach | Hypothesis-Driven Approach |
|---|---|
|  Evaluate all expressed genes |  Evaluate genes thought to be related to cancer based on the scientific literature |
| **PROS** Unbiased, less likely to miss major/important genes or pathways | **PROS** Focuses on pathways or genes that have higher likelihood of being successful candidates due to decades of work |
| **CONS** Requires large amount of data or large number of patients to establish reasonable statistical power, high likelihood of false positive associations, biostatistically complex, small differences may be missed due to stringent biostatical corrections, large amount of data may be overwhelming | **CONS** Biased approach that may miss important genes/pathways, relies heavily on existing knowledge base that is often limited and incomplete |

Genes may or may not be active. Proteins are the products of active genes. Proteins are the critical biochemicals that determine how a cell functions.

## Proteomics

Proteomics is another omics science that is being used to discover cancer biomarkers. The proteome refers to all of the proteins expressed by a cell, tissue, or organism at a given time; proteomics is the study of the proteome. Proteins are useful as cancer biomarkers because they represent the product of an active gene. As noted in the previous section, genes may or may not be active. Moreover, even after a gene is transcribed into RNA, it does not always get translated into protein. Proteins are actually the critical biochemicals that determine how a cell functions.

### Proteins Are the Products of Active Genes

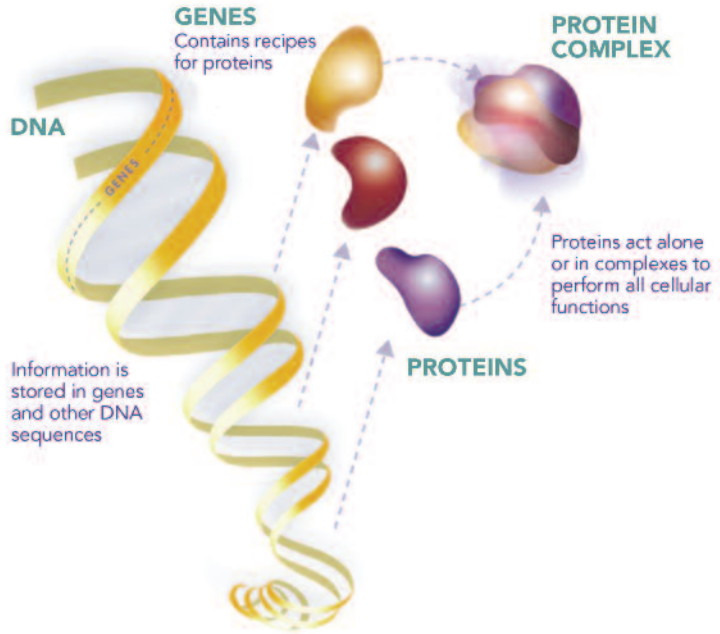



Image Credit: U.S. Department of Energy Genome Programs; http://genomics.energy.gov.

*DNA contains genes that may be transcribed into messenger RNA and translated into proteins. Proteins act alone or in complexes to perform all cellular functions.*

Because cancer cells often release proteins into bodily fluids, protein biomarkers are not necessarily confined to tumor tissue. Researchers are studying protein biomarkers in bodily fluid that is near the tumor (such as breast fluid), as well as in the blood. The blood has the advantage of being easily accessible, although it also tends to have lower levels of tumor proteins than sites closer to the actual tumor. Prostate specific antigen is an example of a protein biomarker obtained from the blood.

Image courtesy Dorie Hightower, National Cancer Institute

*Proteomics: Protein Pattern Analyzer*

One area of proteomics research that is generating increasing attention is the detection of tumor antigens. Antigens are substances (often proteins) that can stimulate immune responses. Some tumors produce protein antigens that stimulate the immune system to produce antibodies. These antibodies, which are themselves proteins, circulate in the blood and can be detected by a variety of techniques. The presence of circulating antibodies against tumor antigens may be used as a biomarker for certain types of cancers because individuals without the tumors lack tumor antigens and therefore lack the antibodies. Many people hope that antibodies against tumor antigens can help detect cancers in the early stages when they can be most effectively treated.

As in genomics, some research in proteomics is being directed at finding a protein signature for cancers. Protein signatures would consist of multiple proteins as opposed to single proteins, some of which may be increased and others decreased. Protein signatures would predict something important about the cancer such as prognosis, prediction of drug response, risk of recurrence, etc.

**Metabolite:** a molecule produced by metabolism, a process of chemical reactions that change one molecule into another molecule for the purposes of storage, use in the body, or elimination

## Metabolomics

Metabolomics refers to the study of the metabolome – or all of the metabolites produced in a cell, tissue, or organism at a given time. A metabolite is a molecule produced by metabolism: the chemical reactions that change one molecule into another molecule for the purposes of storage, use in the body, or elimination. For instance, when our bodies break down proteins from the foods we eat, one of the metabolites produced is uric acid. Levels of uric acid in the blood are a biomarker for a number of different conditions and diseases.

Some cancer cells produce altered metabolites that may serve as biomarkers. Metabolites can be found in cancerous tissue or bodily fluids – one readily-accessible source of metabolites is the urine. This is a useful option for biomarker identification because urine is easily obtained and does not require the removal of tissue from the body.

### *Challenges With Biomarker Discovery in the "Omic" Sciences*

Most experts agree that the omic sciences have the potential to transform medical care. However, many of the technologies used to detect biomarkers must still be standardized (ie, performed the same way each time) so that experimental results obtained by one research group can be replicated by another. Additionally, the biomarkers must be validated or compared to accepted clinical and pathologic standards. As we will see in the following paragraphs, each of the omic sciences has its own set of challenges that must be met in order to permit substantial progress in the development of biomarkers.

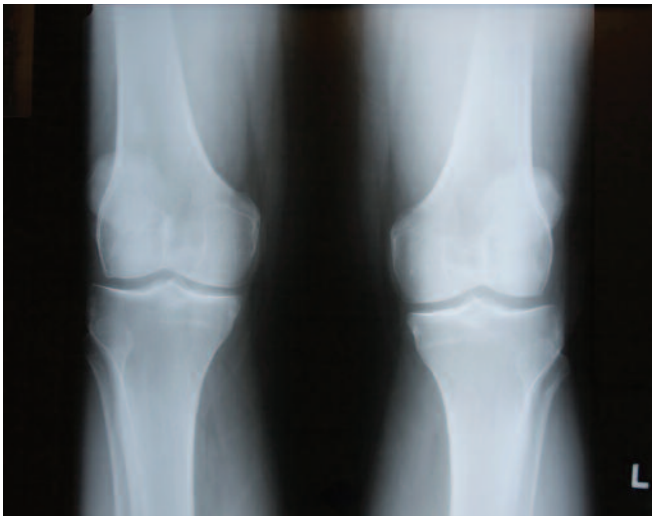
**Genomics.** In genomics, one challenge is whether to use DNA-based tests or RNA-based tests. Each of these molecules has advantages and disadvantages. DNA is more stable than RNA and most cancer-causing mutations occur at the DNA level. However, RNA analysis allows researchers to detect not only changes in the nucleotide sequence of genes, but also secondary changes to the DNA such as the addition of extra chemical groups that can interfere with the gene's function. RNA tests can also detect changes in nucleotide sequence that may occur during transcription, as well as non-sequence related changes known to be important in cancer such as an increase or decrease in the number of copies of a gene. Another concern with genomic-based tests is that non-cancerous cells in a sample can sometimes interfere with the results. Researchers are working to get around these challenges.

**Proteomics.** Many of the challenges in proteomics revolve around technologies or tests used to detect proteins. For example, proteins take on specialized, folded shapes that are critical to their actions. Tests that attempt to detect the presence or function of proteins must maintain this shape, which is a challenge because many common laboratory procedures disturb protein shape. Another challenge is that proteins may exist in tissues at very low levels. No method has yet been developed to amplify or increase the amount of protein in a given sample. A third challenge is that proteins are regulated by means other than just their levels. Proteins are often modified with certain chemical groups that determine their activity, effectively turning them off or on. This means that simply detecting the level of a protein in a tissue sample may not be enough.

**Metabolomics.** In metabolomics, a major challenge is how to extract (get out from the tissue) and analyze the many different types of substances that make up the metabolome. Metabolites can be proteins, DNA, RNA, sugars, organic acids, alkaloids, etc. Suffice it to say that the different chemical natures of these compounds require multiple methods of extraction and analysis. Another challenge in metabolomics is that the compounds of interest are present in the tissue at greatly varying levels. Some are very high, while others are very low. When machines are set to be sensitive enough to detect the less concentrated compounds, the highly concentrated compounds overwhelm the system and interfere with the analysis. However, researchers are busy working on these problems and many believe that metabolomics holds great potential for identifying novel biomarkers.

***Methods of Biomarker Discovery: Molecular Imaging***

Most of us are familiar with the common imaging techniques used in medicine such as X-rays, ultrasounds, and computed tomography (CT) scans. These techniques allow physicians to see inside the body. Although today's imaging techniques are detailed enough to show the location of a tumor, imaging systems are constantly improving, allowing scientists to view smaller and smaller components of the body in greater detail. Such techniques are being actively explored in laboratory studies, and it is only a matter of time before some of them are routinely used in medicine.




*X-ray Image*

Molecular imaging can be contrasted with imaging techniques such as X-rays, which provide information about areas of our body that can be seen without a microscope. Molecular imaging provides information about cells and molecules that cannot be seen with the naked eye. A formal definition of molecular imaging is as follows: techniques that directly or indirectly monitor and record the spatiotemporal distribution [distribution in space and time] of molecular or cellular processes for biochemical, biologic, diagnostic, or therapeutic applications. For our purposes, we can think of molecular imaging as visual representations of biological events occurring at the level of cells; molecular imaging tells us something about our cells and what they are doing.

Molecular imaging does not typically take the form of a traditional picture. Instead, the representation may appear on a screen as a group of "dots." The dots may be fluorescent chemicals that are linked to cancerous cells by any of several methods. For example, investigators could inject into a person's blood an antibody against a particular protein produced by cancer cells. The antibody would be marked in some way so that it would be visible when it bound to cancer cells (e.g., it might be linked to a fluorescent compound that can be seen on certain machines). After allowing time for the antibody to bind to the cancer cell and the body to rid itself of unbound fluorescent antibodies, investigators may be able to detect the presence of cancer cells because they would be fluorescent. In reality, one of the major challenges with this type of imaging is getting rid of the "background" fluorescence that is unbound by cancer proteins. It would be great if the body would just rid itself of the unbound fluorescent antibodies and leave only the ones that are bound, but it does not. Some of the unbound fluorescence can be taken up by non-cancerous cells and confuse interpretation of the image. Fluorescence and antibodies are only two of several methods being investigated for use in molecular imaging.

| Examples of Macroscopic Imaging Techniques (at the level of the tissue) | Examples of Molecular Imaging Techniques (at the level of the cell) |
|---|---|
| X-rays | Multislice CT (MSCT) |
| Computerized Tomography (CT) | PET |
| Positron Emission Tomography (PET) | MRI |
| Ultrasound | Diffuse Optical Tomography (DOT) |
| Magnetic resonance imaging (MRI) | Fluorescence-Mediated Tomography (FMT) |
| | Lymphotropic Nanoparticle-Enhanced MRI (LNE-MRI) |
| | Multiphoton Microscopy (MPM) |

One biomarker avenue that is being explored with molecular imaging is the quantification of circulating tumor cells. We have already noted that the number of tumor cells in the blood is a sensitive biomarker for tumor progression and spread. Unfortunately, it is difficult to measure very low levels of circulating tumor cells, which could be important in identifying a cancer in its early stages. Imaging these cells in the blood may be one way around this problem.

Another important area of imaging research is the use of labeling techniques to mark the cancer. It is very difficult to distinguish a normal cell from a cancerous cell using imaging techniques. In contrast, pathologists looking under a microscope can tell the difference between cancerous and normal cells. Fortunately, cancer cells show many biochemical differences from normal cells that may be useful in developing imaging techniques to view them. For example, cancer cells induce the formation of new blood vessels and break down the matrix material that normally surrounds them. If these changes can be "marked" somehow in the body, it may be possible to track the behavior of cancer cells.

In addition to the new technologies being designed for molecular imaging, some of the existing imaging strategies currently in clinical use are being combined to provide more detailed representations of what is going on in the body. For example, the integrated use of CT and PET scanning techniques has been found to improve the accuracy of diagnostic lung cancer staging – how far the cancer has developed and how much it has spread to other areas. This example demonstrates the importance of integrated computer analysis of various technologies. As we will see in the next section, computers are also being used to help discover new biomarkers.

*"At present, molecular imaging systems enable doctors to see where a tumor is located in the body. Ultimately, it is hoped that some of these systems will also help doctors to visualize the expression and activity of particular molecules, cells and biological processes that influence the behaviour of tumors and/or responsiveness to therapeutic drugs."*

— Weissleder R, Pittet MJ. *Nature* 2008;452:580.

### *Methods of Biomarker Discovery: In Silico*

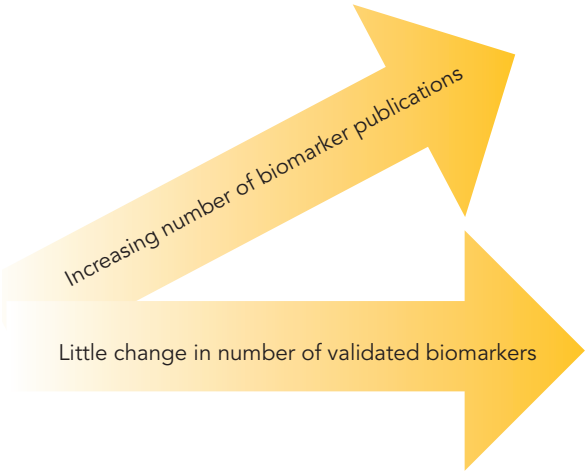
The use of computer technology to search for biomarkers is referred to as *in silico* analysis, which contrasts with *in vivo* (which means "in the living body") and *in vitro* (which means "in a test tube") methods. Clinical studies that incorporate genomic analyses are typically published in biomedical journals. Most of these journals require that the investigators make public the genomic data that they have obtained, allowing the entire research community to access it. Anyone can then conduct a computerized search of the genomic data sets for potential biomarkers – this type of analysis is referred to as *in silico*.

*In silico* analysis typically begins with the large, publically-available information on the sequences of genes that are expressed in cancer cells. Information is extracted from these so-called libraries and is analyzed by the computer to look for patterns. For instance, a study may use *in silico* analysis to determine the location of mutations in a certain tumor suppressor gene.

The advantages of *in silico* analysis are that it is less expensive than some other methods and avoids the need for large-scale clinical trials that can take many years to complete. It also permits an investigator to search for a biomarker in one data set and attempt to validate in another data set. However, the utility of *in silico* analysis depends on the quality of the data collected in the clinical trials. It can also be difficult to compare results across different data sets because of the differences in genomic methods. For these reasons, *in silico* analysis of biomarkers is often considered an initial step that is followed by validation in cancer cells and eventually cancer patients.

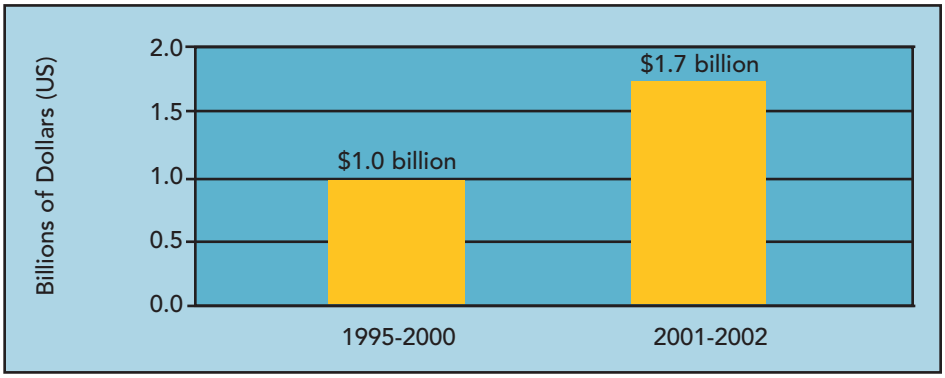
## Increasing the Number of Biomarkers in Clinical Use

In Chapter 1, we described the expanding interest in biomarkers and the ever-increasing number of scientific articles devoted to the topic. It is surprising then to learn that the number of biomarker tests approved by the United States Food and Drug Administration has not kept pace with the increased research. In fact, the number of approvals for protein biomarkers in the blood has actually decreased over the past decade, despite an increase in scientific publications. Additionally, few of the biomarkers that have been approved by the United States Food and Drug Administration (FDA) have become standard practice in cancer medicine. In fact, the FDA has approved very few biomarkers discovered by genomics, proteomics, or *in silico* analyses for clinical use. One example is MammaPrint® – this test is FDA approved. Many biomarkers have been approved only for research use and are not typically reimbursed by health care insurers.




Why this gap? Although various groups have different ideas, the time and cost of developing a biomarker may be partly to blame. It has been estimated that the cost of developing a new drug – from its discovery to its availability to patients – increased from $1 billion in the late 1990s to $1.7 billion in 2001-2002. The entire process can take 10 to 15 years. Others note that the basic science is not being adequately translated into forms that could benefit patients.

## Amount of Money Needed to Develop a New Drug in the United States




Data from Gilbert J, Henske P, Singh A. Rebuilding Big Pharma's Business Model. *In Vivo*, the Business & Medicine Report, Windhover Information, Vol. 21, No. 10, November 2003.

As we look to the future, there is reason to be optimistic about the value of biomarkers in cancer, but also reason to examine the challenges associated with bringing a new biomarker into routine clinical use. The challenges with discovering biomarkers and, eventually, developing clinical tests for biomarkers, go hand-in-hand with the challenges facing other medical products. In some cases, it may be possible to target new cancer biomarkers with a new drug. Thus, the hurdles facing new drug development are relevant in the context of biomarkers – they can potentially be developed together. The US FDA has issued several reports that explain the problem of translating basic scientific findings into medical products that can be used clinically. They have outlined a number of different challenges and opportunities that can perhaps be addressed in the coming years to help deliver on the promise that biomarkers hold for better, more effective cancer treatments. Some of these challenges are listed in the following table.

## FDA: Challenge and Opportunity on the Critical Path to New Medical Products Highlights from the Executive Summary

- "The current medical product development path is becoming increasingly challenging, inefficient, and costly."
- "During the last several years, the number of new drug and biologic applications submitted to FDA has declined significantly; the number of innovative medical device applications has also decreased."
- "In contrast, the costs of product development have soared over the last decade. Because of rising costs, innovators often concentrate their efforts on products with potentially high market return."
- "In FDA's view, the applied sciences needed for medical product development have not kept pace with the tremendous advances in the basic sciences. The new science is not being used to guide the technology development process in the same way that it is accelerating the technology discovery process."
- "Not enough applied scientific work has been done to create new tools to get fundamentally better answers about how the safety and effectiveness of new products can be demonstrated, in faster time frames, with more certainty, and at lower costs."
- "A new product development toolkit — containing powerful new scientific and technical methods such as animal or computer-based predictive models, biomarkers for safety and effectiveness, and new clinical evaluation techniques — is urgently needed to improve predictability and efficiency along the critical path from laboratory concept to commercial product."

Source: United States Food and Drug Administration. Challenge and opportunity on the critical path to new medical products. March, 2004. Available at: http://www.fda.gov/downloads/ScienceResearch/SpecialTopics/CriticalPathInitiative/CriticalPathOpportunitiesReports/ucm113411.pdf. Accessed February 2, 2010.

**Excerpt taken from Oversight of Advanced Diagnostic Tests and Proposed IVDMIA Guidance. A letter to the FDA Commissioner, Available at: http://geneticalliance.org/statements.ivdmia.guidance. Accessed April 28, 2010.**

*The translation of basic science to clinical cancer medicine is an important issue for many advocates. Some advocates support the need for change in the FDA's policies and stances, but disagree with the methods being implemented. For instance, a letter to the FDA Commissioner signed by many advocacy organizations states the following, "We understand that adapting regulatory policies and procedures to the scientific challenges presented by genetic and genomic testing will require creative thinking. It is critically important that we move forward with a regulatory oversight framework that is clear and consistent and recognizes that there are a variety of technological approaches to advanced diagnostic tests. Ultimately, the most critical aspect of any test is risk to the patient." The letter goes on to oppose the proposed* In Vitro *Diagnostic Multivariate Index Assay (IVDMIA) guidance proposed by the FDA on the grounds that they do not focus on patient risk.*

## Ensuring Clinical Relevance of Biomarker Research

In response to the lack of major new developments in biomarkers for breast cancer, the National Breast Cancer Coalition Fund (NBCCF) convened a conference in November 2005 to redirect and reinvigorate breast cancer biomarker research. This conference brought together consumers, clinicians, basic science researchers, industry representatives, and government regulators. The group developed consensus recommendations related to the future of biomarker research in breast cancer, which are available at the NBCCF website: http://www.stopbreastcancer.org/.

The group identified 5 major principles designed to provide the framework for their recommendations:

1. Research on biomarkers and their clinical use must be patient-centered.
2. Biomarker research must aim to substantially improve patient outcomes by accurately identifying those likely to benefit from specific interventions and sparing those who will probably not benefit from these interventions.
3. Research on biomarkers must be conducted in an environment of social responsibility in which resources are shared as part of a social network.
4. The research community must foster and encourage innovative ideas and approaches toward making substantial improvements in patient lives.
5. Stakeholders must adopt and abide by agreed upon standards and guidelines for conducting research, reporting results, and the clinical use of biomarkers.

Within these general principles, the conference panel developed a list of 6 priorities in breast cancer biomarker research:

- Priority 1. Develop and adopt standards and guidelines for the different stages of the "bench to bedside" continuum to ensure that only biomarkers with clinical utility make their way into routine clinical practice.
- Priority 2. Improve access to biological specimens including associated clinical data and research study information.
- Priority 3. Strengthen the role of regulatory agencies, particularly the FDA, in ensuring the responsible and evidence-based clinical use of biomarkers.
- Priority 4. Promote synergistic collaboration across research disciplines and among industry, academia, and consumer advocates.
- Priority 5. Educate all stakeholders, including clinicians and consumers, in all aspects of biomarker research and use.
- Priority 6. Enact legislation to protect patients against discrimination on the basis of biomarker information.

These recommendations from the NBCCF have the potential to guide policy, particularly as changes are now being recommended and entertained by the FDA. As can be seen from this chapter, economic and policy-based challenges add to the scientific and technological challenges with developing novel cancer biomarkers. How these challenges are met will likely form the outline of cancer medicine in the coming decades.

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# CHAPTER 6. PATHWAY-BASED ANALYSIS OF BIOMARKERS

We now know that cancer cells from one person can show dozens of mutations that may be different from the dozens of mutations shown by cancer cells from another person. A third person may have yet a different set of mutations. How is it possible to develop a drug to target all of these different mutations?

The discovery of so-called cancer pathways suggests that we may not have to. This line of research is based on the observation that cancer-causing mutations tend to occur along relay channels in the cell that regulate a handful of important behaviors such as cell replication and cell death. A pathway is a little like a relay race in which one runner (usually a protein) hands off the baton to the next, and so forth. At the end of the race, the cellular switch for some important behavior is thrown on or off. By intervening with a drug at the on/off switch, we may be able to avoid having to make a drug to stop every single runner in the race. Because the pathways are interconnected, targeting a single cancer pathway is probably not a "cure all." However, it is a promising avenue of research that has the potential to yield significant treatments.

## Gene Alterations in Cancer

Before beginning our discussion of cancer pathways, it may be helpful to review some relevant information about cancer and cancer cells. Cancer is caused by alterations in our genes. These alterations can be ones we are born with (inherited/germline) or ones that we accumulate throughout our lives (not inherited/somatic). Inherited alterations such as SNPs or mutations affect all of our cells, whereas non-inherited mutations, called somatic mutations, often affect single cells. Inherited mutations account for only about 10% of cancers. However, as we will see in the next section, inherited mutations may combine with non-inherited mutations over time to cause cancer.

**Some Causes of Gene Alterations in Cancer**

| Cause | Example | Type of Cancer |
|---|---|---|
| Heredity | Paget's disease | Bone |
| Diet | High levels of meat consumption | Colorectal cancer |
| Hormones | Estrogen | Breast cancer |
| Chemicals | Cigarette smoke, asbestos | Lung cancer |
| Radiation | High levels of sunlight | Skin cancer |
| Viruses | Human papilloma virus | Cervical cancer |
| Bacteria | Helicobacter pylori | Stomach cancer |
| Random | Errors in cell replication | Multiple |

For more information about causes of cancer, you may want to visit the National Cancer Institute's website on Understanding Cancer: http://www.cancer.gov/cancertopics/understandingcancer/cancer/allpages

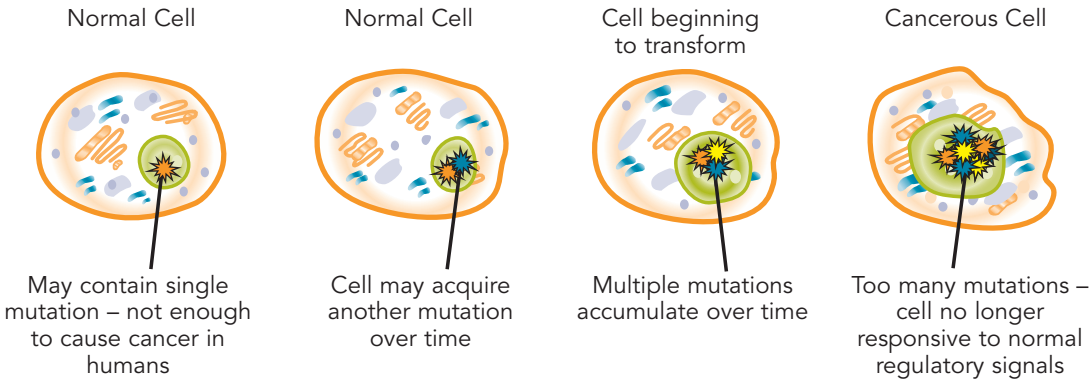
Non-inherited mutations – called somatic mutations – are caused by environmental factors such as sunlight or exposure to carcinogens in cancer smoke. Non-inherited mutations may also be caused by random errors that occur during cell division and replication. Because cells divide and replicate so many times during our lives, there is a high potential for some random mistakes to occur. In a person who has lived to be 80 years old, 10 million billion cells in his or her body have copied themselves correctly.

## Multi-Hit Theory of Cancer

An alteration in one single gene is not typically enough to cause cancer in humans. Instead, cancer is believed to occur when we accumulate multiple alterations in genes that are part of pathways critical to cell growth and the regulation of normal cell behavior.

When a cell accumulates 4 to 6 such mutations, it may begin to replicate out of control, eventually resulting in cancer. These cells lose their shape and become unable to perform their normal functions. Because cancerous cells are very good at replicating and growing, they crowd out normal cells in the tissue, preventing them from performing their functions. Some cells can escape into the blood and be transported to distant locations where they begin to replicate uncontrollably. In this case, the tumor is said to be metastatic.

**Multiple Gene Alterations in Critical Cell Pathways Can Lead to Cancer**




*This graphic shows how multiple mutations can lead to cancer. The left cell shows that a single mutation is not enough to cause cancer in humans; similarly, in the second cell, two mutations are still not enough to cause cancer. As cells accumulate multiple critical mutations over time – typically 4 to 6 – the cell may begin to transform into a cancerous cell. The cell on the far right has accumulated a critical number of mutations in pathways important to cell behavior or fate and has become cancerous. The cell has lost its normal shape and the nucleus, depicted as the green circle in the middle, has become enlarged to take up more and more space in the cell. If this cancerous cell continues to replicate – make more copies of itself – cancer will result.*

## Why Are Pathways Important in Cancer?

When cells communicate with their environment or with one another, they often do so through a chain of events that involves many molecules and interactions – also called pathways. These pathways are designed to provide information to the cell and often influence some aspect of the cell's behavior. Activation of cellular pathways can alter a cell's behavior by influencing whether certain genes are turned on (expressed) or off (suppressed). It can also influence a cell's behavior by altering the levels of a key protein that regulates a critical cellular process. In cancer, we are interested in the cellular pathways responsible for cell growth and survival. When these critical pathways are disrupted, cancer can result.

An example of how a pathway may influence gene expression is shown in the following graphic. Cells can release proteins that affect the behavior of other cells. These include proteins such as growth factors and hormones. Some of these proteins released into the space around cells bind to receptors in the cell membrane. These receptors are often proteins as well. Binding may cause a change in the receptor protein, such as a change in shape. This change may lead to the release of another protein that was bound to the receptor. The released protein may go on to interact with other cellular components, including other proteins. Some of the proteins may be chemically altered, which affects their behavior. Eventually, a protein that normally influences gene expression is activated or inactivated. The resulting change in gene expression may then change something important in the cell, such as causing it to proliferate.

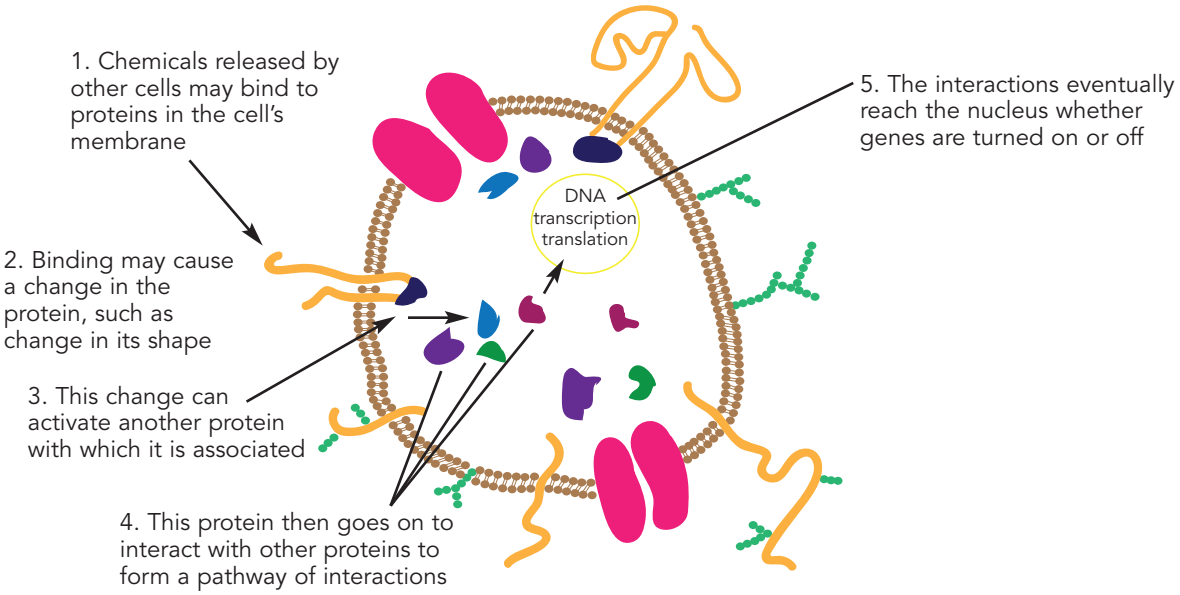
**Cell proliferation:** an increase in the number of cells as a result of cell growth and cell division.

As we noted previously, a cellular pathway is a little like a relay race in which one runner hands off the baton to the next runner. The baton represents the information that is being carried to the cell nucleus, such as "a growth factor bound to its receptor." This information is passed from one runner – protein – to the next until it eventually reaches the finish line, represented by changes in gene expression in the cell nucleus.

Note that it is not always desirable for genes to be "turned on" or expressed. In some cases, you want genes to be turned off, as in the case of growth factors or proteins that signal for unrestrained growth in cancer. In other words, when it comes to genes, expression or "on" does not always equal good and lack of expression of genes or "off" does not always equal bad.

For a good description of cancer pathways, you may want to visit the DNA Learning Center website called Inside Cancer sponsored by Cold Spring Harbor Laboratory (http://www.insidecancer.org/).

## Example of a Cellular Pathway




*Cells communicate with their environment and with one another in several different ways. One method of communication is for a cell to release chemicals that affect other cells. The chemicals that cells release are often proteins, which include growth factors and hormones. These chemicals bind to proteins called receptors, which may be located on the outside of cells. This binding initiates a series of events designed to affect some aspect of the cell. These events occur along multiple pathways and involve many different proteins and other chemicals inside the cell. The activation of these pathways often results in altered gene expression or protein metabolism. That is, the proteins involved in the pathways eventually carry information to the cell nucleus where genes are either activated or suppressed. Alternatively, pathways may affect key proteins that regulate important aspects of cellular behavior such as survival and growth.*

## Why Study Cellular Pathways in Cancer?

When scientists first started studying the genetic basis of cancer, many hoped that they would be able to find a single genetic error that could then be corrected with a drug. In some cases, this has been possible; for example, the Philadelphia chromosome we discussed earlier leads to the production of a faulty protein. This faulty protein is targeted by the drug imatinib, which inactivates it and effectively treats cancer in some patients.

However, as scientists obtained more information about various cancers, it became clear that not all were characterized by a single genetic mutation. In fact, most cancers show a wide variety of genetic mutations or aberrations. There is no simple way to target all of these mutations with a single drug. Moreover, there would be no guarantee that a drug developed for one person's cancer, based on his or her cancer's genetic profile, would work against another person's cancer.

In order to get around this problem, scientists have begun focusing on common pathways that are affected by the various mutations. As we will see in the next section, cancer cells are distinguished from normal cells by several prominent features, including unchecked proliferation and growth, and the ability to avoid death. By targeting pathways that mediate these processes, it may be possible to develop treatments that are effective for a larger group of cancers.

**Six Hallmarks of Cancer Cells**

1. Self sufficient growth signals
2. Insensitivity to signals that inhibit growth
3. Ability to evade programmed cell death (apoptosis)
4. Ability to replicate indefinitely
5. Ability to generate sustained blood supply (angiogenesis)
6. Ability to invade tissue and metastasize (spread to other areas)

From Hanahan D, Weinberg RA. Hallmarks of cancer. *Cell.* 2000;100: 57–70.

As we will see in the next section, these hallmarks of cancer have been refined into five prominent features that distinguish cancer cells from normal cells. By targeting pathways that mediate these processes, it may be possible to develop treatments that are effective for a larger group of cancers.

**The Utility of Targeting Pathways in Cancer: An Example from the National Human Genome Research Institute (http://www.genome.gov/27530687)**

*Imagine a thousand people from all across the United States travelling towards the front door of a single building in Chicago. How would you keep all of these people from entering the building? If you had limitless resources, you could hire workers to go out and stop each person as he or she drove down the highway, arrived at the train station or waited at the airport. That would be the one-target, one-drug approach. But if you wanted to save a lot of time and money, you could just block the door to the building. That is the pathway-based strategy that many researchers are now pursuing to design drugs for cancer and other common diseases.*

## Which Cellular Pathways Are Important In Cancer?

If we think about the things that cancerous cells do that normal cells do not, we can zero in on a handful of general processes. For instance, cancerous cells replicate more frequently than normal cells. This observation led scientists to study the pathways that are important in cell proliferation – the cell cycle and pathways stimulated by signals from the environment telling cells to replicate. Cells that are so busy replicating require a lot of resources. This observation led scientists to study cell pathways that provide support for cells that are rapidly dividing and growing. Another important difference between normal cells and cancer cells is that normal cells are limited in the number of times they can replicate, whereas cancer cells are not. This observation led scientists to study how cancer cells overcome the normal limits on replication. Finally, when the DNA of normal cells contains critical damage, the cell self-destructs. The fact that cancer cells do not self-destruct led scientists to focus on the normal pathway leading to cell death.

In the following sections, we explore each of these general processes that are important in allowing cancer cells to grow, proliferate, and avoid self destruction. These processes have been termed hallmarks of cancer.

In our discussion of these pathways, we list a number of biomolecules that are probably unfamiliar to you, including PTEN, MEK, RAS, and others. These abbreviations generally refer to the chemical names of the various biomolecules. Although all the different molecules in all of the different pathways may seem very confusing, it is not necessary to memorize them or even learn which pathway

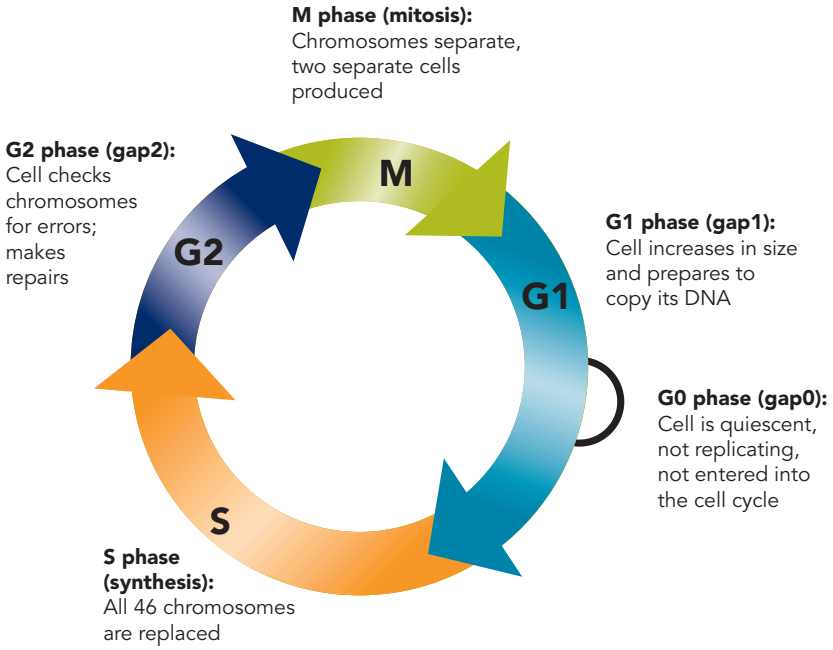
they belong to. Instead, we include these names simply to illustrate that each pathway has many biomolecules in it. That is, even though studying cancer pathways is less complex than studying thousands of genes, the pathways are not exactly simple. Additionally, as advocates, you are likely to hear these abbreviations in your discussions with clinicians and basic scientists. It may be advantageous to know that these abbreviations refer to biomolecules within the cell that are involved in one of the hallmarks of cancer pathways.

**Cell cycle:** A series of steps during which the chromosomes and other cell material double to make two copies. The cell then divides into two identical cells, each receiving one copy of the doubled material. The cell cycle is complete when each cell is surrounded by its own outer membrane.

### *1. The Cell Cycle*

The cell cycle is the process by which cells replicate. It consists of a series of events during which the chromosomes and other cell materials double to make two copies. The cell then divides into two identical cells, with each receiving one copy of the doubled material.

The cell cycle consists of 4 major phases characterized by different cellular events. Many traditional cancer chemotherapies specifically target one or more phases of the cell cycle.




*This figure shows the various phases of the cell cycle. When cells are not replicating, they are said to be quiescent and are in the G0 phase. When cells are stimulated to replicate, they enter the G1 phase where they can then proceed through each stage of the cycle sequentially: S phase, G2 phase, and M phase. However, in order to proceed to each subsequent phase, certain checkpoints must be passed. These checkpoints ensure that the replication process is proceeding correctly. Certain molecules can stop the procession of cells through the cell cycle.*

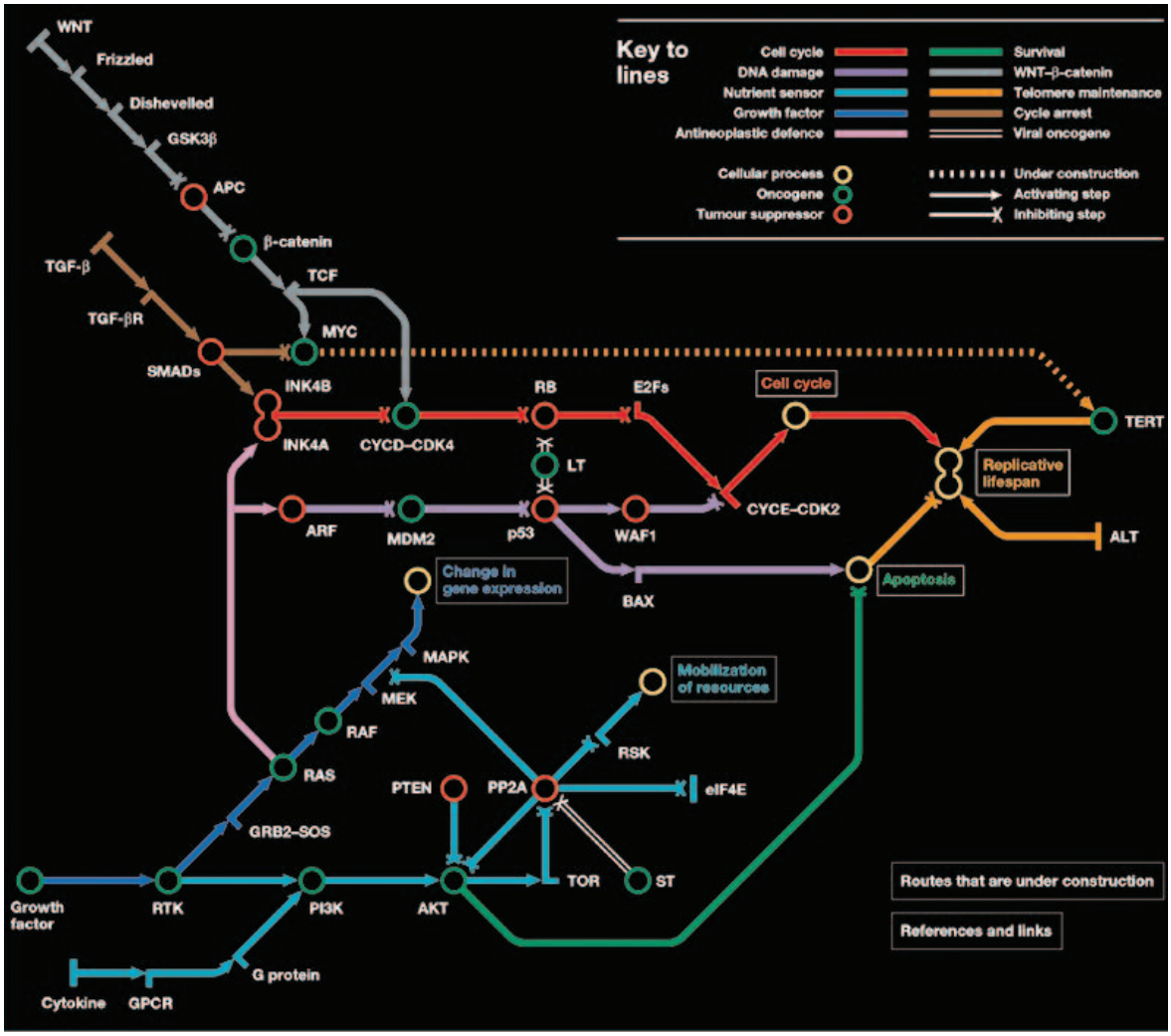
Whether or not a cell enters the cell cycle is determined by proteins that evaluate signals coming from outside and inside the cell. These proteins essentially weigh the stop and go signals, and either permit the cell to enter the cell cycle or cause it to remain in its resting state. Many of these regulatory proteins are referred to as tumor suppressors. In addition to the initial stop/go determination, the cell is regulated at different points in the cycle called checkpoints. These checkpoints are designed to ensure that the appropriate steps have been taken in the appropriate order.

**Oncogene:** A mutated form of a gene involved in normal cell growth. In their normal, unmutated state, onocgenes are called proto-oncogenes, and they play roles in the regulation of cell division. Some oncogenes work like putting your foot down on the accelerator of a car, pushing a cell to divide. When the oncogene is activated, it is similar to having the accelerator stuck to the floor.

Two of the main pathways that block progression through the cell cycle are the retinoblastoma (RB) pathway and the p53 pathway. Mutations in these pathways are common in many cancer cells. The following "subway" map of cancer pathways is useful in visualizing how disruptions in one of the many stops along the pathways can influence the cell cycle. RB and p53 are located just left of center on the subway map. By using your finger to trace the lines, you can see that the pathway by which RB inhibits the cell cycle includes E2Fs and CYCE-CDK2, whereas the pathway by which p53 inhibits the cell cycle includes WAF1 and CYCE-CDK2.

As can be seen on the subway map, RB and p53 can be influenced by several different pathways, some of which are inhibitory or inactivating (denoted by X's on the subway map) and some are activating (denoted by arrows on the subway map). Examples of these pathways include the WNT/frizzled pathway (upper left corner), the transforming growth factor-ß (TGF- ß) pathway (second to upper left corner), and the RAS–INK pathway (RAS in lower right quadrant with purple arrow up to INK and ARF). As you can see by all of the subway stops on this map, there are multiple places along each of these pathways where mutations may occur. Ultimately, these mutations can influence the development of cancer by de-regulating the cell cycle.

## Subway Map of Cancer Pathways (Hahn & Weinberg)




*This figure shows various cancer pathways. Note the complexity of the pathways and their interconnectedness.*

**Tumor suppressor gene:** Tumor suppressors are genes that keep specific other genes from being overexpressed. These tumor suppressor genes function like the brakes of a car. When tumor suppressor genes are mutated, certain other genes are activated or expressed uncontrollably, as if the brakes are not working and allow the car to speed down a hill.

### *2. Signals That Tell Cells to Proliferate*

One of the hallmarks of cancer is the ability of cancer cells to proliferate. Under normal conditions, cells receive signals from their environment – some of which may tell the cell to proliferate. However, the proliferation pathways are normally controlled by regulatory mechanisms that prevent the cell from replicating out of control. Mutations in the proliferation pathways or the proliferation control mechanisms can result in cancer.

**Growth factor:** A substance made by the body that can regulate cell division, growth, differentiation, and survival.

As their name suggests, growth factors are substances that can stimulate cell growth. However, the body produces numerous growth factors that have a variety of different roles, including the promotion of cell maturation and survival, as well as a reduction of cell proliferation. In cancer, the focus is typically on growth factors that stimulate the proliferation pathways.

One of the cellular proliferation pathways under study in cancer is called the RAS pathway. The RAS pathway can be activated when a growth factor binds to its receptor and activates proteins known as GRB2 and SHC. This can initiate the following series of protein interactions: SOS interacts with RAS, which interacts with kinases such as RAF and MEK, which interact with the kinase MAPK. MAPKs can enter the nucleus where they chemically modify proteins that control the transcription of genes involved in cell growth and survival.

**Kinase:** A type of enzyme that causes other molecules in the cell to become active. Some kinases work by adding chemical groups called phosphates to other molecules, such as sugars or proteins.

Another pathway by which growth factor signals can stimulate growth is the PI3K pathway (PIK stands for phosphatidylinositol 3-kinase). The PI3K pathway activates a kinase called AKT that is involved in cell growth and survival. This pathway regulated by a tumor suppressor enzyme known as PTEN. PTEN normally blocks the activation of AKT and thereby regulates the cell cycle, translation, and apoptosis. PTEN is mutated in many non-inherited cancers and in several inherited conditions. It is interesting to note that RAS activation can also cause PI3K activation, leading to the conclusion that the RAS and PI3K pathways are interconnected.

### *3. Mobilization of Cell Resources*

**Ribosome:** A ribosome is a cellular particle made of RNA and protein that serves as the site for protein synthesis in the cell. The ribosome reads the sequence of the messenger RNA (mRNA) and, using the genetic code, translates the sequence of RNA bases into a sequence of amino acids.

Cancer cells that are growing and dividing rapidly need supply chains. Among these cellular support systems are enzymes involved in nutrient metabolism and enzymes that regulate oxidative potential. Oxidative potential is the ability of the chemical to oxidize or lose electrons. One pathway involved in biosynthesis includes the enzyme PP2A and the kinase TOR. Ribosomal S6 kinase (RSK) is an important regulator of ribosome assembly that is regulated by PP2A and TOR. PP2A also activates factor 4E (eIF4E), a protein that is involved in the synthesis of ribosomes, the sites of protein synthesis within cells. Defects in any of these molecules are frequently associated with cancer.

**Telomere:** The ends of a chromosome. Each time a cell divides, the telomeres lose a small amount of DNA and become shorter.

**Telomerase:** An enzyme in cells that helps keep them alive by adding DNA to telomeres.

### *4. Number of Times Cells Can Replicate*

The lifespan of a cell is normally regulated by structures on the ends of our chromosomes called telomeres. Human telomeres consist of proteins and a repeating 6-nucleotide sequence: TTAGGG. Telomeres are synthesized by an enzyme known as telomerase. Each time a cell divides, the telomeres become shorter. When telomeres become too short and are not replaced by telomerase, the cell can no longer divide and eventually dies. Thus, telomeres limit the number of times a cell can divide and replicate.

**Telomeres on a Chromosome**

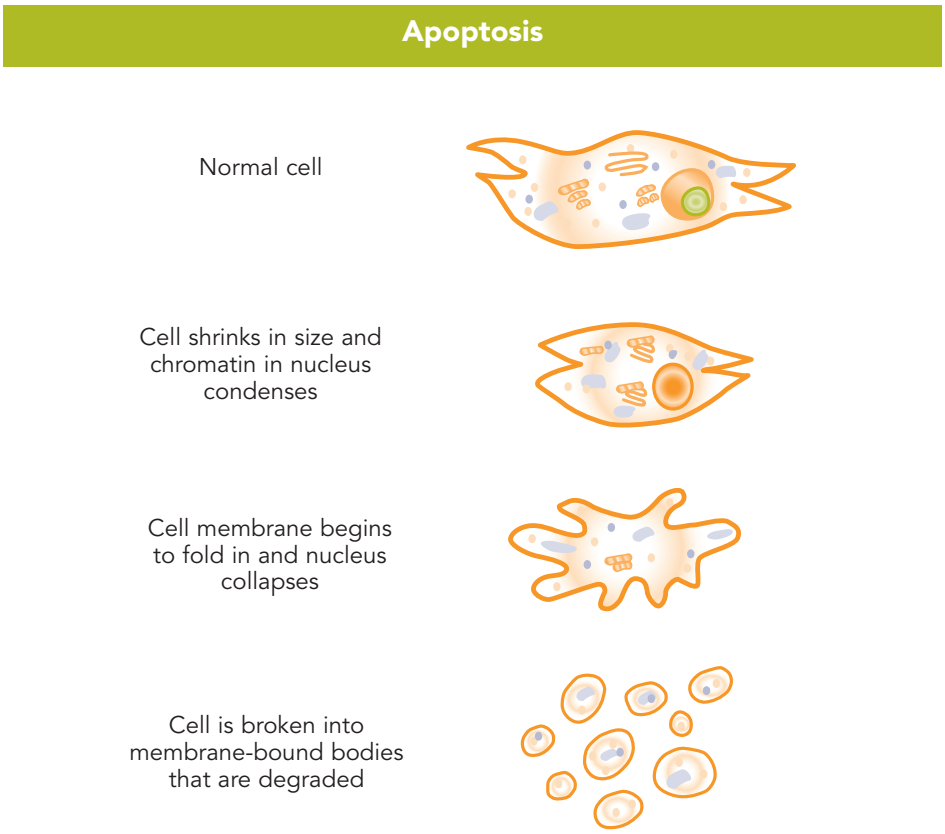
*A chromosome illustrating the telomeres (purple) on the ends of each arm.*

Unlike normal cells, cancer cells can replicate themselves indefinitely. In order to do this, they must overcome the limitation on cell division imposed by telomeres. In cancer cells the telomeres do not get shorter as the cells divide; in fact, the telomeres may even become longer.

Cancer cells maintain their telomere length in at least two different ways. The most common way is for the cells to produce more telomerase. Telomerase consists of a protein component and an RNA component. Up to 90% of all human cancer cells increase the activity of TERT, which is the protein component of telomerase. A smaller portion of cancer cells use another pathway to lengthen telomeres, although researchers don't know exactly what this mechanism is.

### *5. Apoptosis – Programmed Cell Death*

Apoptosis refers to the normal process by which cells actively destroy themselves when they are unneeded in the body, at the end of their lifespan, or damaged in a critical way. Apoptosis is controlled by a genetic program and is often referred to as programmed cell death. The major steps of apoptosis are well characterized and distinguish this form of cell death from necrosis, which is a passive form of cell death caused by mechanical trauma to the cell or exposure to toxic substances.




*When cells need to be removed from the body, they are typically eliminated through apoptosis – programmed cell death. The major steps of apoptosis are shown here. First the cell shrinks in size and chromatin in the cell nucleus condenses. Chromatin is the complex of DNA and proteins (histones) that condenses to form a chromosome during cell division. The membrane then begins to fold in and the nucleus collapses. The cell is then broken into membrane-bound compartments that are then degraded by other cells.*

It is critical that the apoptosis pathways in our cells work correctly. When they do not, cancer can result. In fact, disruptions in apoptosis pathways are nearly always necessary for the development of cancer. After all, if cancerous cells simply self-destructed, they would not be able to replicate and form tumors.

There are several methods by which cancer cells can overcome the normal process of apoptosis. One way is through inactivation of the p53 pathway. The p53 gene functions as a tumor suppressor. When activated, the p53 gene promotes apoptosis. Cancer can result when there are mutations in the p53 gene because cells don't undergo apoptosis. More than 50% of all cancers exhibit mutations in the p53 gene.

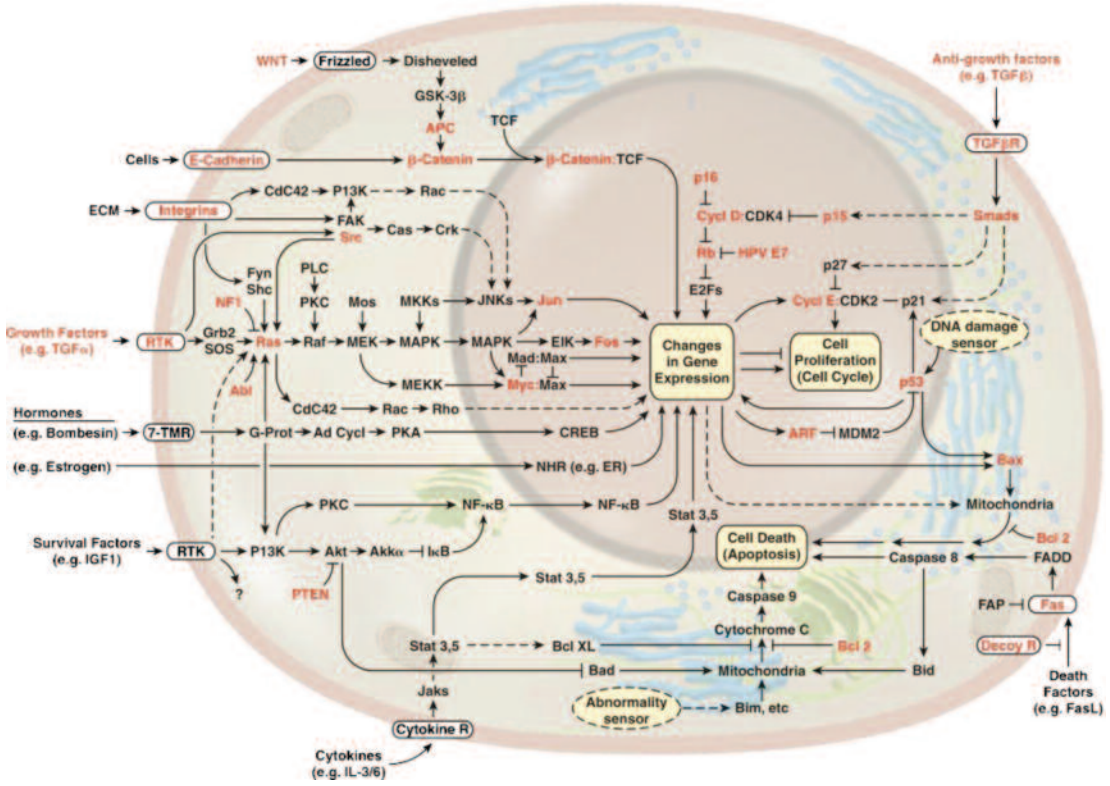
The p53 pathway involves multiple genes. When p53 is activated, it goes on to increase the transcription of many genes that promote apoptosis such as p21/CDKN1A, BAX, FAS, PUMA, BCL-2 and hTERT.

Another method by which cancer cells can overcome the normal process of apoptosis is by increasing the activity of pathways that interfere with this process. One such pathway is mediated by growth factors and involves the activation of a kinase (an activating protein) known as PI3K.

Informative slide shows and videos on apoptosis are available at the following website: http://www.researchapoptosis.com/apoptosis/multimedia/index.m.

Overall, it appears that a manageable number of common cell pathways may represent the most expedient targets for cancer drug development. However, despite this apparent simplicity, the pathways are still complex. They still consist of many players, as shown in the following graphic. Thus, it is not just a simple matter to work out all of the molecules that participate in these pathways and the precise roles that they play.

## Cell Signaling Pathways Relevant to Cancer




Reprinted from *Cell*, Vol 100/1 Hanahan D, Weinberg RA. The Hallmarks of Cancer Review. Pages 57-70, 2000, with permission from Elsevier.

## Biomarkers in Cancer Pathways

How does all of this pathway information relate to biomarkers for cancer? If biomarkers can be identified that signal disruption of certain cancer pathways, they can be used to guide development of new drugs. Individual cancers could then be segregated according to the pathways affected and could be treated with a drug that is effective for that pathway. Some scientists have already begun developing gene-expression pathway signatures for cancer cells studied in test tubes. The next step is applying these findings to actual cancer cells and eventually matching the drug with the cancer, and providing patients with the treatments from which they are most likely to benefit.

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# CHAPTER 7. ETHICAL, LEGAL, AND SOCIAL ISSUES WITH CANCER BIOMARKERS

Imagine that you are applying for a job as a lifeguard on a public beach. You would probably take it for granted that your DNA would not be analyzed as part of the application process. Of course, you would not want to be denied a job based on your genes. However, suppose that you had a genetic sequence that put you at increased risk for skin cancer. Would the presence of this biomarker change your mind about wanting your DNA analyzed?

This example illustrates one important ethical, legal, and social issue; namely, that individuals do not face employment discrimination based on their DNA. This example also illustrates a situation in which it may be to our advantage to know whether we have a certain cancer biomarker. Nevertheless, we probably would not want our prospective employer to know. In 2008, the Genetic Information Nondiscrimination Act known as "GINA" was signed into law. This act prohibits employers from discriminating against individuals based on genetic information. The law does not cover all biomarkers because, as we saw in Chapter 1, not all biomarkers fall into the category of "genetic information" as defined by the law. As non-genetic biomarkers are discovered and become integrated into clinical use, broader laws may be needed to counter such discrimination.

## Basic Principles of Medical Ethics

The ethical issues surrounding biomarkers may be considered within an existing framework of basic principles of medical ethics. These basic principles were developed by bioethics professionals and are often used to consider the ethics of other medical practices or procedures. When a new ethical dilemma arises, it may be useful to consider the five basic principles described in the following table.

| Principle | Definition | Explanation |
|---|---|---|
| Beneficence | Duty to do more good than harm | Who will benefit and in what ways? |
| Non-malfeasance | Duty not to cause harm | Who might be harmed? How might this information be misused? |
| Individual rights | Respect for an individual's right to be his/her own person and choose his/her own course of action | Are rights of all individuals considered and respected? |
| Privacy | Control over one's body and personal information; freedom from interference with personal choices | Protection of confidentiality; are there limits to this? What information is needed to save another person's life? |
| Justice/equity | Fair, equitable treatment for all | Are the interests of all in the community considered and is potential discrimination prevented? Are resources allocated fairly? |

Due to the multifaceted nature of bioethics questions, these principles are often in conflict with one another. In particular, the principles of privacy and individual rights are often in conflict with the principle of beneficence; that is, what is good for the group is not necessarily good for an individual vice-versa. An example of this can be seen with tissue samples provided as part of a research study. It is important to ensure that the person's name not be linked to the sample he or she provided. In contrast, knowing something about the person's clinical status or course may be useful in linking biomarkers to disease behavior and new therapeutics that may help others with the disease at some point in the future. In this case, the need to ensure confidentiality may be in conflict with beneficence. Because tissue provision for research is a critical issue for many advocates, we will discuss this in greater detail later in this chapter.

## The ELSI Program

In addition to the five principles of medical ethics just discussed, ethical considerations pertaining more specifically to genetic information were developed in conjunction with the Human Genome Project. This is referred to as the ethical, legal, and social implications (ELSI) research program. The following table outlines some of the major ethical issues that have come about as a result of knowing the entire human DNA code. As can be seen from this table, the issue of confidentiality is common to the ELSI list and the aforementioned principles of medical ethics.

**Ethical Issues Related to Human Genome Research as Identified by the ELSI Program**

| Issue | Example |
|---|---|
| **Fairness in the use of genetic information** by insurers, employers,courts, schools, adoption agencies, and the military, among others | *Who should have access to personal genetic information, and how will it be used?* |
| **Privacy and confidentiality** of genetic information | *Who owns and controls genetic information?* |
| **Psychological impact and stigmatization** due to an individual's genetic differences | *How does personal genetic information affect an individual and society's perceptions of that individual?*<br>*How does genomic information affect members of minority communities?* |
| **Reproductive issues** including adequate informed consent for complex and potentially controversial procedures, use of genetic information in reproductive decision making, and reproductive rights | *Do healthcare personnel properly counsel parents about the risks and limitations of genetic technology?*<br>*How reliable and useful is fetal genetic testing?*<br>*What are the larger societal issues raised by new reproductive technologies?* |
| **Clinical issues** including the education of doctors and other health service providers, patients, and the general public in genetic capabilities, scientific limitations, and social risks; and implementation of standards and quality-control measures in testing procedures | *How will genetic tests be evaluated and regulated for accuracy, reliability, and utility? (Currently, there is little regulation at the federal level.)*<br>*How do we prepare healthcare professionals for the new genetics?*<br>*How do we prepare the public to make informed choices?*<br>*How do we as a society balance current scientific limitations and social risk with long-term benefits?* |
| **Uncertainties** associated with gene tests for susceptibilities and complex conditions (e.g., heart disease) linked to multiple genes and gene-environment interactions | *Should testing be performed when no treatment is available?*<br>*Should parents have the right to have their minor children tested for adult-onset diseases?*<br>*Are genetic tests reliable and interpretable by the medical community?* |
| **Conceptual and philosophical implications** regarding human responsibility, free will vs genetic determinism, and concepts of health and disease | *Do people's genes make them behave in a particular way?*<br>*Can people always control their behavior?*<br>*What is considered acceptable diversity?*<br>*Where is the line between medical treatment and enhancement?* |
| **Health and environmental issues** concerning genetically modified foods and microbes | *Are genetically modified foods and other products safe to humans and the environment?*<br>*How will these technologies affect developing nations' dependence on the West?* |
| **Commercialization of products** including property rights (patents, copyrights, and trade secrets) and accessibility of data and materials | *Who owns genes and other pieces of DNA?*<br>*Will patenting DNA sequences limit their accessibility and development into useful products?* |

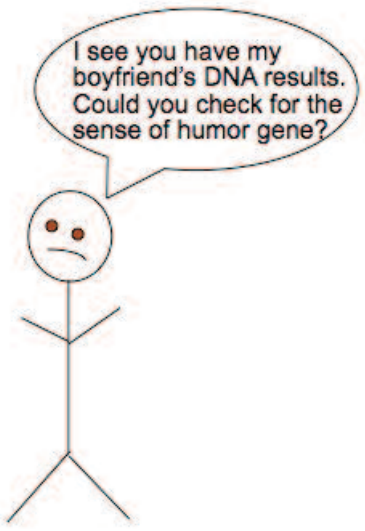
From: U.S. Department of Energy Office of Science, Office of Biological and Environmental Research, Human Genome Program. Ethical, Legal, and Social Issues.

## Practical Ethical Concerns for Advocates Regarding Tissue Samples

In addition to the more abstract guidelines and issues just discussed are the practical ethical concerns that advocates face each day. Many of these issues surround tissue provided as part of a research study.

***Identifiability of Tissue Samples***

The degree to which a tissue sample can be linked with the person who provided it is a major ethical issue. Tissue samples that are accompanied by information about the specific person are typically more useful to researchers than those that are not. For instance, if a researcher discovers that a biomarker is only present in half the tissue samples, it would be important to know why. Maybe the biomarker is only evident in individuals who are younger than 60 years of age. If the researchers don't have any information about the age of the person providing the sample, they wouldn't be able to draw this conclusion. More often, the useful information pertains to the clinical course of disease. Is the biomarker associated with a greater or lesser incidence of remission? Does the biomarker predict response to chemotherapy? More aggressive disease? Longer or shorter duration of survival? Is it only present in individuals with diabetes? These questions can only be answered if clinical information is linked to the tissue samples.




However, knowing more information about the individual who provided the tissue sample can increase the risk of breaching privacy and confidentiality. For example, when we provide tissue for research, we don't want our medical histories or disease information to be inadvertently passed on to our medical or life insurance companies, employers, or our friend who works in the office at the university where the studies are being conducted. As noted earlier, we do not want to be discriminated against based on information provided in conjunction with the tissue sample or contained within the tissue sample itself.

As you can see, there is a real potential for conflict between the need to maintain individual privacy and the need to enable research progress. Advocates can provide an important perspective here and can help determine how to balance these issues. It should also be noted that these concerns apply not only to tissue collected as part of an ongoing research study, but also tissue that is contained within a repository or tissue bank. For an overview of definitions that may be useful to advocates in discussing tissue identification issues, you may want to review the Recommendations of the National Bioethics Advisory Commission available on the internet at the following address: http://bioethics.georgetown.edu/nbac/hbm.pdf. The Executive Summary of this long document is especially useful.

In the early 1950s, a 30-year-old woman named Henrietta Lacks was treated for cervical cancer at Johns Hopkins Hospital in Baltimore. The treatment procedure involved sewing radium to her cervix. During this procedure, the physician took a sample of Henrietta Lacks' tumor and sent it to Dr. George Gey – the head of tissue culture research at Johns Hopkins. The tissue was taken without Ms. Lacks' consent or knowledge, as was standard procedure at the time.

Although Ms. Lacks died from her cancer, her cells did not. These cells proved to be immortal; that is, they did not die like most other cells. Researchers had been trying for decades to get cells to live outside the body so they could study them. However, they had been unsuccessful until the cells of Henrietta Lacks. Indeed, the cells that Ms. Lacks unwittingly provided are still alive today and are known as HeLa cells. These cells have been studied in thousands of experiments. They were used to help eradicate polio and were flown on space missions.

Although Dr. Gey tried to keep the identity of Henrietta Lacks secret, some people guessed that HeLa stood for a person's name and eventually Ms. Lacks was identified. This historical example demonstrates the issues of consent and confidentiality (identifiability) that have become so important to us today. More information about Ms. Lacks' cells can be found in articles and a book by Ms. Rebecca Skloot. The following link is from an article that appeared in the *New York Times*: http://www.nytimes.com/2001/11/17/arts/cells-that-save-lives-are-a-mother-s-legacy.html?]

***Potential for Harm at the Time of Tissue Provision***

Tissue samples are typically collected as part of the diagnostic procedures for cancer. That is, we may provide a blood sample or sample of bone marrow, breast, prostate, or other solid tissue to determine whether cancer is present. In some cases, part of this tissue can be set aside for research. However, in other cases, we may be asked (or may decide on our own) to provide a second tissue sample for research. For instance, researchers may want to know whether a biomarker that was present at diagnosis is still present after three months of treatment. Although many of the tissue sampling procedures are relatively benign for the individual, others are not. The procedure may cause pain and distress and may involve the risk of harm to the individual. It is always important to weigh the potential benefit to be gained through research against the risk to the individual.

***Access to Research Results***

When we provide tissue samples as part of a research study, we may naturally be curious what the researchers find out. We may want to know about the results of the study as a whole, as well as about our individual results. Let's consider each of these in turn.

The overall results of a research study usually end up published in the scientific literature in a medical or scientific journal. These journal articles may be difficult for individuals to understand unless they are medical professionals. Therefore, it may be advantageous to make sure there is a pathway for study participants to receive information about the study results presented in an understandable format. Advocates who participate in study groups may be able to assist with this by ensuring that such a pathway is part of the study planning. However, it is also important to ensure that this information is described to study participants only after the study has been published in the scientific literature because this ensures that results have been deemed acceptable through the review of scientific peers and ensures that other researchers are not able to obtain a "scoop" on the results.

Additionally, many advocates believe that it is important for individuals who provide their tissue for a scientific study to be informed as to whether that study is still progressing or whether it has been abandoned. Although general information is typically provided on the government clinical trials website (clinicaltrials.gov), it is important to ensure that the information is regularly

updated so that it is current. For a thorough discussion of the issues related to communication of study results to participants, please see Chapter 8 of the booklet entitled *Understanding Tissue Pathology Research*, available at the Research Advocacy Network website: http://www.researchadvocacy.org/publications/pdf/pathology_report.pdf.

Even when studies are published in the scientific and medical literature, the results do not always support immediate changes in clinical practice. For instance, some studies have obtained positive results for new treatments that are later disproven. An example of this occurred in the late 1980s and early 1990s, when beneficial effects were noted with high-dose chemotherapy followed by autologous bone marrow transplantation for the treatment of metastatic breast cancer. High-dose chemotherapy is a very intensive treatment that destroys patients' immune systems. In order to help the immune system recover, bone marrow or stem cells from the patient's own blood are extracted and then re-infused after the chemotherapy.

Initial studies that were not well controlled suggested that this intensive and difficult treatment was beneficial for women with metastatic breast cancer. As a result, the treatment was prematurely accepted as a new standard of care and more than 41,000 women underwent the procedure. Insurance companies were pressured to pay for this treatment out of fear that they would be sued if they did not. As the results of well-controlled trials became available, evidence suggested that this intensive treatment was not superior to the previously standard therapy. Additionally, a case of scientific misconduct was uncovered that added to the controversy.

Although 20 years have passed since this initial controversy, the role of high-dose chemotherapy in breast cancer treatment has not been resolved. Some argue that research conducted over the past few decades warrants a new look at this therapy. Nevertheless, the historical example provided important lessons for many involved; namely, that newer treatments are not always better treatments and new treatments must be adequately tested before becoming accepted as standard of care. For more information on the premature acceptance of high-dose chemotherapy in breast cancer, you may want to visit the following websites: National Cancer Institute Press Office (http://www.hhs.gov/news/press/1996pres/960528.html) and an article by M. Mello and T. Brennan (http://content.healthaffairs.org/cgi/content/full/20/5/101).

The other question is whether individuals who provide tissue should expect to receive individual results. That is, does my tissue contain a given biomarker? The current thinking is that participants in a research study should not expect to obtain individual results. An exception to this is when the tissue contains a biomarker that is known to be clinically significant that was perhaps missed on the first examination of the tissue. That is, if the tissue contains a biomarker for response to a known drug that could benefit the person, the results should ethically be shared with the individual and his or her doctor. However, typically, research is undertaken to determine whether or not a biomarker is significant and the clinical relevance can remain undetermined for many years.

***Conflicts of Interest, Commercialization and Other Financial Considerations***

Tissue research has the potential to lead to commercial gain for sponsoring companies. Additionally, some groups may seek to purchase tissue samples for their research. The relationship between individuals who provide tissue and individuals who stand to gain from the research results is important to consider, and advocates should be aware of the potential for conflict.

## Tissue Banks or BioBanks

Tissue banks or biobanks are facilities for storing and maintaining a collection of tissues for future use. Some tissue banks maintain tissues or organs for use as transplants (e.g., skin, liver, kidney, etc.) or in medical materials. However, the tissue banks we discuss here are those that collect, store, and distribute biological materials and associated data (e.g., clinical information) for the purposes of basic

science and clinical research. These tissue samples are often referred to as biospecimens.

Tissue banks are critical because improperly stored tissue will degrade or deteriorate so that it is not useful for research. Additionally, the storage and handling of biospecimens is expensive and requires a great deal of expertise. Standardized protocols must be followed to make sure that the biospecimens are consistently treated the same way and stored in a manner that ensures their preservation for research.

Tissue banks vary in several important respects, including whether they are public or private, the uniformity of biospecimen collection and storage, how the data is shared, and how the resource is protected. We will consider each of these in turn.

***Public vs. Private Tissue Banks***

Tissue banks can be owned and run by individual investigators, companies, universities, or other private interests. In such cases, access to tissue specimens may be limited. For instance, investigators who collect tissue for a particular research project may feel intense ownership of the biospecimens. Some universities maintain tissue banks that are solely for use by researchers at that university. In these cases, the investigators or institutions may be unwilling to share tissue with others or to allow advocates to have a say in what is done with the tissue.

The closed nature of private tissue banks has led to the establishment of a number of national or public tissue banks such as the Susan G. Komen for the Cure Tissue Bank and the Cancer and Leukemia Group B (CALGB) Tissue Bank. These resources aim to make research on biospecimens publicly available based on submission of accepted research protocols.

Some advocates have even formed their own tissue banks in order to protect the rights of those who provide tissue for research and to ensure that they have a say in what is done with that tissue. An example is the Inflammatory Breast Cancer Foundation's Biobank (http://www.ibcresearch.org/diagnosed/biobank/). Other advocates are working with the National Cancer Institute to develop best practice standards for tissue banks that take into account some of the concerns outlined in this chapter.

***Uniformity of Tissue Collection and Storage***

An important issue in the use of biospecimens for research is whether the tissue samples have been collected and stored the same way. Each tissue bank often has its own methods and procedures, making it difficult to get the same information or results with tissue from different tissue banks.

In an attempt to increase the uniformity of tissue samples, the Office of Biorepositories and Biospecimen Research at the National Cancer Institute has established a program known as the Biospecimen Research Network. This organization has generated recommendations for best practices in the collection and handling of tumors and other biospecimens, which ultimately may become accepted common practice among tissue banks. The guidelines can be found at the following website:
http://biospecimens.cancer.gov/researchnetwork/default.asp.

***How is the Data Shared?***

Many people believe that it is important to share the data derived from biospecimens with other researchers. However, some researchers or institutions may have an interest in keeping the data under their control. For instance, an investigator or company that has identified a protein of interest in cancer may want to be the first to develop a drug against that protein. They may not want to share the data until their research and drug development is well established. However, even in such cases, this may not mean that the data will never be shared, but rather that there may be a delay in data sharing. Many researchers will eventually wish to publish the results of their studies in scientific and medical journals as support for their products, although this cannot be guaranteed.

The ethics of data sharing related to biospecimens revolve around whether and how the data should be shared, as well as how decisions about data sharing should be made. Some tissue banks have policies on data sharing, whereas others do not.

The Susan G. Komen Tissue Bank is an example of a resource in which data is required to be shared. Requirements include the publication of research results in scientific journals and presentation of results at scientific meetings. Additionally, digital data generated from tissue samples are made available on the internet. The Komen Tissue Bank also requires researchers to follow established, standard methods for reporting certain types of data, such as data obtain from microarray analysis.

***How is the Resource Protected?***

Given the importance of tissue banks to the individuals who provided the tissue, the investigators who want to conduct research on the tissue, and the public who may benefit from the tissue-based research, it is critical to adequately protect the tissue bank from inadvertent damage. This damage could come from natural disasters such as floods or hurricanes, which damaged tissue storage facilities at Baylor University in Texas and Tulane University in New Orleans. Damage could also come in the form of power outages that could cause tissue to thaw and be rendered useless for research.

When determining where to physically house the tissue specimens, it is important to select a location that provides physical protection to the tissue. Although we cannot foresee all possible events that could damage the tissue, it makes sense to store tissue in locations where it is protected from natural disasters common to the area (e.g., not in basements in areas prone to flooding and not in buildings likely to collapse in an earthquake). Similarly, storage facilities should have back-up electrical generators to prevent sample thawing.

As you can see, there are a number of important issues related to tissue research and biobanks. For more information on tissue banks and biospecimens, please see the following resources:

- Booklet entitled *Understanding Pathology and Tissue Research*, available at: http://www.researchadvocacy.org/publications/posters.php
- BioBank Central website: http://www.biobankcentral.org
- National Cancer Institute website: http://www.cancerdiagnosis.nci.nih.gov/specimens

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# CHAPTER 8. HOW CAN ADVOCATES USE THIS INFORMATION

As cancer advocates, we encounter the term biomarkers on a regular basis: in our communications with physicians and researchers, in our readings, and in the popular press. It is important for us to understand what biomarkers are and what they are used for. It is also important for us to be aware of the controversies surrounding some of the current biomarkers, as well as the challenges inherent in developing validated biomarkers and biomarker tests. Knowing these basics will help us to more confidently interact with medical professionals and researchers, and to gain credibility for our viewpoints.

Within the areas of biomarker use and research, a number of issues may be particularly relevant for advocates. These include the following:

- Ethical conflicts that arise with tissue samples
- Regulation of biomarker tests
- Reliability, validity, and clinical utility of biomarker tests and the controversies over some of the current biomarker tests that result when these concepts are in question
- Lack of rapid progress in the development of biomarkers using genomic or other omic technologies
- Development of policy – the FDA proposes to work with all relevant stakeholders including government, private industry and academia. Advocates have the opportunity to become involved with these decisions and to help guide policy, particularly as changes are now being recommended and entertained by the FDA
- Access to biomarker testing for the un- or under-insured (e.g., Onco*type*DX® – many insurance companies do not pay for this expensive test)
- Determining how best to disseminate study results to individuals who provide biospecimens for clinical research (e.g., what is the best format, timing, etc.)

In the following sections, we briefly review issues not already covered in earlier chapters.

## Regulation of Biomarker Tests

***Regulatory Requirements for Biomarker Tests***

Government regulations for biomarker tests are different depending on whether the test is sold as a product, such as a test kit provided to physicians, or whether the test is sold as a service to be conducted by the company's laboratory.

The United States Food and Drug Administration (US FDA) must approve tests sold as kits to be used by the physician or a laboratory with which the physician is associated. The amount of data required for the FDA to approve these tests varies with the test's intended use and its potential for harm. Tests designed to determine whether someone is pregnant are not associated with as much potential for harm as a cancer diagnostic test, and thus the two are regulated by the FDA at different levels.

Tests sold as services do not require FDA approval. For example, the Onco*type*DX® test that we discussed previously is widely used in the United States and is not FDA approved. Instead, these tests are regulated under the Clinical Laboratory Amendments of 1988 (CLIA). The CLIA regulations are conditions that all laboratories must meet to be certified to perform testing on human

biospecimens. These regulations were intended to ensure quality laboratory testing. Even though the tests sold as services don't require FDA approval, individual components or ingredients in the tests may require FDA approval. The FDA also regulates who can purchase key ingredients in the tests. These ingredients can only be purchased by diagnostic device manufacturers, clinical laboratories that are qualified to perform highly complex tests, and organizations that use the ingredients to make tests for non-medical uses.

More information about CLIA regulations can be found at the Centers for Disease Control and Prevention (CDC) website: http://wwwn.cdc.gov/clia/default.aspx.

**Excerpt taken from Railey, E. Docket: 2006D-0347 - Public Comment regarding FDA Regulation of IVDMIAs. Submitted: January 17, 2007. Available at: http://www.fda.gov/ohrms/dockets/DOCKETS/06d0347/06D-0347-EC13-Attach-1.pdf. Accessed April 28, 2010.**

The regulation of biomarker tests is an important issue for many advocates. The following quotation outlines the view of one advocate on FDA regulation of assays or tests: "We also acknowledge that because the information provided by these assays leads to crucial decision-making on the part of the patient and the physician, it is imperative to ensure that genetic and genomic tests are both scientifically accurate and can be reliably performed by the testing laboratory. We recognize that there is a very fine balance to be achieved, protecting patient safety while still enabling patient access and promoting scientific innovation."

As noted previously, test regulations are designed to ensure that tests are accurate and clinically useful and that they do not cause harm. Even if physically taking the test does not harm someone (e.g., taking a swab of cells from someone's cheek or drawing blood), there is the potential that the test's results could lead to harm. For instance, if a biomarker test indicates that someone likely has cancer when they do not, they may undergo unnecessary biopsies and other procedures. On the other hand, if a test has a relatively good chance of identifying cancer at an early stage, it may be very clinically useful. It may, in fact, prevent harm.

Determining whether the clinical utility of a biomarker test outweighs its potential to harm requires data. The FDA assesses data provided by the manufacturers and/or developers of biomarker tests. In the following section, we consider an example of a biomarker test that showed early promise, but that later faced questions about its validity.

### *OvaCheck® Example*

OvaCheck® is a blood test for the early detection of epithelial ovarian cancer. This test uses proteomics technology to identify a protein signature characteristic of ovarian cancers. It is planned that this test would be made available through two national diagnostic laboratories: Quest Diagnostics and Laboratory Corporation of America (LabCorp).

Data supporting an earlier version of OvaCheck® was published in 2002, showing nearly 100% sensitivity and specificity for detecting ovarian cancer. However, questions about whether the results were reproducible soon followed. In 2004, the Society for Gynecologic Oncologists issued a position statement concluding that more research was needed to validate the test's effectiveness before offering OvaCheck® to the public. As of 2010, OvaCheck® has not yet been cleared by the FDA and it is not yet clinically available.

Experience with OvaCheck® highlights the possibility that chance and bias may lead to conclusions about tests that are not replicable. You may remember that omics sciences focus on multiple parts as opposed to individual parts. A major question in the analysis of omics results is what rules of evidence are required to decide whether a biomarker test is accurate and reliable? Experts are attempting to address this issue and to design, conduct, and interpret clinical studies on biomarkers in a way that will lead to greater assurance of validity.

## Reliability, Validity, and Clinical Utility of Biomarker Tests – Controversies Over Current Tests Result When These Conditions Are Not Met

The example of OvaCheck® we just considered shows how a test that initially appears to be valid and reliable may not hold up to further study. Some of the major issues for advocates related to the validity, reliability, and clinical utility of biomarker tests are listed below.

- We are making clinical decisions based on biomarker tests and we need to ensure that they are accurate and reliable. Moreover, we need to ensure that the tests have clinical utility – that they predict something of clinical importance.
- A high false positive rate can lead to unnecessary tests and biopsies, as is the case for prostate specific antigen (PSA). The following link from the *New England Journal of Medicine* presents an editorial and video roundtable describing this controversy: http://content.nejm.org/cgi/content/full/NEJMe0901166.
- One point of controversy with PSA used to screen the general population of men for prostate cancer is that a recent large study did not find differences in the rates of death from prostate cancer between men who received annual PSA testing and those that received usual care. Thus, one may question whether PSA screening of the general population has clinical utility.

- False negatives can prevent someone from obtaining a treatment that may help them. This is a concern with HER2 testing, which is used to determine whether a person's breast cancer should be treated with trastuzumab. Someone with a false negative result for HER2 may not receive the potentially beneficial drug trastuzumab, which is typically only used in women who have HER2 positive breast cancer. The controversy is summarized in an article published in the *Journal of the National Cancer Institute*, available online at the following link: http://jnci.oxfordjournals.org/cgi/content/full/99/14/1064. Published citation: Tuma RS. Inconsistency of HER2 test raises questions. *J Natl Cancer Inst.* 2007; 99(14):1064-5.
- The reliability and validity of biomarker testing may be influenced by the conditions under which the test is conducted. This is particularly true for tests based on the omic sciences, as described in Chapter 5 in the section on Challenges with biomarker discovery in the omic sciences. Does the testing laboratory use standardized methods? Is the laboratory accredited? These are important considerations, as mistakes may be more likely when the tests are conducted by laboratories that do not follow strict guidelines and do not test high volumes of samples.

  The Genentech website contains a series of questions and answers about HER2 testing, one of which is entitled "How can I help ensure an accurate HER2 test result?" (http://www.herceptin.com/hcp/HER2-testing/faqs.jsp). This question and answer provides an overview of some of the issues that advocates should be aware of with regard to HER2 testing.

  Essentially, it is important to ensure that testing is undertaken by a qualified laboratory that has expertise in the particular test being performed. For some biomarkers, all testing is performed at a central laboratory, which helps ensure consistency and validity. As advocates, we may be interested in leading or participating in discussions about testing for new biomarkers as they are discovered. Can we apply some of the lessons we've learned from the testing of current biomarkers such as HER2? Is it necessary to have a central laboratory perform biomarker testing in order to ensure accuracy? These are questions that informed advocates can help address.

Statistical models are typically used to make sense of the results of omics research. However, these models can be influenced by a variety of factors other than just the presence or absence of disease.

## Lack of Rapid Progress in the Development of Biomarkers Using Omic Technologies

As we discussed earlier, few biomarkers are in clinical use today that have been developed using omic technologies. This has led many to question why this is the case. Why aren't there more cancer biomarkers? Are our rules and regulations too strict? Is science failing to progress rapidly enough? What problems are preventing the translation of basic science progress into clinically useful cancer biomarker tests? Are there cost issues and, if so, how can we get around these?

The FDA recognizes that there is a gap between the technological advances and clinical products and has sought to hasten progress, as discussed in Chapter 5. In the following text, we consider a few potential reasons for the lack of useful cancer biomarkers in the clinic.

### *Reproducibility of Omic Results*

In the OvaCheck® example, we have seen how biomarker tests based on the omic sciences may give initial results that are difficult to reproduce. This problem is not limited to OvaCheck®, but is a consideration for all of the omics sciences.

The problem arises because of the large numbers of different genes, proteins, or other groups of molecules being used as predictors. Whereas more traditional biomarker tests, such as PSA testing, measure only one protein, tests based on proteomics measure numerous proteins simultaneously. In proteomics, the protein signature is used to distinguish a group of individuals with cancer from those without cancer. Statistical models are used to make these distinctions, and they can be influenced by a variety of factors. The model may give results that appear to be real but are actually due to chance. Scientists and biostatisticians are working on ways to get around these problems.

***Cost of Biospecimen Research***

The search for cancer biomarkers usually involves analyzing biospecimens from individuals with cancer. Often, this research may be conducted as part of a clinical trial for a new treatment. For instance, investigators may be conducting a clinical trial to determine whether a certain chemotherapy combined with radiation produces better results (such as longer survival) than chemotherapy alone. In this case, there is the potential that biopsies taken as part of the trial could be used for genomic, proteomic, or other omic analyses to search for biomarkers.

However, the search for biomarkers is not the major question under study, but rather a sort of "bonus" study – often referred to as correlative science. Costs are associated with obtaining, transporting, processing, storing, and analyzing the biospecimens. Who should pay for these added costs? The overall trial may be paid for by the government or a company, neither of which may be inclined to pay extra costs. A related issue is that, even when sponsors do pay for the bonus study, the main study investigators may receive the money instead of the pathology department that is doing the basic science work.

Another related issue is that the biomarker search may not be included in the contract that details how the study is to be conducted. It may be difficult to change the contract, particularly if the biomarker research is not directly relevant to the primary study.

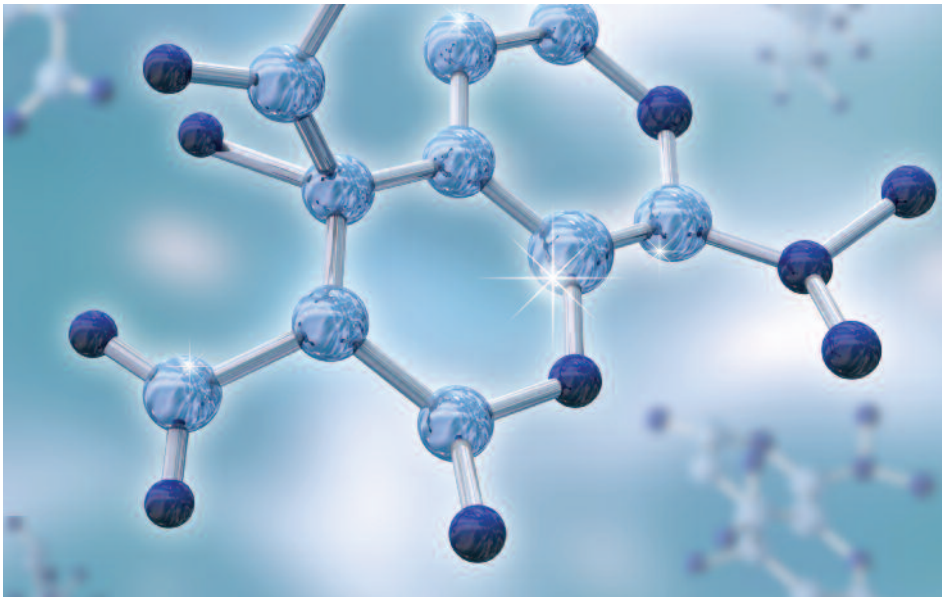
***Lack of Tissue for Research***

The increased knowledge about human genes and the proteins they encode has increased the need for tissue samples in research. Without these samples, it is difficult to identify differences between cancerous tissue and normal tissue that could be targets for new medicines or could help identify which treatments or drug doses would be most beneficial for individuals with a given genetic pattern. Researchers working on many different cancer types lack adequate tissue to address these important questions. In fact, several professional groups have identified the lack of access to pathological tissue specimens as a primary barrier to the development of cancer diagnostics, preventives, and therapies. For a more detailed discussion of this issue, please see the booklet entitled *Understanding Pathology and Tissue Research* available for download from the Research Advocacy Network website: www.researchadvocacy.org.

The lack of tissue availability has led to the question of whether tissue consent should be made mandatory for participants in clinical trials. Of course, participation in clinical trials is voluntary and individuals may or may not decide to participate if the provision of tissue samples for research is included as a pre-condition for that consent. Some have proposed that the following circumstances may influence whether they favor inclusion of tissue as a mandatory provision:

- Source of the tissue (e.g., tissue type, previously gathered or newly obtained, procedure required to obtain)
- Intended use of the tissue (e.g., decision-making within the trial, analyzing the results of the trial, correlative studies, future unspecified research)

Advocates should be aware that this is currently an important but controversial issue for investigators as well as advocates.



***Lack of Adequate Translation of Basic Science Research to the Clinic***
Even if biomarkers are developed through basic science research, they must be "translated" into tests that are clinically useful. One barrier to translation, according to the National Institutes of Health, is the increasing complexity of conducting clinical research. The heavy reliance on study designs that have been useful in the past may also be an issue. Some have proposed that we need to develop and implement a new model of how potential biomarkers get translated into clinically useful tests, while still retaining the requirements of validity and reliability.

In 2006, the National Institutes of Health launched an initiative designed to help enhance the translation of basic science research into clinical medicine and back again (i.e., the translation is bidirectional – it works both ways). This model is useful because it allows practitioners to help guide the research and bring practical issues to the attention of the basic science researchers. More information about this initiative can be found at the NIH Common Fund website: http://nihroadmap.nih.gov/clinicalresearch/overview-translational.asp. Translation of basic science results into clinical medicine is an important issue for many advocates and one in which many advocates are actively involved.

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# GLOSSARY

**Angiogenesis:** The generation of new blood vessels from existing blood vessels. Tumor angiogenesis is the growth of new blood vessels that tumors need to grow. This is caused by the release of chemicals by the tumor.

**Apoptosis:** Programmed cell death. In adults, apoptosis is used to rid the body of cells that have been damaged beyond repair. Apoptosis also plays a role in preventing cancer. If apoptosis is for some reason prevented, it can lead to uncontrolled cell division and the subsequent development of a tumor.

**Biobank:** A facility for storing and maintaining a collection of tissues for future use.

**Biomarker:** A characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacologic responses to therapeutic intervention.

**Biorepository:** See biobank.

**Biospecimen:** Samples of biological material, such as urine, blood, tissue, cells, DNA, RNA, and protein from humans, animals, or plants. Biospecimens are stored in a biobank (biorepository) and are used for laboratory research. If the samples are from people, medical information may also be stored along with a written consent to use the samples in laboratory studies.

**CA-125:** Cancer antigen (CA) 125. A substance that may be found in high amounts in the blood of patients with certain types of cancer, including ovarian cancer. CA-125 levels have been used as a biomarker to help monitor how well cancer treatments are working or if cancer has come back.

**Cancer:** Diseases in which abnormal cells divide without control and can invade nearby tissues. Cancer cells can also spread to other parts of the body through the blood and lymph systems.

**Cancer pathway:** A group of molecules in a cell that work together to control one or more cell functions, such as cell division or cell death, that are critical to cancer.

**Cell cycle:** A series of steps during which the chromosomes and other cell material double to make two copies. The cell then divides into two identical cells, each receiving one copy of the doubled material. The cell cycle is complete when each cell is surrounded by its own outer membrane.

**Cell proliferation:** An increase in the number of cells as a result of cell growth and cell division.

**Chromatin:** The substance within a chromosome consisting of DNA and protein.

**Chromosomes:** Compacted structures of long strands of DNA located inside almost all of our cells.

**Codon:** A three nucleotide sequence of DNA or RNA that corresponds to a specific amino acid.

**Diagnostic biomarker:** A biomarker whose presence aids in the diagnosis of disease.

**False negative:** A negative test result is found when the condition being tested is actually present.

**False positive:** A positive test result is found when the condition being tested is actually absent.

**Gene Expression:** The process by which a gene gets turned on in a cell to make RNA (ribonucleic acid) and proteins.

**Gene:** A piece of DNA that contains the information for making a particular biochemical, usually a protein.

**Genetic variation:** An alteration in the normal sequence of a gene.

**Genome-wide association:** An approach that involves rapidly scanning markers across the complete sets of DNA, or genomes, of many people to find genetic variations associated with a particular disease.

**Genomics:** The study of the complete genetic material, including genes and their functions, of an organism.

**Growth factor:** A substance made by the body that can regulate cell division, growth, differentiation, and survival.

**HER2/neu:** A protein involved in normal cell growth. It is found on some types of cancer cells, including breast and ovarian. Cancer cells removed from the body may be tested for the presence of HER2/neu to help decide the best type of treatment. HER2/neu is a type of receptor tyrosine kinase. Also called c-erbB-2, human EGF receptor 2, and human epidermal growth factor receptor 2.

**Histone:** A protein that provides structural support to a chromosome. In order for very long DNA molecules to fit into the cell nucleus, they wrap around complexes of histone proteins, giving the chromosome a more compact shape.

***In silico*:** On the computer. Used to indicate research that is performed via computer as opposed to, for instance, cells in a test tube or a living creature.

***In vitro*:** In a test tube. Used to indicate research that is performed in a test tube, usually on cells.

***In vivo*:** In the body. Used to indicate research that is performed in the body, such as a clinical trial of a new cancer treatment.

**Kinase:** A type of enzyme that alters the function of other molecules in the cell. Some kinases work by adding chemical groups called phosphates to other molecules, such as sugars or proteins.

**Messenger RNA:** A single-stranded RNA molecule that is complementary to one of the DNA strands of a gene. The mRNA is an RNA version of the gene that leaves the cell nucleus and moves to the cytoplasm where proteins are made.

**Metabolite:** A molecule produced by metabolism, a process of chemical reactions that change one molecule into another molecule for the purposes of storage, use in the body, or elimination.

**Metabolomics:** Study of the metabolome or all of the small molecule metabolites in a cell, tissue, or organism under given conditions.

**Metastatic:** Having to do with metastasis – the spread of cancer from the primary site (place where it started) to other places in the body.

**Molecular imaging:** techniques that directly or indirectly monitor and record the spatiotemporal distribution of molecular or cellular processes for biochemical, biologic, diagnostic, or therapeutic applications.

**Mutation:** A change in the nucleotide base sequence of DNA that occurs in <1% of the population. Usually used to refer to a change that has deleterious effects on the organism.

**Oncogene:** A gene that is a mutated (changed) form of a gene involved in normal cell growth. Oncogenes may cause the growth of cancer cells. Mutations in genes that become oncogenes can be inherited or caused by being exposed to substances in the environment that cause cancer.

**Oxidation potential:** The ability of a material to oxidize or lose electrons.

**Pathway** (see cancer pathway)

**Pharmacodynamics:** Study of the biochemical and physiological effects of drugs.

**Pharmacokinetics:** The activity of drugs in the body over a period of time, including the processes by which drugs are absorbed, distributed in the body, localized in the tissues, and excreted.

**Predictive biomarker:** A biomarker whose presence helps determine how well a treatment will work for that person.

**Prognosis:** The expected course of disease, independent of any treatment.

**Prognostic biomarker:** A biomarker whose presence aids in the determination of disease prognosis.

**Prostate specific antigen (PSA):** A protein made by the prostate gland and found in the blood. PSA blood levels may be higher than normal in men who have prostate cancer, benign prostatic hyperplasia (BPH), or infection or inflammation of the prostate gland. PSA levels have been used as a biomarker in prostate cancer screening and in monitoring diagnosed prostate cancer.

**Protein:** A molecule made up of amino acids linked together. Proteins are the basis of body structures such as skin and hair and of substances such as enzymes, cytokines, and antibodies.

**Proteomics:** The study of the structure and function of proteins, including the way they work and interact with each other inside cells.

**Ribosome:** A cellular particle made of RNA and protein that serves as the site for protein synthesis in the cell. The ribosome reads the sequence of the messenger RNA (mRNA) and, using the genetic code, translates the sequence of RNA bases into a sequence of amino acids.

**RNA:** Ribonucleic acid. RNA contains information that has been copied from DNA (the other type of nucleic acid). Cells make several different forms of RNA, and each form has a specific job in the cell. Many forms of RNA have functions related to making proteins.

**Sensitivity (pertaining to a test):** Likelihood of obtaining a positive result when the target is actually present. In our examples, this is the likelihood that the biomarker will be present when the person does have the given condition (pregnancy or cancer). The likelihood that you do have hCG in your system if you are a pregnant woman is very high.

**SNP:** Single nucleotide polymorphism. An inherited difference in one base pair of the DNA sequence that occurs in at least 1% of the population.

**Specificity (pertaining to a test):** Likelihood of obtaining a negative result when the target is not present. In our examples, this is the likelihood that the biomarker will be absent when the person does not have the given condition (pregnancy or cancer). The likelihood that you do not have hCG in your system if you are not pregnant is very high.

**Telomerase:** An enzyme in cells that helps keep cells alive by adding DNA to telomeres.

**Telomere:** The ends of a chromosome. Each time a cell divides, the telomeres lose a small amount of DNA and become shorter.

**Tissue:** A group or layer of cells that work together to perform a specific function.

**Tissue bank:** A facility for storing and maintaining a collection of tissues for future use.

**Transfer RNA:** Each tRNA molecule has two important areas: a three nucleotide region that pairs with the codon and a region for attaching a specific amino acid. During translation, each time an amino acid is added to the growing chain, a tRNA molecule forms base pairs with its complementary sequence on the messenger RNA (mRNA) molecule, ensuring that the appropriate amino acid is inserted into the protein.

**Transcription:** The process by which a cell makes an RNA copy of a sequence of DNA that is a gene.

**Translation:** The process by which the genetic code carried by messenger RNA (mRNA) directs the production of proteins from amino acids.

**Tumor:** An abnormal mass of tissue that results when cells divide more than they should or do not die when they should. Tumors may be benign (not cancer), or malignant (cancer). Also called neoplasm.

**Tumor marker:** A substance that may be found in tumor tissue or released from a tumor into the blood or other body fluids that may be used as a biomarker for the presence of a tumor.

**Tumor suppressor gene:** Genes that keep specific other genes from being overexpressed. These tumor suppressor genes function like the brakes of a car. When tumor suppressor genes are mutated, certain other genes are activated or expressed uncontrollably, as if the brakes are not working and allow the car to speed down a hill.

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## Why was this guide developed?

As advocates try to work within the system to advance research it is important to understand the basic tenets of the science. By gaining a better understanding, advocates can identify and illustrate the issues and problem-solve to support solutions. The emerging science and issues in research involving biomarkers were the motivation for developing this manual. We hope that this information will be helpful to advocates and others interested in advancing the science and improving care for cancer patients.

## About Research Advocacy Network

Research Advocacy Network is committed to improving patient care through research. Our goals are to get results of research studies (new treatments) to patients more quickly, to give those touched by the disease an opportunity to give back and to help the medical community improve the design of its research to be more attractive to potential participants. Because research holds the hope for improvements in treatment, diagnostics and prevention, we are dedicated to patient focused research. We believe dissemination of research results to the medical community and patients can have a major impact on clinical practice.

The Research Advocacy Network (RAN) is a not for profit (501 c 3 tax exempt) organization that was formed in 2003 to bring together participants in the research process with the focus on educating, supporting, and connecting patient advocates with the medical research community. While there are many organizations addressing the needs of patients with specific diseases, political advocacy, cancer education and fundraising, Research Advocacy Network focuses on advancing research through advocacy and providing the patient perspective to the research dialogue.

RAN works with advocates and organizations to effectively integrate advocates into research activities. Please learn more about us at ourWeb site at www.researchadvocacy.org or contact us about our work by e-mailing us at info@researchadvocacy.org or by phone 877-276-2187 or FAX at 888-466-8803.


## Funding

Funding for the development and printing of this material is part of the Patient Advocacy and Care Translation (PACT) Core of the Komen Promise Grant "*Comprehensive Biomarker Discovery Project for Bevacizumab in Breast Cancer*" at Indiana University Melvin and Bren Simon Cancer Center, Bryan Schneider, M.D. Principal Investigator

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*Advancing Patient-Focused Research*

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