Molecular Diagnostics in Cancer

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Chapter 1: Introduction to Molecular Diagnostics

When Maria was diagnosed with invasive breast cancer, her doctor recommended several tests to help determine the best course of treatment. These tests, known as molecular diagnostics, showed that Maria's tumor would likely benefit from a medication designed to reduce the effects of estrogen. The test also showed that, if Maria did indeed take the anti-estrogen therapy, she was unlikely to need chemotherapy as well. In this way, Maria was able to make a more informed decision about her treatment options.

This example illustrates one use of molecular diagnostics—to help patients select treatments for cancer. Although a modest number of molecular diagnostics are currently available, researchers are working to develop more such tests for different cancer types. Molecular diagnostics are a fundamental component of precision medicine, the approach in which each person receives the best treatment for his or her particular health condition based on an analysis of DNA, RNA, protein, or related molecules. In this chapter, we discuss the many different uses of molecular diagnostics and their contribution to precision medicine.
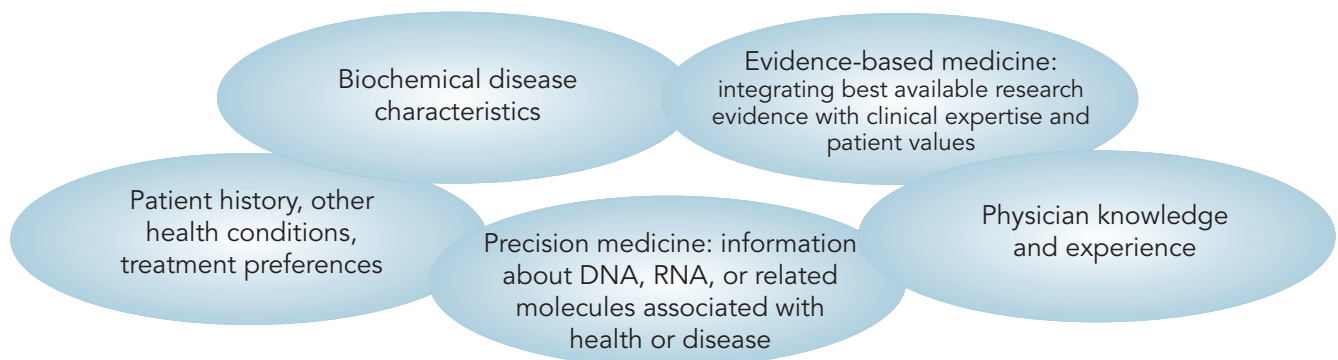
Precision Medicine and Molecular Diagnostics

Ideally, treatments for cancer would be 100% effective and safe. Each person would receive a treatment only if needed, and the treatment would be specifically suited to his or her cancer. In the ideal case, the treatment wouldn't affect any other tissues in the body, which would reduce the possible side effects. In other words, the treatment would be precise. Although there is no perfect treatment available today, molecular diagnostics (combined with targeted therapies) are moving us closer to this ideal.

Molecular diagnostics are part of the so-called "personalized" or "precision" medicine revolution in healthcare. Doctors have been trying to deliver personalized medicine for centuries, but the ability to do so is becoming more precise with improved diagnostic and therapeutic tools. The term "precision medicine" is now being used to refer to a relatively new and evolving field that provides important information that can be used to select the best treatment for each patient. The information in precision medicine comes from DNA, RNA, proteins, or related molecules that we will learn about in the next chapter. As tests that detect these molecules, molecular diagnostics are a fundamental component of precision medicine.

Personalized Medicine
Healthcare professionals use information from a variety of sources to provide care tailored to each individual patient

Biochemical disease characteristics

Evidence-based medicine: integrating best available research evidence with clinical expertise and patient values

Patient history, other health conditions, treatment preferences

Precision medicine: information about DNA, RNA, or related molecules associated with health or disease

Physician knowledge and experience

Molecular diagnostics are tests that detect genetic material, proteins, or related molecules that provide information about health or disease. These tests are most commonly run on samples of blood, saliva, or tumor tissue. Depending on the type of test, a molecular diagnostic may also be referred to as a gene panel, a gene signature panel, a gene signature test, or a gene expression panel, but molecular diagnostics is the broader term encompassing many different types of tests that examine DNA, RNA, and proteins.

EXAMPLES OF MOLECULAR DIAGNOSTICS IN CANCER*

Test	Associated Health Condition(s)
Human papilloma virus (HPV)	Cervical cancer
BRCA1 and BRCA2 genes	Breast, ovarian and cervical cancers
CA125 protein	Ovarian cancer
Prostate Specific Antigen (PSA)	Prostate cancer
HER2/neu gene	Breast cancer
DNA mismatch repair genes (MSH2, MLH1, MSH6, PSM2)	Lynch syndrome (colon cancer)
KIT gene	Acute myelogenous leukemia and GIST tumors of the gastrointestinal tract
EGFR, ALK, and KRAS genes	Lung cancer

*Molecular diagnostics are most commonly conducted on samples of blood, saliva, or tumor tissue.

How Are Molecular Diagnostics Used?

Risk Assessment

Molecular diagnostics can be used to determine whether a person is at risk for a certain type of cancer. When used this way, the tests may also be referred to as molecular profiling or molecular risk assessment. These tests help a person determine how likely he or she is to develop cancer. They can also be used to help decide whether a person should undergo more intensive screening or take preventive measures.

An example of a molecular diagnostic test used for risk assessment is the blood test for the BRCA1 and BRCA2 genes. Alterations in either of these genes can increase the risk of breast, ovarian, and several other cancers. Many of us have heard the story of Angelina Jolie, the actress, filmmaker, and special envoy of the United Nations High Commissioner for Refugees. Angelina Jolie wrote several editorials for the *New York Times* explaining that she has a mutation in the BRCA1 gene. Her mother, grandmother, and aunt all died of cancer, and Ms. Jolie, chose to have her breasts and ovaries removed to prevent cancer of these organs. She stresses that the actions she took may not be right for everyone but that they were right for her.

Ms. Jolie's public acknowledgment of her BRCA1 mutation and her choice of preventive surgery helped raise awareness of the BRCA mutations and their potential to cause cancer. However, it is important to note that not everyone with a mutation in the BRCA1 or BRCA2 gene will get cancer. Moreover, many people diagnosed with breast cancer do not have either of these mutations and preventive surgery may not be the best choice for these individuals. Alternatives to preventive surgery are available, and each individual should discuss the options with her family and physician prior to making a decision.
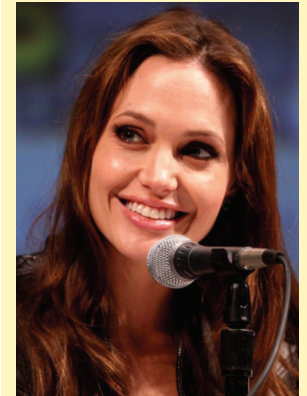
Angelina Jolie has been public about her BRCA1 mutation.

Photo credit: "Angelina Jolie by Gage Skidmore 2" by Gage Skidmore. Licensed under CC BY-SA 3.0 via Wikimedia Commons.

Differential Diagnosis

Molecular diagnostics can also be used to help diagnose cancers. They can help differentiate cancer from benign tumors (i.e., growths that are not harmful) and can further help identify the type of tissue in which the cancer originated (e.g., breast, lung, skin, etc.). Molecular diagnostics can even help classify different cancer subtypes that affect the same tissue. These analyses may help estimate the aggressiveness of the cancer. For example, there are many different types of blood cancers. Molecular diagnostics are widely used to determine blood cancer subtypes. One blood cancer, known as acute myelogenous leukemia (AML), is classified into poor, intermediate, or favorable risk categories based on an evaluation of chromosomes. Patients whose disease is classified as intermediate risk then undergo molecular testing for several different mutations. Still other AML subtypes such as acute promyelocitic leukemia are detected by other molecular diagnostics. The cancer's molecular subtype may have implications for treatment.

Prognosis

Prognosis refers to the natural course of disease in the absence of treatment or a predicted outcome of the results from medical treatment. Some cancers are naturally more aggressive than others and knowing this may help patients and physicians determine which treatment to select. An example is the gene known as FLT3. Alterations in this gene indicate an aggressive cancer in people diagnosed with AML. Notably, over the past several years, scientists have developed medications designed to inhibit FLT3 and some of these are undergoing clinical studies. If these medications prove useful, the presence of FLT3 alterations, as detected by a molecular diagnostic, may be useful in predicting treatment response. This example shows how a single molecular diagnostic may have more than one use.

Molecular diagnostics can also be used to evaluate the likelihood that an existing cancer will recur after treatment—another aspect of prognosis. Several molecular diagnostics are available to predict the likelihood of breast cancer recurrence in women with early-stage, node-negative, estrogen receptor-positive, invasive breast cancer who will be treated with hormone therapy. These tests examine multiple genes in cells obtained from a sample of the breast tumor.

Prediction of Treatment Response

As just described, molecular diagnostics can also help predict whether patients will respond to cancer treatments. An example is the test for HER2/neu gene overexpression in a person's breast cancer tumor tissue. The HER2/neu gene is a piece of DNA that directs cells to make a protein known as human epidermal growth factor receptor 2 (HER2). Approximately one-fourth of all breast cancers have too many copies of this gene, which cause too much of the protein to be produced. The extra protein makes cells grow and divide rapidly. A drug known as trastuzumab inhibits the activity of the HER2 protein and may be used to treat breast cancers with overexpression of HER2/neu. Testing for HER2/neu is another example of a molecular diagnostic with more than one clinical use because the results of the test can also help determine how aggressive the cancer is (i.e., prognosis).

Pharmacokinetics

When you swallow a pill, the medication must be absorbed and distributed throughout the body so that it can reach its intended site of action—in our case, the cancerous tissue. After some period of time, the body breaks down and eliminates the medication, leading to a need for more medication. The process of absorption, distribution, metabolism (break down), and elimination of drugs is called pharmacokinetics. The rate at which these processes occur depends on a variety of factors, including genetics. Due to genetic differences, some people metabolize drugs faster than others, which has major implications for certain cancer therapies. One example of this is a medication known as irinotecan that is used for the treatment of colon cancer. Individuals with a genetic pattern designated UGT1A1*28 metabolize irinotecan more slowly than those without this pattern. In order to prevent the medication from accumulating in the body, these individuals must be given a lower dose than normal.

> **Pharmacokinetics** is the process of drug absorption, distribution, metabolism (break down), and elimination by the body.
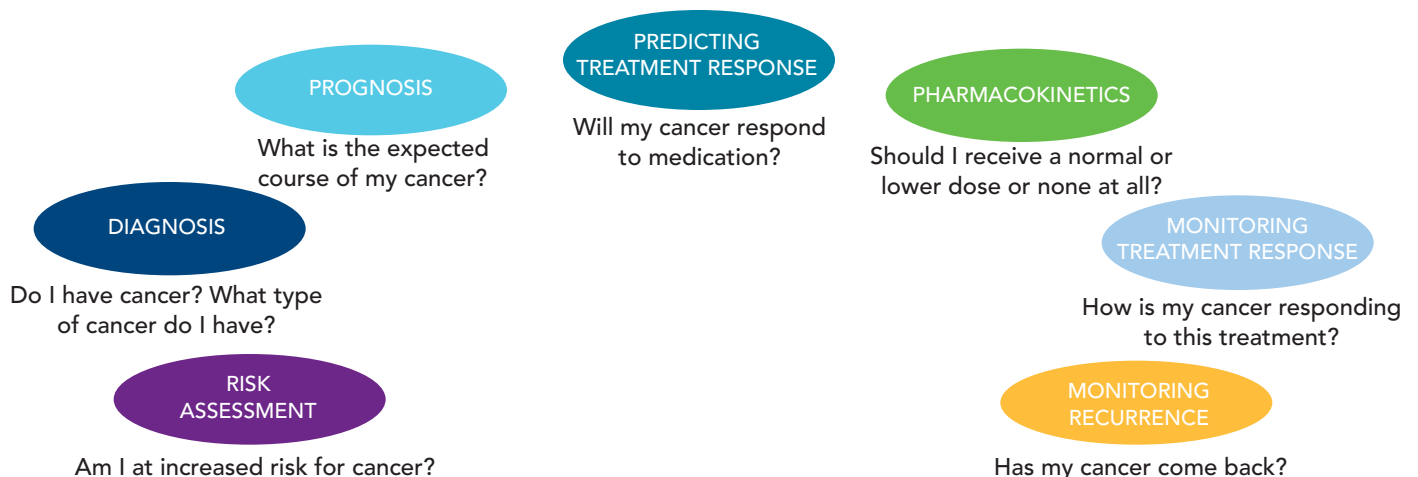
Monitoring Treatment Response

Naturally, patients and physicians want to know as soon as possible whether a treatment is working. Molecular diagnostics that can be repeated multiple times over the course of treatment, such as simple blood tests, have been developed for colon and several other cancers to examine treatment response. Some cancers can develop resistance to medications, as in the case of chronic myelogenous leukemia, a type of blood cancer. This cancer is often treated with a medication known as imatinib, which inhibits a protein made by an abnormal combination of genes. People with chronic myelogenous leukemia may need to take imatinib for years, and in some people, the sequence of genetic material can change over time. This change may lead to reduced medication effectiveness. Consequently, patients who no longer respond to imatinib may undergo molecular diagnostic testing to determine whether the gene has changed.

Monitoring Recurrence in Patients Without Symptoms of Cancer

Patients whose cancer has been successfully treated are typically monitored at regular intervals for signs of recurrence. For some cancers, molecular diagnostics can aid in determining whether a cancer has recurred. For breast, prostate, and ovarian cancers, the use of molecular diagnostics to monitor recurrence in patients without symptoms of cancer is controversial. In the future, scientists are likely to develop better molecular diagnostics that can more accurately monitor recurrence in patients whose disease has been successfully treated.

CLINICAL USES OF MOLECULAR DIAGNOSTICS

PROGNOSIS
What is the expected course of my cancer?

PREDICTING TREATMENT RESPONSE
Will my cancer respond to medication?

PHARMACOKINETICS
Should I receive a normal or lower dose or none at all?

DIAGNOSIS
Do I have cancer? What type of cancer do I have?

MONITORING TREATMENT RESPONSE
How is my cancer responding to this treatment?

RISK ASSESSMENT
Am I at increased risk for cancer?

MONITORING RECURRENCE
Has my cancer come back?

Molecular diagnostics and precision medicine are changing the face of medicine, although we are still at an early stage in the game. Undoubtedly, molecular diagnostics will play an ever increasing role in cancer management for the foreseeable future.

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Chapter 2: Genes, Proteins, and Chromosomes: The "Molecular" in Molecular Diagnostics

It was the early 1950s and researchers were working assiduously throughout the world to discover the structure of DNA. Among these was the chemist Dr. Rosalind Franklin, who was refining her X-ray techniques to produce distinct images of DNA. However, Dr. Franklin was uncomfortable in the British laboratory, where women were not even allowed in the cafeteria. She left for Paris, leaving behind an image she called Photo 51, which showed two strands of DNA apparently forming a helix. Dr. Franklin's colleague Maurice Wilkins obtained the photo and showed it to his fellow scientists James Watson and Francis Crick. Watson and Crick had also been studying DNA and were on the verge of determining its structure. They instantly recognized that the photo depicted the missing piece of evidence in their DNA model, and the rest is history.

Discovery of the structure of DNA was one of the most valuable scientific advances of the century. Knowledge of this structure has led to monumental advances in our understanding of cancer and forms the basis of molecular diagnostics. In this chapter, we discuss DNA and other molecules detected by molecular diagnostics.

Chromosomes

Chromosomes are the cell structures that contain DNA—our genetic information. Chromosomes are located in a part of the cell known as the nucleus. Humans have 23 pairs of chromosomes, for a total of 46 overall. These include 22 pairs of chromosomes called autosomes that are numbered 1 through 22 and look the same in males and females. Chromosome number 23 is the sex chromosome, which is different in males and females: females have two X chromosomes, whereas males have one X and one Y chromosome.

Chromosomes are the structures inside cells that contain the genetic information.

HUMAN CHROMOSOMES
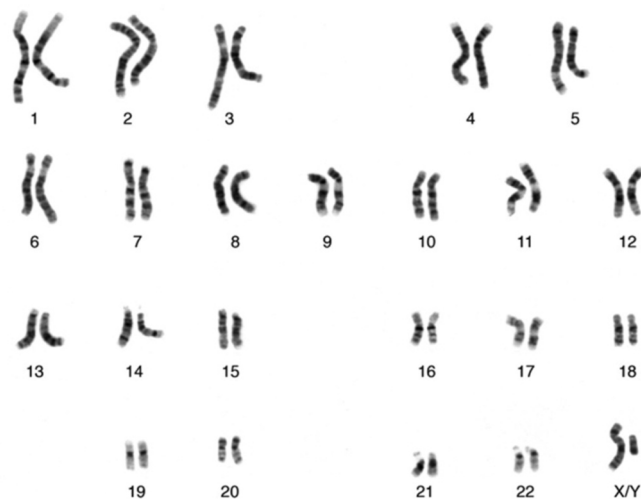
Humans have 22 pairs of chromosomes. The 23rd set determines a person's sex: females have two paired X chromosomes and males have an X and a Y. This set of chromosomes is from a male because the 23rd set of chromosomes are XY.

Image credit: Darryl Leja, National Human Genome Research Institute, www.genome.gov.

DNA

DNA, short for deoxyribonucleic acid, is the molecule in chromosomes that actually encodes the genetic information. DNA is made up of two strands of chemical building blocks called nucleotide bases that link precisely with one another in pairs. When these nucleotide bases pair with one another, they form a twisting double helix pattern that has been compared to a twisted ladder. The individual nucleotide bases are known as adenine, thymine, guanine, and cytosine, which are usually abbreviated as capital letters: A, T, G, and C. Importantly, A only pairs with T and G only pairs with C. When DNA strands bind because they contain nucleotide base pairs throughout their entire length, they are known as complementary strands. For example, the nucleotide base sequence of GGG is complementary to the nucleotide base sequence of CCC.

> **DNA,** or deoxyribonucleic acid, is the molecule in chromosomes that contains the genetic information that is passed on from one generation to the next.
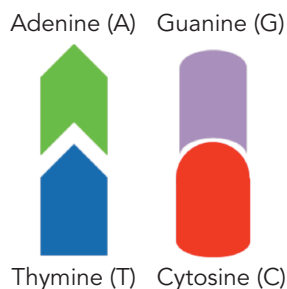
STRUCTURE OF DNA
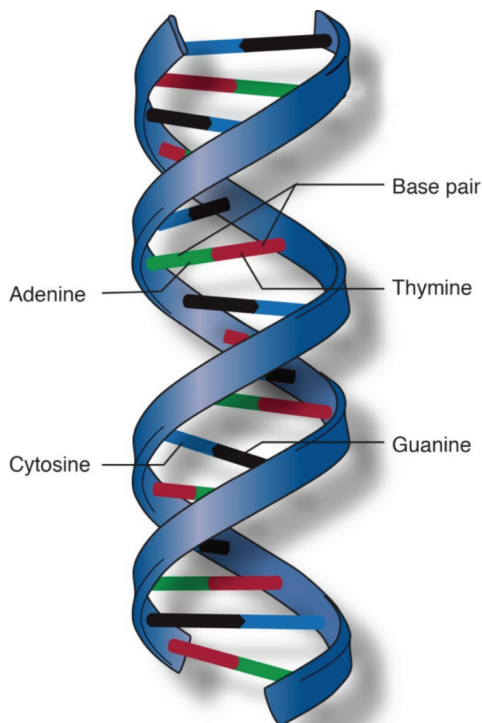
This graphic shows the double helix, twisted ladder structure of DNA and the individual nucleotide bases that pair to form the rungs of the ladder (adenine, thymine, guanine, and cytosine).

Image credit: Darryl Leja, NHGRI, www.genome.gov.

NUCLEOTIDE BASE PAIRING IN DNA

Each nucleotide base in DNA pairs with only one other base: A (adenine) pairs with T (thymine) and G (guanine) pairs with C (cytosine).

> Two DNA strands that can bind because they contain nucleotide base pairs throughout their entire length are said to be complementary. For example, the nucleotide base sequence of AAAGA is **complementary** to the nucleotide base sequence of TTTCT.

Genes

Genes are stretches of DNA that tell the body to make a specific protein. Genes are passed on from one generation to the next and contain all of the information needed for life. Amazingly, the four nucleotide bases in DNA are arranged in different sequences and lengths to make up all of our different genes. Variations in the sequence of chemical building blocks of DNA in a gene explain why one person has curly hair just like her mother and another person is tall like his father.

Genes are pieces of DNA that contain the information for making a specific protein.

CHROMOSOME, DNA, AND GENE
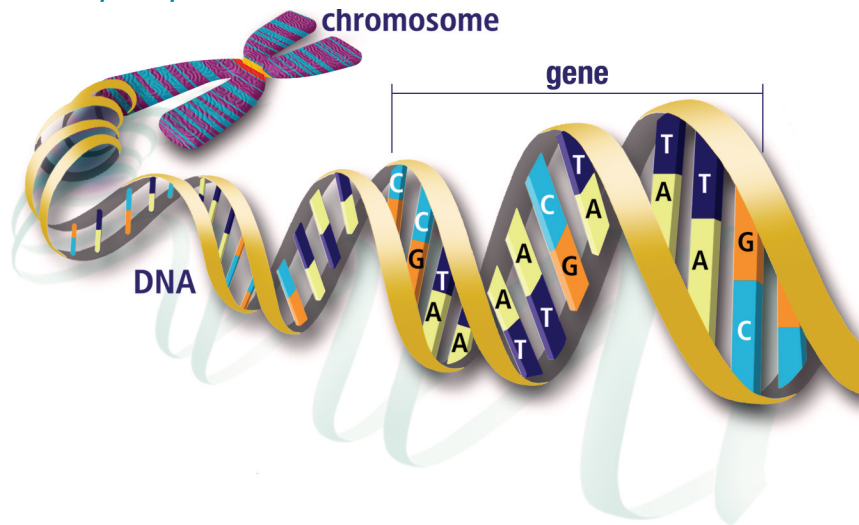
This graphic shows the relationship among chromosomes, DNA, and genes. Chromosomes are made up of DNA, and certain stretches of DNA make up genes.

Image credit: Office of Biological and Environmental Research of the U.S. Department of Energy Office of Science, www.science.energy.gov/ber/.

RNA

One of the most interesting features of DNA is that its double strands can be unzipped and copied. RNA or ribonucleic acid is a molecule that helps in this process. DNA is copied to make proteins, the workhorses of cells that carry out the instructions encoded in the DNA. RNA or ribonucleic acid plays essential roles in copying DNA so that its sequence of nucleotide bases can be translated into proteins.

The structure of RNA is very similar to DNA—it also contains a series of four nucleotide bases—except that one of these bases is different from DNA: RNA uses a nucleotide base called uracil (U) instead of the thymine (T) in DNA. We really do not know why this substitution takes place, but the A in DNA pairs exclusively with the U in RNA, just as it does with the T in DNA.

RNA or ribonucleic acid is a molecule inside cells that helps make proteins from DNA. Three different types of RNA help in this process: messenger RNA, transfer RNA, and ribosomal RNA.

Proteins

As we have seen, the DNA in genes consists of a series of nucleotide base pairs arranged in a specific order. Each set of three nucleotide bases encodes a biochemical known as an amino acid. As described in the next section, these amino acids are strung together to make proteins.

We obtain amino acids from the protein in foods we eat. Our bodies break down the protein in fish, meat, beans, seeds, nuts, and other foods into individual amino acids that the body can then use to build proteins that it needs to survive and function. The following table lists some general types of proteins in our bodies.

Proteins are biochemicals made up of individual amino acids. The body uses proteins to make up structures such as hair and nails and carry out the functions of life.

EXAMPLES OF PROTEINS IN THE BODY

Protein Type	Description
Antibodies	Bind to specific foreign particles to protect the body
Enzymes	Carry out nearly all chemical reactions within cells 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

DNA ENCODES INFORMATION NEEDED TO MAKE PROTEINS
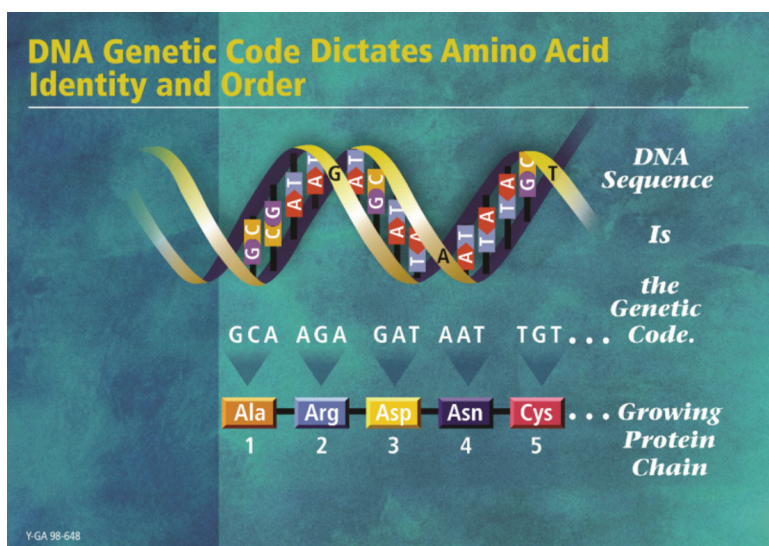
The sequence of base pairs in DNA is copied by RNA, which spells out the 3-letter code for an amino acid (shown the genetic code in the graphic). The amino acids are shown as colored blocks in the graphic, with their abbreviated names: Ala for alanine, Arg for arginine, Asp for aspartic acid, Asn for asparagine, and Cys for cysteine. There are many more amino acids that are not shown in this graphic. The amino acids link together to form a growing protein chain, which eventually becomes a full-fledged protein capable of carrying out its specific function in the cell.

Image credit: Office of Biological and Environmental Research of the U.S. Department of Energy Office of Science, www.science.energy.gov/ber/.

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

The unique double-helix structure of DNA allows it to unwind or unzip 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 RNA, which may then be translated into a protein.

Gene expression can be stimulated by chemical and physical processes that originate both inside and outside the cells. For example, when you cut your finger, signals are sent to cells in the skin that initiate the repair process, which includes turning on genes to make proteins. Internal chemicals such as hormones can also stimulate gene expression.

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.

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 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 RNA. It can be likened to putting your hand into a substance such as wet concrete that hardens into a mold.

> **Gene expression** is the process by which a gene gets turned on in a cell to make RNA (ribonucleic acid) and proteins.

TRANSCRIPTION
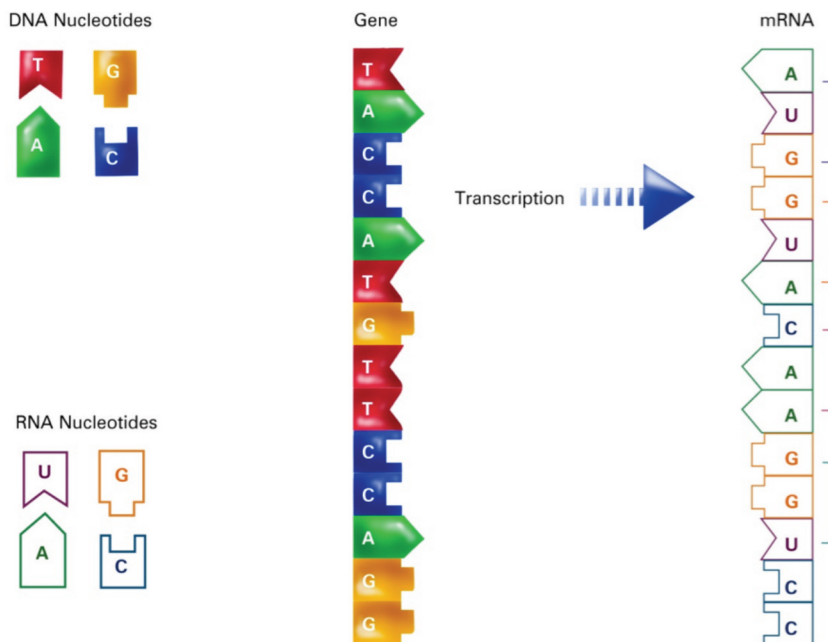
This graphic shows the basic process of transcription. The DNA molecule unzips and the gene on one strand is copied to mRNA. Copying occurs by generating a strand of mRNA whose nucleotide bases pair with those of the DNA. The only exception is that RNA uses a nucleotide base called uracil instead of 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 (TACCAC . . .). 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.

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

Step Two: Translation

During the second step of gene expression, known as translation, the information that is contained in the mRNA is translated into amino acids by a structure within the cytoplasm called a ribosome. The ribosome reads the sequence of nucleotide bases, with three nucleotide bases coding for a particular amino acid. This sequence of three nucleotide bases 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
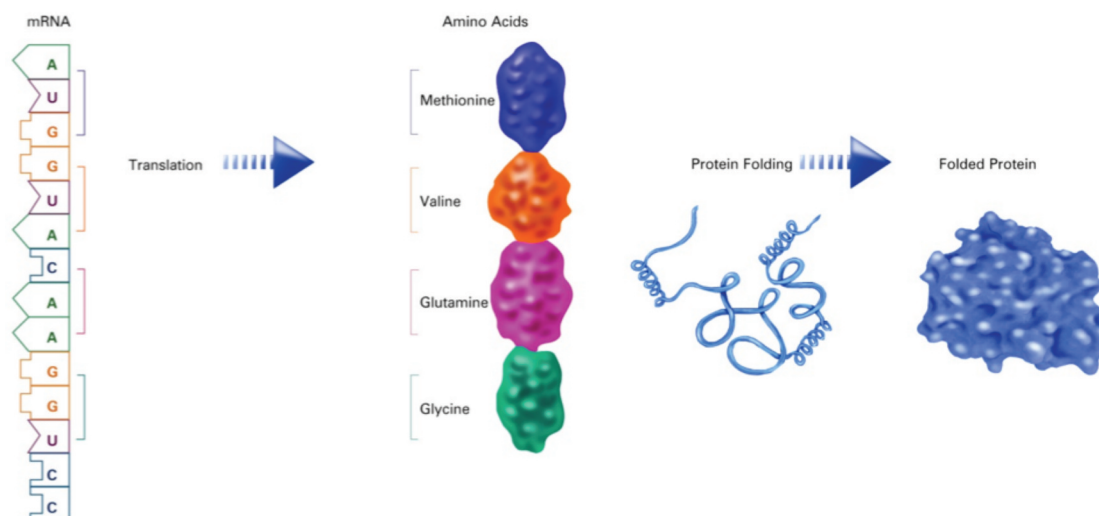
This graphic shows the basic process of translation. The mRNA strand shown on the left moves out of the cell nucleus onto a ribosome. Here each set of three nucleotide bases is translated into a single amino acid as shown in the center. The spelling of the nucleotide bases tells the cell which amino 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.

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

Many excellent sources are available for more information on how cells copy DNA and express genes. A few of these sources are listed below.
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Chapter 3: Molecular Biomarkers in Cancer

It was 1960 and Dr. Peter Newell and his graduate student David Hungerford were working at the University of Pennsylvania School of Medicine in Philadelphia trying to uncover abnormalities in the chromosomes of patients with blood cancers. Looking under a microscope, the investigators noticed an unusual, small chromosome in the white blood cells from two patients. Eventually it was recognized that this so-called "Philadelphia chromosome" was present in the cancer cells of nearly all patients with chronic myelogenous leukemia and that it was the cause of the disease.

Discovery of the Philadelphia chromosome is one of the greatest success stories in cancer because it led to a deep understanding of the basic, molecular cause of chronic myelogenous leukemia. This understanding, in turn, led to the development of a medication known as Gleevec® (imatinib) that targets the protein produced by the abnormal chromosome and is highly effective in treating the cancer. The Philadelphia chromosome is an example of a molecular biomarker—the "stuff" for which molecular diagnostics test. In this chapter we define and discuss the different types of molecular biomarkers.

> **Molecular biomarkers** are alterations in chromosomes, DNA, RNA, proteins, and related molecules that indicate a particular biological condition such as cancer.

What is a Molecular Biomarker?

Certain alterations in chromosomes, DNA, RNA, proteins, and related molecules have the potential to cause cancer or other diseases. When such alterations are linked with a particular state of disease or health, they are known as molecular biomarkers.

Biomarkers are associated with many different states of health and disease—not just cancer. For example, a high level of the protein called human chorionic gonadotropin in the urine is a biomarker for pregnancy. Additionally, biomarkers are not always molecular. Certain patterns of brain activity may be biomarkers for neurologic disease and high blood pressure can be a biomarker for heart disease and stroke. More information about biomarkers can be found in Research Advocacy Network's publication *Biomarkers in Cancer*, available at: http://researchadvocacy.org/general-resources]

Molecular Alterations Can Be Inherited or Acquired

Molecular alterations can be inherited (i.e., passed on from one generation to the next) or acquired during the course of life. Whether or not a genetic alteration can be passed on from one generation to the next depends on the type of cell in which it occurs. Genetic alterations can occur in two types of cells: germ cells and somatic cells. Germ cells are the reproductive cells: in humans, eggs are the female reproductive cells and sperm are the male reproductive cells. Somatic cells are the non-reproductive cells, or all other cell types that aren't eggs or sperm cells.

> **Germ cells** are reproductive cells; in humans, eggs are the female reproductive cells and sperm cells are the male reproductive cells.

> **Somatic cells** are the non-reproductive cells; in humans, all cells that are not eggs or sperm are somatic cells.

Germ cells: a sperm cell fertilizing an egg

Alterations in DNA are often referred to as mutations. The word *mutation* is usually but not always used to refer to DNA changes that, under certain circumstances, can have negative health consequences. However, sometimes DNA changes that are "good" for the organism are called mutations, as in the case of DNA changes that allow living organisms or plants to better adapt to their environment. Thus, there is ambiguity in the meaning of the word mutation.

> **Mutations** are alterations in DNA. The word mutation is usually but not always used to refer to DNA changes that, under certain circumstances, can have negative health consequences.

Somatic mutations can be caused by exposure to the environment.

Mutations that occur in germ cells are called hereditary mutations or germline mutations. Given that embryos can only be formed by eggs and sperm, only mutations in these cells can be passed on to the next generation. Germline mutations confer increased susceptibility to a disease or a change in how the body metabolizes a drug. In contrast, mutations that occur in somatic cells are called somatic or sporadic mutations. Somatic mutations can be caused by exposure to the environment (e.g., sun, radiation, asbestos, or other carcinogens) or spontaneous events within cells. These spontaneous mutations may occur as cells are carrying out their normal functions, such as synthesizing new cells and repairing damage to DNA. Somatic mutations occur in normal tissue that then develops into a cancer.

> **Hereditary or germline mutations** are mutations that occur in germ cells (eggs and sperm).

Germline and somatic mutations can have different health consequences, even if they occur in the same gene. For example, a gene known as P53 has been associated with cancer. Germline mutations in the P53 can cause a disease known as Li-Fraumeni syndrome, which is characterized by an increased risk of different cancer types in various tissues of the body such as breast, bone, and brain. In contrast, somatic mutations in the P53 gene occur in a single tissue such as the skin.

> **Somatic or sporadic mutations** are mutations that occur in somatic cells (non-reproductive cells).]

## Chromosomal Alterations as Biomarkers in Cancer

Specific alterations in chromosomes can be biomarkers for cancer. Usually, the chromosomal alteration is not only a biomarker, but also the underlying cause of the cancer.

Philadelphia chromosome

As discussed in the introduction to this chapter, the Philadelphia chromosome is one of the most well-known chromosomal alterations in cancer. The Philadelphia chromosome occurs when genetic material from chromosome 9 swaps places with genetic material on chromosome 22—a process known as translocation. The combined genetic on chromosome 22 creates a cancer-causing gene known as BCR-ABL. This gene contains the instructions to make an abnormal protein that that causes leukemia. The abnormal protein is similar to our normal proteins except that it cannot shut itself off.

> **The BCR-ABL gene** is formed when pieces of chromosomes 9 and 22 break off and trade places. The ABL gene from chromosome 9 joins to the BCR gene on chromosome 22, to form the BCR-ABL fusion gene. The changed chromosome 22 with the fusion gene on it is called the **Philadelphia chromosome.** The BCR-ABL fusion gene is found in most patients with chronic myelogenous leukemia (CML) and in some patients with acute lymphoblastic leukemia (ALL) or acute myelogenous leukemia (AML).

THE PHILADELPHIA CHROMOSOME AND BCR-ABL GENE
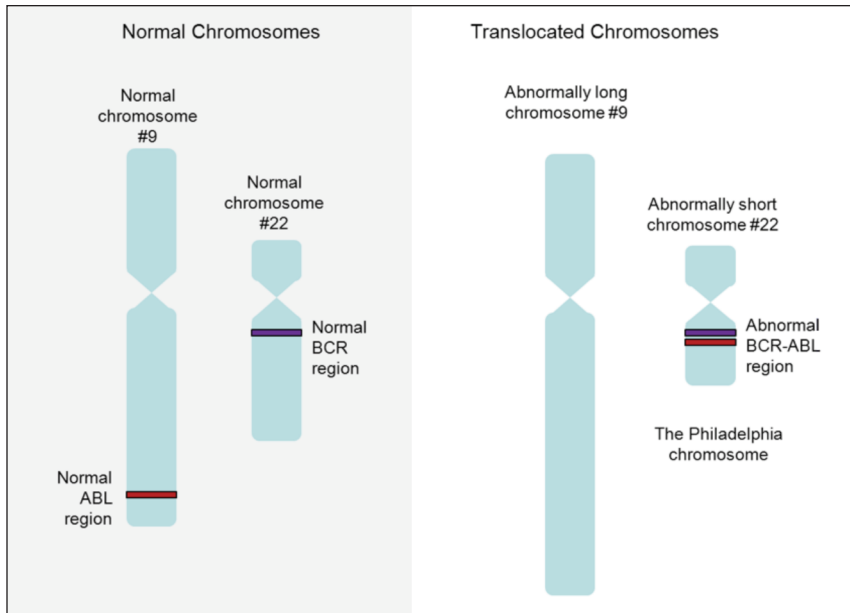
Graphic representation of chromosomes 9 and 22 in their normal, non-cancerous state (left) and after the cancer-causing translocation of chromosomes 9 and 22 seen in chronic myelogenous leukemia (right). This translocation causes an abnormally long chromosome #9 and an abnormally short chromosome #22, known as the Philadelphia chromosome. The Philadelphia chromosome contains the aberrant BCR-ABL region that provides the instructions for a cancer-causing protein.

This BCR-ABL gene provides the instructions to make an abnormal protein that causes leukemia. This abnormal protein is similar to our normal proteins except that it cannot shut itself off.

Molecular diagnostics that test for the presence of the Philadelphia chromosome can be done in several ways. One of the most common methods is to determine whether the RNA sequence for the fusion protein is present in a person's blood; we will learn more about the methods of molecular diagnostics in the next chapter. The presence of this RNA sequence indicates that the body is actively making the fusion protein and allows us to infer the presence of the Philadelphia chromosome. The drug imatinib (Gleevec®) effectively inhibits the overactive protein and decreases progression of the cancer, leading to a complete response in 98% of patients with chronic myelogenous leukemia.

Imatinib is an example of a targeted therapy. Targeted cancer therapies are medications or other substances that treat cancer by interfering with molecules that are specifically involved in cancer cell growth, spread, and progression. These drugs act on cancer cells to prevent them from replicating or proliferating.

> A **targeted therapy** is a medication or other substance that treats cancer by interfering with molecules that are specifically involved in cancer cell growth, spread, and progression.

Other chromosomal abnormalities

Chromosomal translocation, like the one that produces the Philadelphia chromosome, is common in many other types of cancer. Most often, this translocation results in fusion proteins that cannot shut themselves off—in fact, more than 200 such proteins have been identified. Another example of this is a gene known as anaplastic lymphoma kinase (ALK). The ALK gene normally regulates cell growth. However, when the ALK gene fuses with any one of several other genes, it overproduces a protein that removes the brakes from cell growth. This uncontrolled cell growth plays an essential role in some cancers; tumor tissue from about 3-5% of people with non-small-cell lung cancer has this gene abnormality. A targeted therapy known as crizotinib has been developed to inhibit the excessive protein produced by ALK overactivity and may be useful for people with non-small cell lung cancer whose tumor is positive for the ALK fusion gene.

Another chromosomal abnormality that sometimes occurs in cancer is chromosome inversion, or the end to end switching of a portion of DNA within the same chromosome. A third type of chromosomal abnormality is known as copy number variation. This includes deletions (loss of part of a chromosome) and amplifications (part of the chromosome is duplicated). Chromosomal duplication is sometimes also called gene duplication or gene amplification. In this situation, a piece of DNA is abnormally copied one or more times. Duplication in parts of a gene may cause too many copies of the protein to be made, which can then cause overactivity. This is the case for certain breast cancers that are termed "HER positive." HER2 is a protein known as human epidermal growth factor receptor 2. This protein normally helps cells grow. When cancers have too many copies of this gene, it causes them to grow rapidly and invade other tissues. Molecular diagnostic tests are available to test for this mutation. The discovery that HER2 was associated with more rapidly growing breast cancers led to the development of the drug trastuzumab (Herceptin®), which inactivates the HER2 protein. Another drug called lapatinib (Tykerb®) blocks the function of HER2 and other selected proteins.

EXAMPLES OF SOME CHROMOSOMAL ABNORMALITIES ASSOCIATED WITH CANCERS
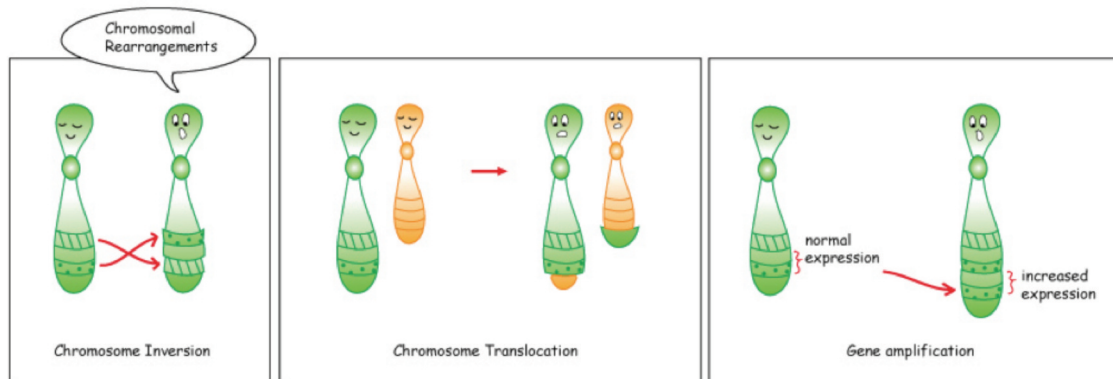
Copyright 2015 by Mina Kang, available under the Creative Commons Attribution-ShareAlike 4.0 International license (CC BY-SA 4.0)

Many of the chromosomal abnormalities in cancer occur in regions containing genes known as proto-oncogenes or tumor suppressors. Proto-oncogenes are genes that normally help cells grow. These genes are needed for development and day-to-day housekeeping and repair. When some mutations develop in proto-oncogenes, they can become oncogenes, or genes that stimulate cells to grow out of control, leading to cancer. In contrast, tumor suppressors are normal genes that slow down cell division, repair DNA mistakes, and initiate cell death when it is needed to prevent cancer. Mutations in tumor suppressor genes can interrupt their function, removing the brakes that normally prevent cancer.

Potential Effects of Alterations in DNA
Alterations in DNA can have one of three consequences:
- No effect/undetectable effect: the alterations in DNA do not affect the structure or function of the resultant protein.
- Activation: the alterations in DNA cause the resultant protein to gain an uncontrolled function.
- Inactivation: the alterations in DNA interfere with the resultant protein causing it to lose its function, which leads to loss of suppression of other genes and their uncontrolled activation.

EXAMPLES OF TUMOR SUPPRESSOR GENES

Gene	Associated Health Condition(s)
P53	Li-Fraumeni syndrome (increased risk for multiple cancer types)
BRCA1 and BRCA2 genes	Breast, ovarian and cervical cancers
CDKi2A (cyclin dependent kinase inhibitor 2A)	Brain cancers, non-small-cell lung cancers, leukemias, melanomas

LOCATION OF TUMOR SUPPRESSOR GENE ON CHROMOSOME 9

Approximate location of tumor suppressor gene known as cyclin-dependent kinase inhibitor 2A
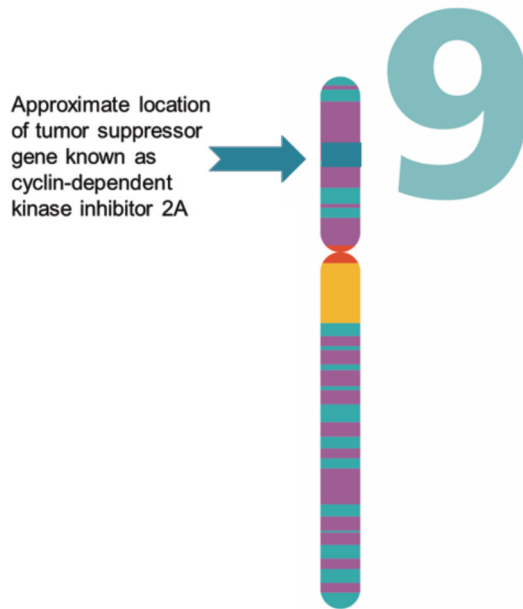
An **oncogene** is a mutated form of a proto-oncogene—a gene involved in normal cell growth. Oncogenes may cause the growth of cancer cells.

A **tumor suppressor gene** is a type of gene that helps control cell growth. Mutations in tumor suppressor genes may lead to cancer.

Chromosome image credit: Genome Management Information System, Oak Ridge National Laboratory

Gene Alterations as Biomarkers in Cancer

Small alterations in certain parts of our DNA can also serve as biomarkers. No two humans have exactly the same DNA sequences in all of their chromosomes and genes unless they are identical twins. In other words, we all exhibit genetic variations. Some of these variations are inherited and others are acquired over the course of our lives.

Several different types of genetic variation can occur. Single nucleotide polymorphisms (SNPs; pronounced "snips") are differences in only one nucleotide base pair (the A, C, G, or T discussed in Chapter 2) in the DNA sequence that occurs in at least 1% of the population compared to everyone else. SNPs account for 90% of all variation in human DNA. SNPs are not exclusively good or bad—some may be beneficial and others harmful, and still others may have no detectable effect at all.

SNPs are variations in one nucleotide base pair of DNA that affect 1% or more of the population.

SINGLE NUCLEOTIDE POLYMORPHISMS (SNPS)

Individual 1

Chr 2 copy1 ...CGATATTCC**T**ATCGAATGTC...
...GCTATAAGG**A**TAGCTTACAG...

Chr 2 copy2 ...CGATATTCC**C**ATCGAATGTC...
...GCTATAAGG**G**TAGCTTACAG...

Individual 2

Chr 2 copy1 ...CGATATTCC**C**ATCGAATGTC...
...GCTATAAGG**G**TAGCTTACAG...

Chr 2 copy2 ...CGATATTCC**C**ATCGAATGTC...
...GCTATAAGG**G**TAGCTTACAG...

Individual 3

Chr 2 copy1 ...CGATATTCC**T**ATCGAATGTC...
...GCTATAAGG**A**TAGCTTACAG...

Chr 2 copy2 ...CGATATTCC**T**ATCGAATGTC...
...GCTATAAGG**A**TAGCTTACAG...

Individual 4

Chr 2 copy1 ...CGATATTCC**T**ATCGAATGTC...
...GCTATAAGG**A**TAGCTTACAG...

Chr 2 copy2 ...CGATATTCC**C**ATCGAATGTC...
...GCTATAAGG**G**TAGCTTACAG...

Individual 5

Chr 2 copy1 ...CGATATTCC**C**ATCGAATGTC...
...GCTATAAGG**G**TAGCTTACAG...

Chr 2 copy2 ...CGATATTCC**T**ATCGAATGTC...
...GCTATAAGG**A**TAGCTTACAG...

Individual 6

Chr 2 copy1 ...CGATATTCC**C**ATCGAATGTC...
...GCTATAAGG**G**TAGCTTACAG...

Chr 2 copy2 ...CGATATTCC**T**ATCGAATGTC...
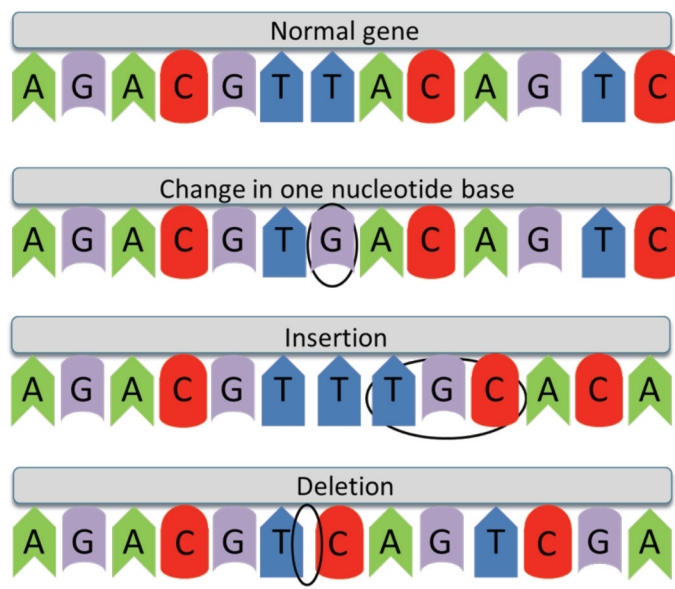...GCTATAAGG**A**TAGCTTACAG...

Image credit: Darryl Leja, National Human Genome Research Institute

SNPs that have potentially negative health consequences are often referred to as mutations, as defined earlier in this chapter. However, SNPs are only called mutations if they occur anew, either by being inherited (germline mutations) or induced in the cancerous tissue by events in the internal or external environments (somatic mutations).

Several different types of mutations can occur, as shown in the following graphic. Single nucleotide bases can be changed to different bases (called point mutations), new nucleotide bases can be inserted into the sequence (called insertions), and nucleotide bases can be deleted (called deletions).

EXAMPLES OF MUTATIONS THAT CAN OCCUR IN GENES

Normal gene
A G A C G T T A C A G T C

Change in one nucleotide base
A G A C G T G A C A G T C

Insertion
A G A C G T T T G C A C A

Deletion
A G A C G T C A G T C G A

Deletions and insertions in DNA are often referred to as *indels*. They are arbitrarily distinguished from deletions and amplifications that occur at the chromosomal level by the number of DNA bases that are involved. The current convention is to use the term indel to describe deletions or insertions that involve 1-50 nucleotides or so and to use the term copy number variation to refer to larger deletions and insertions that typically involve more than 100 nucleotides. Other types of gene mutations are possible, and these are listed on the Genetics Home Reference Page produced by the National Institutes of Health: http://ghr.nlm.nih.gov/handbook/mutationsanddisorders/possiblemutations.

EXAMPLES OF CHROMOSOMAL AND GENE-LEVEL BIOMARKERS ASSOCIATED WITH CANCER

Type of Biomarker	Explanation	Somatic or Germline
Chromosomes		
Translocation	Genetic material from one chromosome switches places with genetic material from another chromosome	Somatic
Inversion	A piece of genetic material switches end to end within the same chromosome	Somatic
Copy number variation (deletion or amplification)	A portion of DNA is lost (deletion) or copied two or more times (amplification); sometimes amplifications (also known as gene duplications) are described as mutations at the gene level	Somatic
Genes*		
Point mutations	A single nucleotide base is substituted for another	Somatic or Germline
Insertions or deletions (indels)	A small portion of DNA is inserted or deleted	Somatic or Germline

*A more complete list of gene mutations is available on the Genetics Home Reference Page produced by the National Institutes of Health: http://ghr.nlm.nih.gov/handbook/mutationsanddisorders/possiblemutations.

Proteins as Biomarkers in Cancer

Protein biomarkers are probably the most common type of biomarker evaluated by molecular diagnostics today. Tumors can shed certain proteins that enter the bloodstream. These proteins can then be evaluated by taking a blood sample and subjecting it to a molecular diagnostic test. Other proteins can be measured in tissue, such as levels of estrogen receptors and HER2 protein (the protein encoded by the HER2/neu gene) in breast tumors.

One example of a protein biomarker is carcinoeymbryonic antigen (CEA). Blood levels of this protein are not used for diagnosis, but rather to monitor the treatment of people already diagnosed with colon cancer, thyroid cancer and cancers of the rectum, lung, breast, liver, pancreas, stomach, and ovaries. In some cases, a CEA test is conducted prior to treatment and then re-taken several times over the course of treatment in order to monitor how well the treatment is working and determine whether the cancer has progressed or recurred. For certain cancers, CEA levels may also be used to determine the prognosis and staging (cancer size and spread away from its initial site). The CEA test is used along with other biomarkers and clinical tests, and for some cancers, is only useful in people whose cancer has spread from its original site (metastasized).

The following table lists some protein biomarkers that are used clinically today.

EXAMPLES OF SOME CANCER BIOMARKERS AND THEIR CLINICAL USES

Cancer Type	Cancer Biomarker	Specimen Type	Clinical Use(s)
Breast	Estrogen receptor (ER), progesterone receptor (PR), human epidermal growth factor receptor 2 (HER2)	Tissue	Predicting treatment response
Testicular	α-fetoprotein	Blood	Diagnosis, prognosis, monitoring treatment response, monitoring disease recurrence
Prostate	Prostate specific antigen	Blood	Monitoring treatment response, monitoring disease recurrence
Colorectal	Carcinoembryonic Antigen (CEA)	Blood	Prognosis, monitoring treatment response (advanced disease), monitoring disease recurrence
Ovarian	CA-125	Blood	Diagnosis, prognosis, monitoring treatment response, monitoring disease recurrence

Sturgeon CM, Duffy MJ, Stenman U-H, et al. National Academy of Clinical Biochemistry Laboratory Medicine Practice Guidelines for Use of Tumor Markers in Testicular, Prostate, Colorectal, Breast, and Ovarian Cancers. Clin Chem 2008;54:12.

More information about the specific clinical uses of different cancer biomarker is available on the internet in two publications from the National Academy of Clinical Biochemistry:
• Sturgeon CM, Duffy MJ, Stenman U-H, et al. National Academy of Clinical Biochemistry Laboratory Medicine Practice Guidelines for Use of Tumor Markers in Testicular, Prostate, Colorectal, Breast, and Ovarian Cancers. *Clin Chem* 2008;54:12. Available at: http://www.clinchem.org/content/54/12/e11.long. Accessed December 20, 2015.
• Sturgeon CM, Duffy MJ, Hofmann BR, et al. National Academy of Clinical Biochemistry Laboratory Medicine Practice Guidelines for use of tumor markers in liver, bladder, cervical, and gastric cancers. *Clin Chem.* 2010 Jun;56(6):e1-48. Available at: http://www.clinchem.org/content/56/6/e1.long. Accessed December 20, 2015.

Companion Diagnostics

The use of molecular diagnostics to ascertain whether patients have a certain biomarker that can then be targeted with a specific therapy is becoming increasingly common as we learn more about the biology of cancer. When used in this way, molecular diagnostics are often referred to as companion diagnostics because they go hand-in-hand with the targeted therapy.

> **Companion diagnostics** are tests that provide information that is essential for the safe and effective use of a corresponding therapeutic product.

Genomics

In 2003, researchers finished sequencing all of the nucleotide bases in human DNA—all 3 billion of them—in a massive effort known as the Human Genome Project. This project ushered in the era of genomics, which is the study of multiple genes working together to perform a specific function. In the past, researchers focused on single genes because they didn't have the capacity to study many genes working simultaneously. This type of study requires sophisticated computer technology and laboratory methods that have only become available in the past few decades. In the next chapter, we will discuss some of these methods.

> **Genomics** is the study of multiple genes working together.

> **The Human Genome Project** was a large-scale scientific effort designed to determine the sequence of the human genome. More information about this project can be found at www.genome.gov/10001772

> **Genome** refers to all of an organism's DNA.
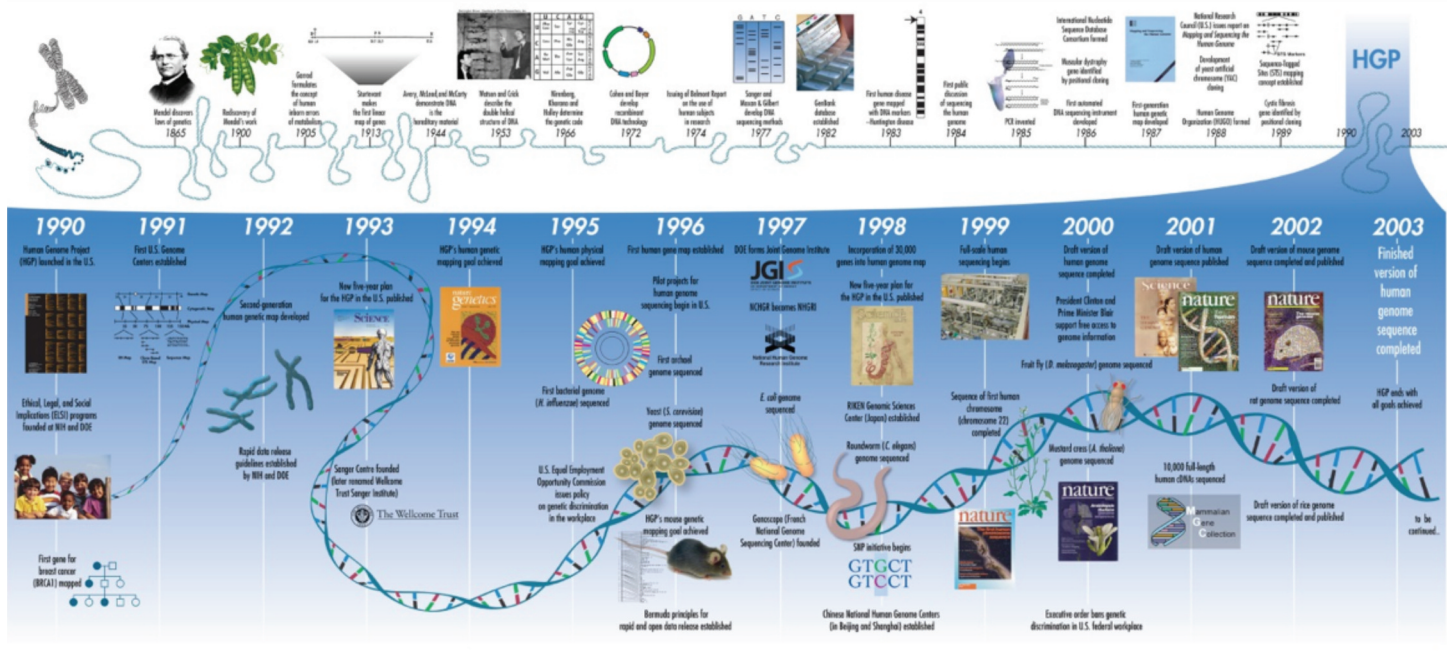
TIMELINE OF THE HUMAN GENOME PROJECT

Image Credit: Darryl Leja, National Human Genome Research Institute

Genomics has led to the realization that cancers are typically associated with changes in multiple genes and proteins. Consequently, molecular diagnostics have been developed to evaluate gene profiles, or assessments of many genes at once. One of the most widely used and best studied gene profile or genomic tests is OncotypeDX® Breast Cancer Assay. This test examines a panel of 21 genes that provides information about breast cancer. It is specified for use in women with early-stage (Stage I or II), node-negative, estrogen receptor-positive invasive breast cancer who will be treated with hormone therapy. The results of this test are given as a score that predicts the likelihood of breast cancer recurrence; the higher the score, the more likely the tumor is to recur. This test can also help predict whether a woman with early-stage, invasive breast cancer that is ER positive and HER2 negative will benefit from chemotherapy added onto her hormone therapy (also called adjuvant therapy).

Proteomics

Proteomic molecular diagnostics detect more than one protein at a time. Researchers are working to develop proteomic tests that will be useful for cancer, but because cells synthesize so many different proteins (each gene can synthesize more than one protein), the work is difficult and complex. Breakthroughs are expected to come from the compilation of large datasets—that is, data listing protein levels in hundreds of thousands of different people with various health conditions involved in many different studies. As more of these datasets become available, researchers will analyze them to determine patterns of proteins that may be associated with cancers, hopefully leading to useful molecular diagnostics based on proteomics.

Communication of Scientific Findings

Breakthroughs in genomics, proteomics, and related disciplines are communicated to the public in scientific articles. These articles are published in scientific or medical journals that are primarily read by other scientists, but may also be reported by the media. The findings are then "translated" by various organizations such as the government, foundations, and advocacy groups into language that can be understood by the general public. Some breakthroughs in science result in new products such as molecular diagnostics and targeted therapies. Once these products are approved or cleared by government agencies, they may be available for patients.

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US National Library of Medicine. Genetics Home Reference. What kinds of gene mutations are possible? Published June 29, 2015. Available at: http://ghr.nlm.nih.gov/handbook/mutationsanddisorders/possiblemutations. Accessed July 2, 2015.

Chapter 4: Methods in Molecular Diagnostics

One night in the spring of 1983, Dr. Kary Banks Mullis was driving his Honda along a winding northern California highway. He was pondering the problem of how to test for mutations in human DNA without using the lengthy cloning techniques available at the time. As he drove, a process began to take shape in his mind that he initially thought would help in his laboratory work. However, as Dr. Mullis continued snaking through the mountains, he realized that he was on to something much, much bigger—a discovery that would earn him the 1993 Nobel Prize for Chemistry. His big idea was a method of amplifying specific DNA sequences from very small amounts of genetic material known as the polymerase chain reaction, or PCR.

PCR is now an indispensable technique in molecular diagnostics. In this chapter, we discuss PCR along with several other important methods and techniques that allow physicians to "see" alterations in our DNA that may underlie health issues and can help researchers identify new biomarkers that can be targeted with precision therapies.

In Vitro Diagnostics

Molecular diagnostics are often referred to as *in vitro* diagnostics. The words *in vitro* are Latin for "in glass" and refer to the glass test tubes in which the tests were originally performed. Today, the phrase *in vitro* diagnostics refers to tests that are conducted on samples taken from the body, such as blood, saliva, or cells from a tumor. *In vitro* diagnostics can be contrasted with *in vivo* diagnostics such as ultrasound, X-rays, and computed tomography (CT) scans, which are performed on a living person and produce an image. Today, all molecular diagnostics are performed *in vitro*, and sometimes the whole field of molecular diagnostics is referred to as *in vitro* diagnostics. Today, the two types of diagnostics, *in vitro* and *in vivo*, are actually coming together. For example, specific molecules in the body are being labeled with chemicals that make them visible with imaging machines, as is being done with the administration of radioactive estrogen to a person combined with imaging to examine estrogen receptors in the living body.

Tissue Sampling

The first step in molecular diagnostics is to obtain tissue or specimen for testing. Tissue samples are collected by different methods depending on the purpose and the type of test. Blood samples are often drawn from veins in the arm. Urine can be studied, as can saliva obtained from the mouth. Samples may be taken from the skin following a local anesthetic. When samples need to be obtained from a solid tissue abnormality or tumor, the simplest and least invasive option is a fine needle biopsy, in which a fine needle is inserted into the tissue and cells are aspirated. If a larger amount of tissue is needed, a core needle biopsy may be used to remove cells and a small amount of surrounding tissue. Surgical procedures may also be used when the removal of an even larger amount of tissue is needed: an incisional biopsy removes a portion of the abnormality and an excisional biopsy removes the entire abnormality or tumor. Cells can also be obtained by scraping tissues that naturally open to the environment, such as the cheek and cervix. Another method involves the use of a flexible, lighted instrument called an endoscope that is inserted into one of the body's natural openings. The endoscope allows the physician to see abnormal areas on the lining of organs and pinch off tiny bits of tissue.
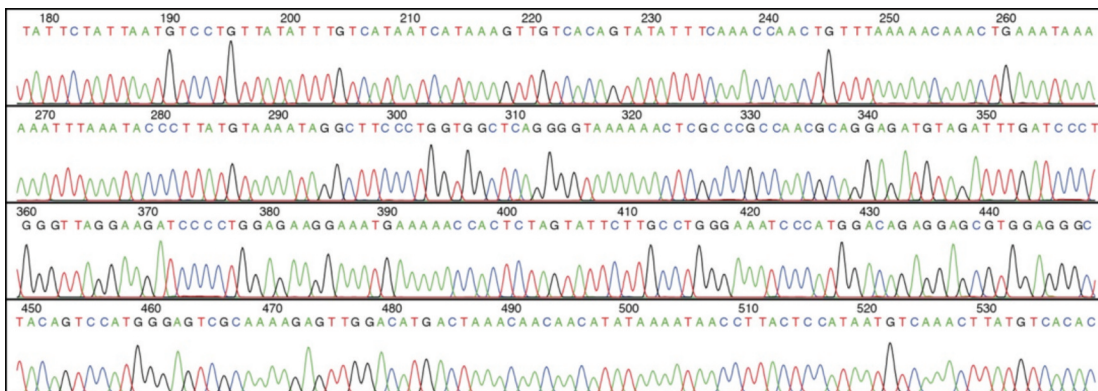
Tissue Storage

Tissue that does not undergo immediate molecular diagnostic testing may be processed and stored for future use. One way of preserving the tissue is to treat it with chemicals such as formalin and embed the sample in wax. Another method is to snap freeze the tissue and store it at −80°C. Tissue type, collection methods, and storage methods can all influence the viability of the tissue for molecular diagnostic testing. Often, tissue that is intended for use in the more distant future, such as for research, is kept in tissue banks. When snap frozen and stored at −80°C, DNA and protein can remain viable for many years, and RNA may last for up to 5 years.

Methods Used to Detect DNA

DNA Sequencing

One method used to detect DNA is to directly determine the nucleotide bases it contains. The first person to develop this method was Fred Sanger, and the method is sometimes known as the Sanger method. With the Sanger method, the DNA is first separated into two strands. Next, one strand is copied multiple times using chemicals that stop the copying process at different places along the DNA strand. This process results in numerous smaller DNA strands of different lengths. The researchers know which nucleotide is on the end of each fragment because of the chemicals they used to stop the copying process. This allows them to assemble the pieces of DNA like a jigsaw puzzle to reveal the sequence of the original DNA strand. A video of this process is available at the DNA Learning Center website: https://www.dnalc.org/view/15479-Sanger-method-of-DNA-sequencing-3D-animation-with-narration.html.

DNA SEQUENCE DATA FROM AN AUTOMATED SEQUENCING MACHINE

DNA sequence data from an automated sequencing machine.

Image credit: Darryl Leja, National Human Genome Research Institute

Today, methods are available that sequence DNA much more quickly and inexpensively. The most popular method used currently is called next generation sequencing, or NGS. In this method, up to 500 million separate sequencing reactions are run at the same time on a slide the size of a Band-Aid®. This slide is put into a machine that analyzes each reaction separately and stores the DNA sequences in a computer. The reaction is a copying procedure similar to the one described for the Sanger method, but does not require the use of altered nucleotide bases.
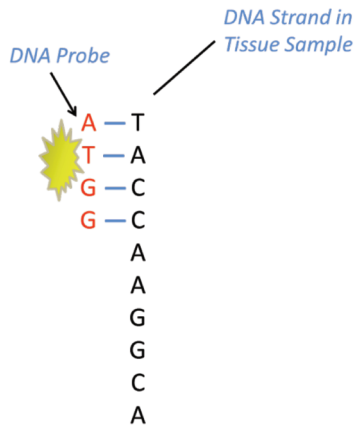
DNA Probes

DNA probes are an important tool in several molecular diagnostics that are used to detect the presence of specific DNA sequences. DNA probes take advantage of the fact that each nucleotide base in DNA binds to only one other base. As we learned in Chapter 2, adenine (A) only binds with thymine (T), and guanine (G) only binds with cytosine (C). The DNA probe is constructed by stringing together nucleotide bases that are complementary to the DNA sequence of interest. For example, if you are trying to detect the sequence CCCTT, you could construct a probe with the sequence GGGAA. Under the right conditions, this probe would bind to CCCTT, but not other DNA sequences. In reality, DNA probes are longer than just a few nucleotides.

DNA probes are attached to fluorescent markers then mixed with tissue samples that have been prepared for the test. The probes are allowed to bind or hybridize with DNA from the tissue then washed off. If the probe finds a matching, complementary DNA sequence, it will stick to it and won't wash off. The presence of the specific sequence in a person's DNA can then be detected as a fluorescent signal. If the person's DNA did not contain the faulty DNA sequence, the DNA probes will wash off and no fluorescence will be detected.

> Complementary DNA strands **hybridize** when their nucleotide bases form pairs—A with T and G with C.

DNA PROBE HYBRIDIZING WITH DNA IN TISSUE SAMPLE

This graphic shows a DNA probe (red letters on the left with a fluorescent marker attached) hybridizing with a complementary DNA sequence in a tissue sample. DNA sequences that are not complementary to the DNA probes do not bind and the probe (along with the fluorescent markers) are washed off. In reality, DNA probes are much longer than just 4 nucleotide bases.

DNA Microarrays

DNA microarrays were developed to detect thousands of genes at once—a feature that is integral to the field of genomics. In DNA microarrays, DNA probes containing selected DNA sequences are "arrayed" or spotted in a grid pattern on a very small glass surface. The DNA microarray actually looks like thousands of tiny dots arranged in precise rows and columns. Each dot contains a single DNA probe like the one just described that is designed to hybridize with the complementary DNA sequence in the tissue sample. Because there are many spots for probes, many different DNA sequences can be detected at the same time. This allows so-called "high throughput," or the analysis of many DNA sequences in parallel.

> **High throughput** refers to the assessment of many things at the same time. For our purposes, it refers to the detection of many different DNA sequences in a single molecular diagnostic.

After the DNA probes are placed in the microarray, a sample containing the person's DNA is prepared for analysis. The double-stranded DNA in the sample is denatured or separated into two complementary single strands. The strands are then cut into smaller fragments and attached to fluorescent dye. The labeled DNA in the sample is placed into the chip and allowed to hybridize with the DNA probes. The microarray is then washed; DNA that has hybridized will not wash off, but DNA that has not hybridized will wash off. Bound and unbound DNA is then detected as fluorescence. If the DNA in the sample has hybridized with the DNA probe, that spot on the array will light up. Computers contain information about which spot corresponds to which DNA sequence and can identify the presence or absence of that sequence in the sample. Depending on the type of technology, the array may use between one and four colors in the detection scheme.

DNA microarrays are also called genome chip, GeneChip® (trade name of a specific product), and gene array. Microarrays can be placed on other surfaces besides glass—bead arrays, capillary arrays, and well arrays all work the same way. That is, DNA probes (whose sequence is, of course, known because you put them in) are attached to the array surface, allowing thousands of genes or even the whole genome to be examined in a single experiment. Some microarray analyses detect different variations of the same gene. For example, more than 800 mutations have been found in the BRCA1 gene, which increases the risk of breast and several other cancers. A single microarray can be used to detect all of these variations.

**Variants of Uncertain Significance in BRCA1 and BRCA2 Genes**

Inherited, germline single nucleotide polymorphisms (SNPs) occur in everyone and account for many of the differences or "variations," between people that we can see, such as eye color, and that we can't see, such as taste and smell. These variations are neither good nor bad. However, when variations in the nucleotide base sequence occur in genes such as BRCA1 and BRCA2, which are known to be associated with an increased risk of cancer, they are more concerning. Nevertheless, not all variations in these genes are associated with cancer. This is because some variations do not affect the structure or function of the protein encoded by the gene. In contrast, other variations may change the amino acid in the resultant protein, but it is unknown if they change the function of the protein. These variations are called "variants of uncertain significance (VUS)". As described earlier, variations that prevent the resultant protein from working as it should may lead to an increased risk of cancer, and are called mutations. Some people also refer to these mutations as deleterious variations. However, as noted previously, some people refer to any variation in these genes as mutations.

Variants of uncertain significance occur in all genes that have been linked to disease. However, BRCA1 and BRCA2 are extremely large genes, which increases the potential number of variations. (For comparison, the smallest gene that encodes a protein contains 500 nucleotide base pairs, and half of all genes contain less than about 25,000 nucleotide base pairs. BRCA1 and BRCA2 each contain more than 80,000 nucleotide base pairs. Although BRCA1 and BRCA2 are large genes, they are not even close to the largest genes, which contain more than 2 million nucleotide base pairs.) Thousands of nucleotide base sequence variations have been found in BRCA1 and BRCA2. Most of these likely have no detrimental effects, but determining this can be very difficult.

> **Variants of uncertain significance** are sequence variations in a gene where the effect of the sequence change on the function of the protein is not known.

The situation becomes even more complex when dealing with genes that don't have the documented links to cancer that BRCA1 and BRCA2 have. Some of these genes have presumed links to cancer, but the research is not yet conclusive. As more patients undergo genetic testing, the more variants of uncertain significance will be identified in known cancer-related genes such as BRCA1 and BRCA2, as well as genes for which the link to cancer is uncertain. Another aspect of variants of uncertain significance is whether they occur as germline mutations or somatic mutations. Germline mutations can be inherited and thus have implications for family molecular diagnostic testing.

It can be stressful for patients to undergo molecular diagnostic testing only to find out that they have a variant of uncertain significance. Such a finding is also challenging for physicians who may not know what to advise patients with regard to preventive strategies. Variants of uncertain significance will lead to false alarms for many patients and unnecessary clinical actions that will likely become more common with the proliferation of molecular diagnostics.

IMAGE OF A DNA MICROARRAY (AKA: AN ARRAY "HEAT MAP")
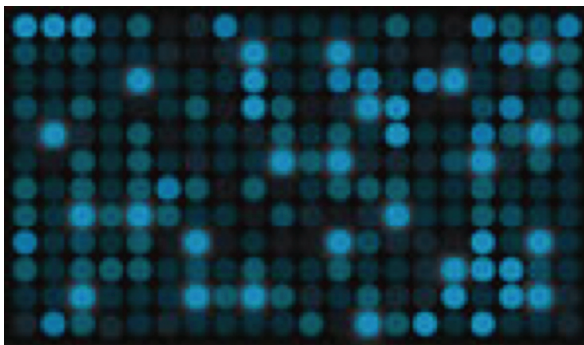
This graphic shows a DNA microarray. Each part of the grid contains a DNA probe. After exposure to a sample containing a person's DNA, some of the probes hybridize and some do not. Those that do hybridize light up, indicating that the specific DNA sequence is present in the sample. The different colors of the dots correspond to different levels of expression. Sometimes different colored dots such as red, black, and green are used in microarrays.

Image courtesy of U.S. Department of Energy Genomics:GTL Program, http://doegenomestolife.org.

Cytogenetic Testing

Cytogenetic testing involves examining the number and structure of chromosomes. This field was pioneered by Dr. Janet Rowling who discovered in the 1970s that chromosomal abnormalities could cause cancer. Although some people do not consider cytogenetic testing a "molecular diagnostic" because it doesn't involve detecting specific molecules such as DNA, we will describe it here so that it can be compared with more modern methods.

> For more information about the process and phases of cell division, you may want to visit the DNA Learning Center site at www.dnalc.org, searching on the phrase "cell division".

Conventional cytogenetic tests involve taking cells from a certain area of the body and growing the cells in a test tube for one day or more. The cells that are dividing are then stopped or essentially "frozen" in the process of division. It is essential that the cells are dividing because only in this stage can the chromosomes be seen using a regular microscope. The dividing cells are then placed on a microscope slide. Each chromosome is evaluated in multiple cells (usually at least 20) by looking under a regular microscope. This type of test is often used to detect Down syndrome, in which the affected individual has an extra copy of chromosome #21. This genetic disease can be detected by simply counting the chromosomes.

Cytogenetics is also used for the typing of blood cancers such as leukemia. One example is the Philadelphia chromosome, which we discussed in Chapter 3. This is an example of a somatic abnormality, or one that occurs in non-germline cells and is not inherited.

Fluorescence In Situ Hybridization (FISH)

FISH, also known as molecular cytogenetic testing, is a way to visualize and document the location of genetic material, including specific genes or DNA sequences within genes. FISH is used to look for the presence, absence, relative positioning, and/or number of specific DNA segments under a fluorescence microscope. Unlike conventional cytogenetic techniques, FISH does not have to be performed on cells that are actively dividing—which makes it more versatile. FISH is particularly helpful in identifying copy number variations, especially translocation and amplifications, such as occur frequently with HER2 in breast and gastric cancers.

The following table lists some examples of actual FISH-based molecular diagnostics and their clinical uses. This table and the others in this chapter are simply designed to demonstrate that the methods described here are used in the real world to detect molecular abnormalities. The test names and the descriptions shown in the tables likely contain some unfamiliar gene names and medication names. For our purposes, it is not necessary to know all of these, but rather to be aware that these methods have tangible applications.

EXAMPLES OF FISH BASED MOLECULAR DIAGNOSTICS AND THEIR CLINICAL USES

Cancer Type	Molecular Diagnostic Name	Clinical Use
Acute myeloid leukemia	Vysis D7S486/CEP 7 FISH Probe Kit	Test detects several deletion patterns in chromosome 7 that may be associated with patient outcome
Breast cancer	DakoCytomation Her2 FISH pharmDx™ Kit	Test designed to detect HER2 positive metastatic breast cancer that may be treated with Perjeta™ (pertuzumab) or Kadcyla™ (ado-trastuzumab emtansine)
B-cell chronic lymphocytic leukemia	Vysis CLL FISH Probe Kit	Test designed to detect several chromosomal abnormalities that may aid in determining prognosis at the time of diagnosis when used in combination with additional biomarkers
Breast cancer	The PathVysion HER-2 DNA Probe Kit (PathVysion Kit)	Test designed to detect amplification of the HER-2/neu gene in breast cancer tissue

FISH uses DNA probes attached to fluorescent dye. DNA in the chromosomes is treated so that the double strands of DNA separate. The DNA probe is then introduced, where it can bind to complementary DNA sequences in the chromosome sample. Once the probes have had time to hybridize, they are viewed under a fluorescent microscope. Hybridization of the DNA probes with the DNA on the chromosomes is visible under the fluorescent microscope.

FLUORESCENCE IN SITU HYBRIDIZATION (FISH)
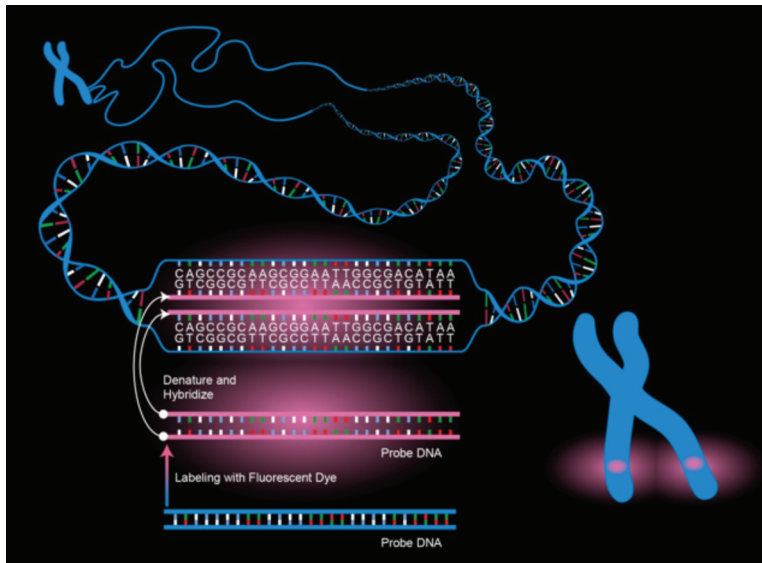
In FISH, DNA probes are labeled with fluorescent dye then exposed to chromosomes whose DNA double strands have been separated or denatured. The probes are allowed to hybridize with the DNA in the chromosomes, then viewed under a fluorescent microscope. DNA that is bound to the probes (indicating the presence of the specific DNA sequence) will be visible as fluorescent light (shown as pink spots on the chromosome in the lower right.

Image credit: Darryl Leja, National Human Genome Research Institute

Primers

Primers are DNA sequences that serve as starting points for DNA copying. The proteins that promote the replication of DNA, called DNA polymerases, cannot begin synthesizing a new DNA from scratch. They can only add to an existing strand of nucleotide bases. This is where primers come in: they begin the process and then DNA polymerases complete it.

Polymerase Chain Reaction (PCR)

PCR is a laboratory method, typically used for research purposes, that amplifies small amounts of DNA so that it can be more easily detected and analyzed. In fact, it has been referred to as "xeroxing DNA." The following figure shows the basic steps in PCR. First, double-stranded DNA is heated to separate the strands. The temperature is then lowered and primers bind to the single strands of DNA. The DNA copying enzyme (DNA polymerase—the P in PCR) then copies each single strand. The sample is again heated to separate the newly created double strands, and the process is repeated. Each time the process is repeated, the number of DNA strands doubles. Within a few hours, PCR can produce a billion copies of the DNA sequence.

POLYMERASE CHAIN REACTION (PCR)
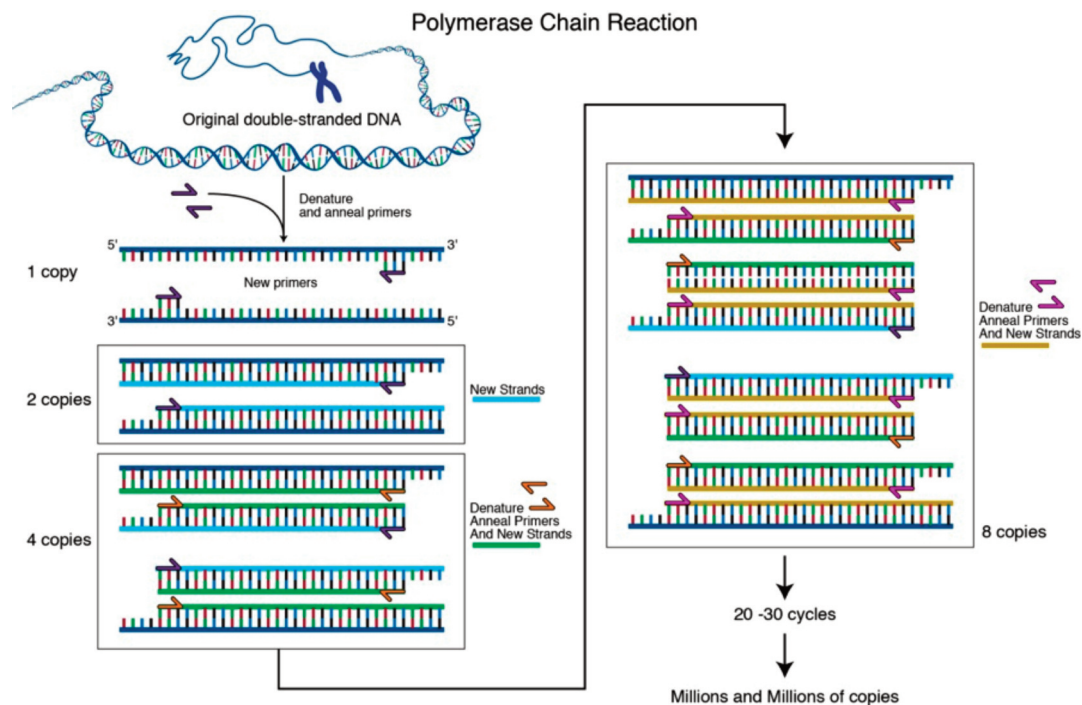
In PCR, the original double-stranded DNA is heated to separate it into single strands. The strands are cooled and exposed to primers, which signal the enzyme DNA polymerase to begin copying. The enzyme makes one copy of each single strand, resulting in two new strands, or 2 full copies of double-stranded DNA. The DNA is again heated to separate the strands and the process is repeated.

Image credit: Darryl Leja, National Human Genome Research Institute

Real-Time PCR

Real-time PCR is similar to regular PCR except that the DNA is not only copied, but also detected or quantified in the same process. In regular PCR the end product is analyzed after the reaction is complete. A number of companion diagnostics used to determine whether cancers are likely to respond to precision therapies utilize real-time PCR.

EXAMPLES OF REAL-TIME PCR MOLECULAR DIAGNOSTICS USED AS COMPANION DIAGNOSTICS

Companion Medication	Molecular Diagnostic	Description
Iressa® (gefitinib)	therascreen® EGFR RGQ PCR Kit	Tests for certain deletions and mutations of the epidermal growth factor receptor (EGFR) gene in non-small cell lung cancer (NSCLC) tumor tissue. The test is intended to be used to select patients who may respond to the Gilotrif® (afatinib) or Iressa® (gefitinib).
Erbitux® (cetuximab); Vectibix® (panitumumab)	The cobas® KRAS Mutation Test	Tests for the certain mutations of the KRAS gene in DNA colorectal cancer (CRC) tumor tissue. The test is intended to be used as an aid in the identification of CRC patients for whom treatment with Erbitux® (cetuximab) or with Vectibix® (panitumumab) may be indicated based on a no mutation detected result.
Mekinist® (tramatenib); Tafinlar® (dabrafenib)	THxID™ BRAF Kit	Tests for certain BRAF mutations in human melanoma tissue. Intended to be used as an aid in selecting melanoma patients who may respond to dabrafenib (Tafinlar®) or trametinib (Mekinist®).

The next section describes a PCR technique called reverse-transcription PCR, which is abbreviated RT-PCR. Most experts agree that real-time PCR—which is different from reverse-transcription PCR—should not be abbreviated RT-PCR. Instead the abbreviation RT-PCR should be reserved for reverse-transcription PCR to avoid confusion. However, not everyone adheres to this convention.

Methods Used to Detect RNA

Several of the methods just described for the detection of DNA have been adapted to detect RNA. RNA tests are useful because they can help determine the degree to which genes are expressed—that is, RNA tests can tell us whether the DNA is actively being made into proteins.

Reverse Transcription-PCR

Reverse transcription (RT)-PCR is similar to PCR except that it detects RNA instead of DNA. As discussed in Chapter 2, RNA is the intermediate chemical that copies and translates the DNA sequence into proteins. RT-PCR uses the same steps as PCR, but instead of DNA, the sample (e.g., from a tumor, blood, urine, etc.) contains a type of RNA.

The ability of RT-PCR to detect genes that are expressed as opposed to genes that are present has made it an important addition to the techniques available for research and medicine. Because RT-PCR can detect even low levels of genes that are activated, it is being used to determine whether cancers have spread to distant regions.

MicroRNAs

MicroRNAs are not a method but rather a type of RNA that was discovered in 1993. MicroRNA or miRNAs are small, single-stranded RNA molecules approximately 19 to 25 nucleotide bases in length that bind to specific parts of larger RNA molecules, preventing them from making proteins. In this way, microRNAs inhibit gene expression. Some of the tests for microRNA utilize quantitative real-time, reverse-transcription PCR. As our understanding of the role microRNAs play in cancer, it is likely that the number of molecular diagnostics that detect them will increase.

Methods Used to Detect Proteins

Molecular diagnostics may also detect proteins directly instead of the DNA that encodes them.

Immunohistochemistry

One of the most common methods used to detect proteins is called immunohistochemistry. This technique takes advantage of the method our immune systems use to rid the body of foreign proteins – namely, antibodies.

EXAMPLES OF IMMUNOHISTOCHEMISTRY (IHC) BASED MOLECULAR DIAGNOSTICS USED AS COMPANION DIAGNOSTICS

Companion Medication	Molecular Diagnostic	Description
Gleevec®/Glivec® (imatinib mesylate)	DAKO C-KIT PharmDx	Tests for identification of c-kit protein/CD 117 antigen (c-kit protein), which aids in the diagnosis of gastrointestinal stromal tumors (GIST) that may be eligible for treatment with Gleevec®/Glivec® (imatinib mesylate)
Erbitux® (cetuximab); Vectibix® (panitumumab)	DAKO EGFR PharmDx Kit	Tests for epidermal growth factor receptor (EGFR) expression in normal and cancerous tissues to aid in identifying colorectal cancer patients eligible for treatment with Erbitux® (cetuximab) or with Vectibix® (panitumumab)
Herceptin® (trastuzumab)	Pathway Anti-HER-2/NEU (4B5) Rabbit Monoclonal Primary Antibody	Tests for c-erbB-2 antigen in breast cancer tissue to aid in the assessment of breast cancer patients for whom Herceptin® treatment is being considered

In immunohistochemistry, a sample in which we are trying to identify a specific protein is placed together with antibodies that bind to that protein. The antibodies are labeled beforehand with some sort of marker, often a fluorescent one that can be seen under a fluorescence microscope. The antibodies are mixed with the sample in a test tube and given time to bind. The sample is then washed off. If the protein of interest is present in the sample, the antibodies will bind and a visible colored label will be seen under the microscope. Unbound antibodies will wash off. Immunohistochemistry can be qualitative (is the protein present?) or quantitative (how much of the protein is present?). Immunohistochemistry is commonly used to detect estrogen receptors.

EPIDERMAL GROWTH FACTOR RECEPTORS IN HUMAN SQUAMOUS CELL CARCINOMA LOCALIZED WITH IMMUNOHISTOCHEMISTRY (DARK BROWN STAINING)]
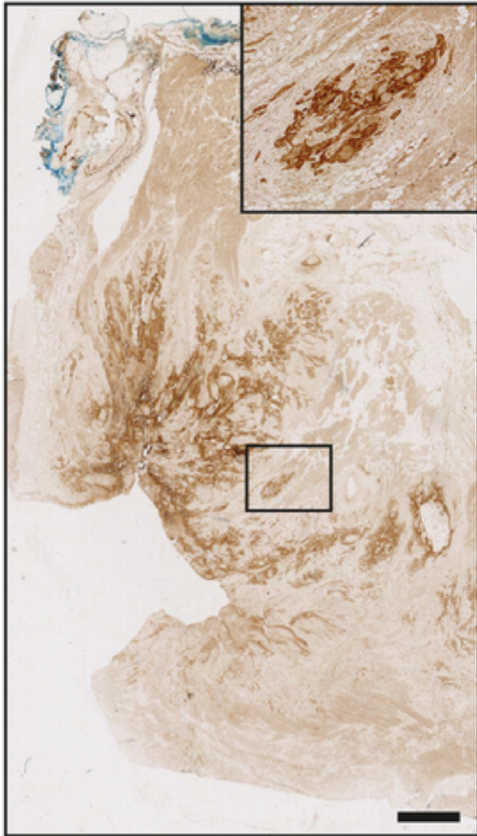

Photo courtesy of De Boer E, Warram JM, Tucker MD, et al. In Vivo Fluorescence Immunohistochemistry: Localization of Fluorescently Labeled Cetuximab in Squamous Cell Carcinomas. Scientific Reports 2015; 5, Article number: 10169.

For complete information on nucleic acid tests and companion diagnostics approved by the FDA, you may want to visit these two FDA websites:

• Nucleic acid-based tests approved FDA: http://www.fda.gov/MedicalDevices/ProductsandMedicalProcedures/InVitroDiagnostics/ucm330711.htm
• Companion diagnostic devices *(In Vitro* and Imaging Tools) approved or cleared by FDA: http://www.fda.gov/MedicalDevices/ProductsandMedicalProcedures/InVitroDiagnostics/ucm301431.htm

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Chapter 5: How Good Are Molecular Diagnostics? Validity, Reliability, and Clinical Utility

Since 1986, the prostate specific antigen (PSA) test has been used along with a digital rectal exam to screen for prostate cancer in men 50 years of age and older. However, in 2012, the U.S. Preventive Services Task Force released a statement unequivocally recommending against use of the PSA test to screen for prostate cancer. Other organizations are also reconsidering their recommendations in light of findings from two large studies in which annual prostate cancer screening combined with digital rectal exams did not save lives. Men who underwent annual screenings were just as likely to die from prostate cancer as men who did not undergo annual screenings.

This real-life example illustrates a major problem with molecular diagnostics: they are not always useful. The PSA test, which measures levels of PSA in the blood, is not specific and it often leads to overdiagnosis and overtreatment. In this chapter, we discuss concepts used to determine the usefulness of molecular diagnostics—essentially, ways to test the tests and determine how good they are.

Analytical Validity

Molecular diagnostics must exhibit two types of validity in order to be useful: analytical and clinical. This section focuses on analytical validity and the next section addresses clinical validity. Analytical validity refers to how well a test measures what it is supposed to measure. For example, a test designed to detect a mutation associated with melanoma should not give a positive result for an unrelated mutation associated with diabetes.

> **Analytical validity** is a measure of how well the test measures what it purports to measure.

Specificity

Two different aspects of validity that good tests must show are specificity and sensitivity, which are really two sides of the same coin. These concepts can apply to analytical validity of the molecular diagnostic test as well as to the clinical validity of the biomarker. Let's consider specificity first.

> **Specificity** is the ability of the test to correctly identify those patients without the condition. Stated another way, a test is specific if it gives a positive result only when the condition is present. For instance, a specific test for a cancer mutation would give a negative result for everyone who does not have the mutation and only give a positive result if someone does have the mutation.

Specificity is the ability of the test to correctly identify those patients without the biomarker or condition. Said another way, a specific test is one that gives a positive result only when the biomarker or condition is present. Returning to our PSA example, approximately 80% of men who have a positive PSA test do not have prostate cancer. This is because high levels of PSA are not specific for prostate cancer; they are also associated with inflammation of the prostate and benign prostatic hypertrophy (enlargement of the prostate)—relatively common health conditions in older men. Thus, one of the problems with the PSA test as a screening tool for prostate cancer is that it lacks specificity. Specificity depends on analytical validity. A poorly reproducible or inaccurate test may be positive in a specimen in which the actual biomarker does not exist. Further, it also depends on the use context. It can relate to specificity between normal or benign conditions and cancer, between different types of cancers, etc.

A lack of specificity is problematic because it can cause mental anguish and can lead people to undergo unnecessary follow-up procedures and treatments that are associated with risks. For example, men with high levels

of PSA and/or abnormal findings on a digital rectal exam may elect to undergo a needle biopsy. Such biopsies can cause stress and anxiety and may be 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, in addition to erectile dysfunction and incontinence. Thus, these tests are not without drawbacks and risks and, as with all medical tests, it is best to minimize the number of patients who undergo them unnecessarily.

It is worth noting here that the PSA test is still used to monitor recurrence of prostate cancer in men who have already been diagnosed. This illustrates an important point about molecular diagnostics: they may have more than one clinical use and their clinical utility may vary with the different uses.

Sensitivity

Sensitivity can be considered the opposite of specificity. Sensitivity is the ability of the test to correctly identify those patients with the biomarker or condition; in other words, it should correctly identify everyone who has the biomarker or condition. With a sensitive test, you can be relatively certain that, if you have the biomarker or condition, you will get a positive result on the test.

> **Sensitivity** is the ability of the test to correctly identify those patients with the biomarker or condition.

SPECIFICITY AND SENSITIVITY
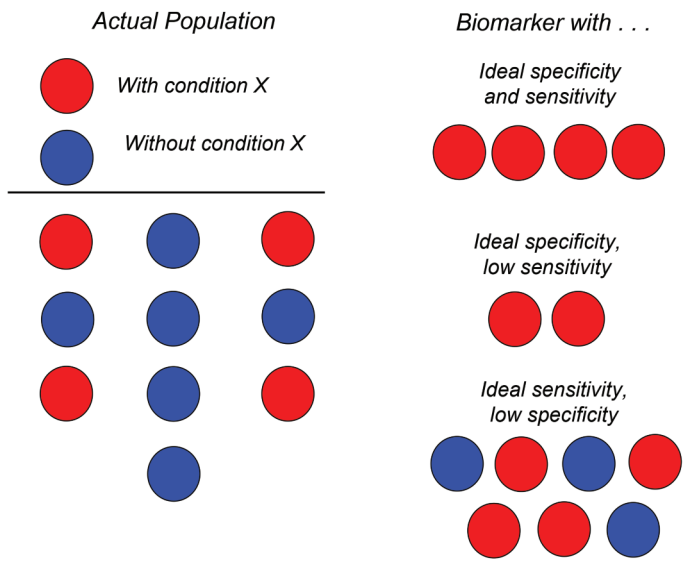
In this imaginary population of 10 people (circles), 4 have the condition (red circles) and 6 do not (blue circles). A biomarker with ideal specificity and sensitivity would be evident in all 4 red people but 0 blue people. A biomarker with ideal specificity but low sensitivity might be evident in 2 red people but 0 blue 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 red people but might also be evident in 3 blue 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.

True and False Positives and Negatives

Results of molecular diagnostics (or any tests, for that matter) can be classified as correct or incorrect. When a test correctly identifies a person with a given biomarker or condition, the result is said to be a true positive. When a test correctly determines that a person does not have a given biomarker or condition, the result is said to be a true negative. This is the ideal situation—we always want the results of tests to be correct or true. However, when a test is incorrect, the results are said to be false. If the test incorrectly identifies a person as having a biomarker or condition, the result is a false positive. If the test incorrectly identifies a person as not having the biomarker or condition, the result is a false negative.

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

Accurate and useful tests have high true positive and true negative rates and low false positive and false negative rates. In the ideal case, the test would identify only those people with the biomarker or condition, but would also identify everyone with the biomarker or condition. In real life, very few molecular diagnostics come close to this ideal.

In molecular diagnostic testing, these terms are often called the positive and negative predictive values (PPV and NPV). A highly specific test will have a high positive predictive value; that is, if it is positive in a patient, it is very likely that patient has the condition. A highly sensitive test will have a high negative predictive value; that is, if the test is negative in a patient, it is very unlikely that the patient has the condition. However, no test is perfect. Depending on the condition, it may be better to have a higher positive predictive value or negative predictive value.

For example, let's presume that 100,000 people undergo cancer screening and 200 of them have a certain cancer. Let's apply a test with high sensitivity, say 90%. In this case 180 of the 200 patients who actually have the cancer will be identified. Let's also give this test what appears to be a high specificity, say 90%. In this case, 10%, or 10,000, of the screened patients will have a false positive test, leading them to worry needlessly and undergo additional screenings and procedures even though they are not necessary. So, in this case, the positive predictive value is only approximately 2%; that is, of the 10,180 positive tests, only 2% were true positives.

The positive and negative predictive values depend on three issues:

- The prevalence of the condition in the population
- The sensitivity of the test
- The specificity of the test

In the example above, if the prevalence were much higher, let's say 50%, occurring in half of the 100,000 people, then we would have detected 45,000 true positives, but would still have had 10,000 false positives. In this case, 45,000 of the 55,000 positives were true positives, and therefore the positive predictive value would have been 82%!

These considerations highlight how important the use context and analytical validity are for a given molecular diagnostic.

Test Reliability

Another aspect of analytical validity is test reliability. Test reliability means that the results of the test are repeatable. If a molecular diagnostic performed on Monday indicates that a cancer is positive for a certain gene, it should also give the same result when conducted on Tuesday. Clearly, unreliable tests are not useful in making diagnoses or treatment decisions.

Reliability of HER2 Molecular Diagnostics

Reliability is an issue with tests used to detect HER2 overexpression in breast cancer. As discussed previously, approximately one quarter of breast cancers overexpress a gene known as HER2/neu. This overexpression causes cells to produce too much HER2 protein. Because the HER2 protein is involved in cell growth and replication, cells that have too much HER2 receive too many signals telling them to grow and replicate. A medication known as trastuzumab inhibits the activity of HER2 protein. However, this medication is only approved to treat cancers that are positive for HER2/neu overexpression, which must be determined by a test (a companion diagnostic).

Two different types of tests are available for this purpose: one based on immunohistochemistry and the other based on FISH. Although many women obtain accurate and reliable results from these tests, guidelines from the American Society for Clinical Oncology (ASCO) and College of American Pathologists (CAP) state that 20% of current HER2 testing may be inaccurate. Some tumors that initially test negative with these diagnostics may actually overexpress HER2/neu and vice versa. These problems with reliability are important because the tests are being used to determine treatment. If a test result is false negative (that is, if a tumor overexpresses HER2/neu but the test gives a negative result), a woman could fail to receive a treatment that could prolong her life. Conversely, if a test result is false positive (that is, if the tumor does not overexpress HER2/neu but the test gives a positive result), a women may receive a treatment that is not as likely to benefit her.

Standardization of Molecular Diagnostics

One reason that the tests for HER2/neu overexpression are sometimes unreliable is a lack of standardization. Ideally, molecular diagnostics 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. Because many molecular diagnostics require precise measurements, complicated equipment, and/or different mixtures of chemicals, reliability can be difficult to achieve.

One feature that can contribute to the variability of results from a molecular diagnostic is the way tissue samples are collected, processed, and stored—so-called pre-analytic factors. These factors can substantially alter the consistency of the tissue and its molecular composition. Consequently, the same tissue sample evaluated in the same molecular diagnostic may give different results depending on how it is collected, processed, and stored. An example of this is when tissue samples are frozen versus fixed with formalin and embedded in paraffin; these two methods may give different results. Differences in pre-analytic factors can also contribute to variability in scientific studies that incorporate molecular diagnostics.

> **Pre-analytic factors** are factors that occur prior to the molecular diagnostic test, including sample collection, processing, and storage, that can affect test outcome.

Ideally, the pre-analytic factors would be standardized, but at the very least, it is important that they are consistently reported. In 2011, experts published recommendations on Biospecimen Reporting for Improved Study Quality (BRISQ). These guidelines describe the pre-analytic details that must be reported anytime human biospecimens are used; this information is designed to help evaluate, interpret, compare, and reproduce the experimental results.

In order to combat the lack of reliability of molecular diagnostics, ASCO-CAP recommends that laboratories adhere to strict tissue sample handling procedures, among other things. These guidelines also recommend that new tests for HER-2, should show 95% agreement with a reference test for HER-2 that has been clinically validated (i.e., the reference test predicts clinical outcome). Stringent laboratory accreditation standards are recommended, along with proficiency testing and competency assessments. Standardization is important not only so that an individual undergoing pathology testing can be confident that his or her results are accurate, but also so that results from different patients and laboratories can be compared.

Sometimes tests that are sold by the manufacturer as kits include an internal standard—a test sample that contains a given, known amount of the biomarker being detected. This standard can then be used to calibrate the test. For example, a test kit might contain an internal standard that consists of 100 micrograms of a protein. When that standard sample is run in different laboratories, they should also find that it contains 100 micrograms. In this way, laboratories can make sure that their test is giving the correct results and that it is comparable to those of other laboratories.

Standardization in laboratory tests may be achieved by requiring laboratories to undergo proficiency testing. For example, blood samples may be sent to participating laboratories for the determination of the substance of interest. The results from all participating laboratories are sent to a central facility where they are evaluated and the laboratory is either certified or not based on its ability to obtain accurate results.

To overcome the problem of reliability, some companies have designed molecular diagnostics that require samples for testing to be sent to the company's own laboratory. In this case, the test can be performed the same way each time and the company has control over the reliability of the results. This is the case for Oncotype® DX, a test that helps predict the likelihood of benefit from add-on (adjuvant) chemotherapy and of breast cancer recurrence. For this test, healthcare professionals obtain samples of breast tumors and then send them to the company's laboratory for analysis.

Clinical Validity

Clinical validity refers to the ability of the test to provide clinically relevant information. Clinical validity depends on close association of the biomarker with a clinically important outcome such as response to a medication or aggressiveness of the cancer. Per our previous example, one reason that the PSA test is not useful as a screening tool for prostate cancer is because it lacks clinical validity; that is, routine PSA testing does not increase a man's lifespan.

> **Clinical validity** is the measure of a test's ability to provide clinically relevant information.

Clinical validity is related to the concept of clinical utility, which is described more fully in a subsequent section. Some experts define clinical utility as a test's ability to provide clinically relevant information—which is the exact definition used for clinical validity. In contrast, other experts believe that clinical utility is a broader concept that involves a practical aspect of usefulness in the clinic.

Many experts, including a committee of the Institute of Medicine, consider clinical validity a measure of whether the assay reliably divides one population into two or more with different biological or clinical characteristics or outcomes. However, if this difference is insufficiently large to justify treating those two groups differently (even if it is statistically significant) OR if knowing the difference is not associated with a therapeutic option for one but not the other that improves clinical outcomes, then it does not have clinical utility. Notably, there may be many different tests or assays for a single biomarker. They may have different analytical and clinical validities and clinical utilities. Each must be tested separately before it is recommended to guide therapy in a specific use context, as described above, for patient care.

How Are Molecular Diagnostics Clinically Validated?

Molecular diagnostics are clinically validated by conducting studies that document the relationship of the test's outcome with an important medical or clinical outcome. For instance, if the molecular diagnostic purports to detect response to therapy, then test results would need to show a relationship with reduced tumor growth, patient survival, or another important variable in a clinical study. Such studies provide scientific proof of the molecular diagnostic's accuracy. Without validation in a clinical study, the test's accuracy must be considered unproven.

Clinical Utility

Clinical utility refers to the overall usefulness of a molecular diagnostic in clinical practice that is determined by weighing its benefits and drawbacks. As discussed earlier, molecular diagnostics must provide some clinically useful information in order to be clinically valid. This information should aid in diagnosis or clinical decision making. A test that could reliably detect 20 common genes associated with a tumor may not be clinically useful if those genes don't predict anything of value for the patient or physician.

> **Clinical utility** *is the overall usefulness of a molecular diagnostic in clinical practice determine by weighing its benefits and drawbacks. This definition is still evolving, with some experts equating clinical validity and clinical utility.*

In addition to being valid and reliable, a molecular diagnostic should also be practical. For example, a test that is extremely difficult to perform or requires rare technical equipment may not have clinical utility in routine hospital use, even if it provides clinically useful information. Practical concerns are an important part of the equation, although not all experts consider them an aspect of "clinical utility." Instead, some experts limit the definition of clinical utility to mean providing clinically useful information. Irrespective of whether you consider practical concerns and drawbacks as part of clinical utility, they are important determinants of how useful molecular diagnostics are.

Potential Reasons for Lack of Clinical Utility in Molecular Diagnostics

- The test doesn't really work.
- The test works but doesn't distinguish one group of patients from another with sufficient magnitude that they would receive different treatments or procedures.
- The test works and distinguishes patient groups adequately, but no better treatments are available for either group.
- Insufficient evidence that the test reliably distinguishes one group of patients from another.

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. Tissue accessibility is part of clinical utility—the more readily accessible the tissue, the more practical and useful the test.

For molecular diagnostics, researchers must often take a sample of the cancerous tissue in order to determine whether a biomarker is present. With blood cancers such as the leukemias, this is not typically an issue, as most people accept having their blood drawn (although children may be an exception). 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 gene 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. A less invasive method that is being intensively studied is the analysis of biomarkers in tumor cells found in the blood, known as circulating tumor cells. Circulating tumor cells break off from tumors and travel through the blood circulation. However, circulating tumor cells occur at extremely low levels, and as yet, the analytical validity and clinical utility of tests on these cells have not been established. Another alternative being investigated is the measurement of blood for circulating tumor DNA. In the future, this method may be an alternative to sampling tumor tissue.

Ease of Use and Interpretation

Another aspect of clinical utility is the ease with which the molecular diagnostic can be used or conducted, and the ease with which results can be interpreted. Tests that are easy to perform and interpret are likely to be more useful than those that aren't. Moreover, tests that are difficult to perform may introduce a greater potential for error. The highly technical nature of some molecular diagnostics can make them challenging to perform, and ease of use is definitely a concern as we will discuss when talking about test regulation in the next chapter.

Weighing Benefits and Drawbacks

Weighing the benefits and drawbacks of molecular diagnostics is an important undertaking for patients as well as physicians, with a variety of factors besides validity and reliability coming into play. As we will discuss in the next chapter, the Food and Drug Administration also weighs the benefits and drawbacks of molecular diagnostics in determining whether a given test should be marketed for clinical use.

The drawbacks to molecular diagnostics can be non-trivial. Returning to our example of PSA screening, the PSA test identifies a substantial percentage of men (17% to 50%) with prostate tumors that will not progress or will remain asymptomatic for the man's lifetime. These men may undergo treatment that they don't need and may even hurt them. Thus, many issues must be considered in determining whether a molecular diagnostic has clinical utility for a given outcome.

Example of Concepts Related to Judging the Quality of Molecular Diagnostics

The concepts discussed in this chapter essentially allow us to determine the quality of molecular diagnostics. These concepts apply to all clinical tests, not just molecular diagnostics. Let's consider these concepts as they apply to a blood test that might be developed for a familiar disease that all of us have experienced: influenza.

JUDGING THE QUALITY OF A BLOOD TEST FOR THE INFLUENZA VIRUS

Test Parameter	Definition	Examples
Analytical validity	Does the test measure what it is supposed to measure?	**Good analytic validity:** The test detects influenza in 100% of people who have the virus and never gives a positive result for people who do not have the virus. **Bad analytic validity:** The test frequently gives a positive result for people who have other viruses such as chicken pox or hepatitis. **Bad analytic validity:** The test frequently gives a negative result for people who actually do have the influenza virus.
Reliability (may be part of analytical validity)	Are the test results repeatable?	**Good reliability:** People who have a positive test result for the influenza virus will also have a positive result if the test is repeated later in the day. **Bad reliability:** Many people who have a positive test result for the influenza virus will have a negative result if the test is repeated later in the day. **Bad reliability:** Many people who have a negative test result for the influenza virus will have a positive result if the test is repeated later in the day.
Clinical validity	Does the test provide clinically relevant information?	**Good clinical validity:** People with a positive test result reliably get symptoms of influenza. **Bad clinical validity:** Many people with a positive test result do not show, or will not develop, symptoms of influenza.
Clinical Utility	Overall, is the test useful in clinical practice?	**Good clinical utility:** The test shows good validity and reliability for the influenza virus and is clinically useful. **Bad clinical utility:** The test does not have good analytical validity, clinical validity or reliability. **Bad clinical utility:** The test requires that the blood sample be sent to Siberia for analysis. **Bad clinical utility:** The test requires people to sit in a sauna for 3 hours prior to giving blood.

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Chapter 6: Challenges with Molecular Diagnostics: Regulatory, Ethical, Legal Issues

> *Should people be allowed to patent genes? What if they discover those genes and link them to a particular cancer? These were the questions brought to the US Supreme Court by the Association for Molecular Pathology in a 2013 landmark suit against Myriad Genetics. At issue were the DNA sequences of BRCA1 and BRCA2 mutations, which are associated with an increased risk of breast cancer and several other cancers. These mutations were discovered by scientists at Myriad Genetics and the company obtained patents that prohibited anyone else from testing for them. The Supreme Court ruled that Myriad Genetics' patents were invalid because naturally occurring substances such as DNA sequences cannot be patented, and the BRCA1 and BRCA2 mutations occur in nature.*

This example illustrates one of many important ethical/legal issues associated with molecular diagnostics and the genes or other biomarkers they are designed to detect. These issues challenge lawyers, philosophers, ethicists, judges, advocates, and citizens to balance the potential for medical advances, protection of privacy and individual rights, and commercialization aspects of molecular diagnostics. Another challenge with molecular diagnostics is their regulation. As we saw in the last chapter, it is critical that the tests be as accurate and reliable as possible, yet accessibility is also important. In this chapter, we discuss challenges with molecular diagnostics, including regulatory, ethical, and legal issues.

Regulation of Molecular Diagnostics

Molecular diagnostics are regulated by the US Food and Drug Administration, but the regulations differ depending on whether they are sold as kits or services. Tests are considered kits, or medical devices, if they are marketed as products. Kits can also be used as part of clinical studies. Kits contain all of the necessary materials for physicians to conduct the test in their offices or affiliated laboratories. In contrast, if hospitals or diagnostic laboratories analyze tissue samples using their own chemicals and procedures, the molecular diagnostic is considered a service. Similarly, if a tissue sample must be sent to a company's laboratory to be analyzed by them, the molecular diagnostic is considered a service.

Regulation of Molecular Diagnostic Kits (Medical Devices)

Molecular diagnostics sold as kits are classified as medical devices by the United States Food and Drug Administration (FDA). The amount of oversight the FDA exerts depends on the kit's intended use and its risks. The classification scheme for molecular diagnostics is the same as that for other medical devices, and ranges from Level I to Level III. The levels denote the amount of regulatory control by the FDA needed to provide reasonable assurance of the device's safety and effectiveness. Level I medical devices include items such as dental floss, medical tongue depressors, and enema kits. Level II medical devices include condoms and pregnancy tests that you buy at the store.

Molecular diagnostics for cancer are classified as Level III because they provide information that can have a high emotional impact and may alter the course of life. These molecular diagnostics are required to undergo validation procedures to make sure that they are accurate and clinically useful.

What Are Molecular Diagnostic Kits?
If a molecular diagnostic is sold as a kit, it means that the test is sold along with all of the materials necessary for physicians to conduct the test in their offices or affiliated laboratories. There is no need to make or synthesize anything additional.

Before medical devices like molecular diagnostic kits can be approved for marketing, they must be either approved or cleared by the FDA. The regulatory route that a medical device is allowed to follow depends on whether or not it is similar to a medical device already legally marketed for the same use. If a medical device is "substantially equivalent" to an existing product, it may follow the so-called "510(k)" pathway. This pathway is named for a section of the Food, Drug, and Cosmetic Act. This pathway does not require the manufacturer to provide the FDA with data showing the device's safety and effectiveness, but rather that it is sufficiently similar to an already-approved medical device. Medical devices that follow the 510(k) pathway and are given the green light for marketing by the FDA are said to be "cleared."

In contrast, medical devices that are not substantially equivalent to an already-approved product must follow the pre-marketing approval (PMA) pathway. This pathway requires that the manufacturer show evidence of the product's safety and effectiveness, which often involves clinical studies. Even if a product qualifies for the 510(k) pathway, the manufacturer can choose to follow the more rigorous PMA pathway. Medical devices that follow the PMA pathway and are given the green light for marketing by the FDA are said to be "approved."

Molecular Diagnostics Regulated as Services

Molecular diagnostics sold as services are regulated under the Clinical Laboratory Improvement Amendments (CLIA) of 1988. According to these regulations, all laboratories performing molecular diagnostics as services must be certified to conduct testing on human tissue samples. The essential ingredients needed to conduct the tests are regulated by the FDA. These ingredients may only be sold to organizations that are diagnostic device manufacturers, qualified clinical laboratories, or organizations that use the ingredients to make tests for non-medical purposes (such as academic laboratories). More information on CLIA regulations may be found at the following website: http://wwwn.cdc.gov/clia/. These regulations include general as well as specific information on each type of test, including information about test accuracy, validity, and the range or span of test result values that are reportable.
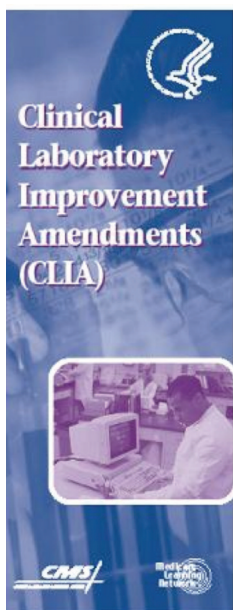
Many experts believe that CLIA accreditation is a relatively low regulatory hurdle. For molecular diagnostics sold as services, there is no mechanism by which to gain the FDA's stamp of approval. In contrast, for medications, a clear approval process exists: the product must be tested in Phase I, III, and III studies designed to document efficacy and safety. If the medication "passes" all three sets of trials, the FDA will officially approve the medication for one or more medical conditions. Insurance companies use these official FDA approvals as a basis for reimbursement.

The lack of molecular diagnostics regulation can have serious repercussions for patients. Studies examining the clinical validity of the tests are not required and, although some manufacturers conduct these studies, others do not. If the molecular diagnostic has not been sufficiently studied, there is no way to know whether its use is beneficial for patients. In some cases, the test results could lead patients to be assigned to a less than optimal treatment. Conversely, lack of regulation may inadvertently keep patients from receiving a clinically valid molecular diagnostic that may be beneficial. Without an approval process for molecular diagnostics, insurance companies often will not reimburse them, making it difficult for patients to afford. Moreover, physicians may lack confidence in the molecular diagnostic and may not recommend it.

Notably, the FDA does not use the terms analytical validity, clinical validity, and clinical utility. However, because molecular diagnostics are devices, the FDA does not require that they are "safe and effective" in the manner they require for therapeutic products such as medications. Instead, a molecular diagnostic must have excellent analytical validity and must do what the manufacturer claims, which is basically clinical validity. Consequently, a molecular diagnostic that is approved or cleared by the FDA may or may not have clinical utility and may or may not be worth using. Several decades ago, the FDA issued a discretionary decision that if a laboratory-developed test (LDT, or "home brew") is used in a CLIA-approved laboratory (CLIA does NOT approve the test, just the laboratory), and if the test is not sold to other sites for use, then it can be used to care for patients, and the laboratory can bill and get reimbursed for it.

These serious problems affect the use and acceptance of molecular diagnostics and are issues that interest many advocates. We will revisit them in the next chapter.

Ethical Challenges with Molecular Diagnostics

Molecular diagnostics are associated with ethical issues directly related to the tests and their findings, as well as issues related to genomics research and human tissue (the samples on which molecular diagnostics are conducted).

Ethical Issues with Molecular Diagnostics for Cancer Risk

Testing for cancer risk genes poses several ethical questions. First, mutations are hardly ever a guarantee that a person will get cancer. Instead, most cancer genes increase the risk of getting cancer by a certain percentage over a given time period—numbers that can be difficult for people to understand. Some people overestimate their risk, whereas others underestimate it. Thus, some people who are informed of an increased cancer risk will worry needlessly.

Moreover, we don't know how to prevent most cancers, so the question is whether it is ethical to provide people with risk information upon which they cannot act. Notable exceptions are the BRCA1 and BRCA2 genes—as we saw in the first chapter, women with these mutations may elect to have their breasts and/or ovaries removed, whereas others may elect to undergo more frequent screening. Increased screening can detect the tumor at an earlier stage when it is more treatable. The same is true for a genetic condition known as hereditary nonpolyposis colorectal cancer, also called Lynch syndrome. This syndrome increases the risk of colon, rectal, and several other cancers. Increased screening may be useful in detecting the cancer early or even in removing growths before they develop into cancer.

Notably, one unintended consequence related to the Supreme Court ruling described at the beginning of this chapter is that any laboratory can now do a laboratory-developed test for genetic susceptibility such as BRCA1 and BRCA2. Additionally, any commercial lab can make a panel of genetic markers to compete with Myriad Genetics even if those markers are genes about which we know very little. Today, some patients are undergoing genetic testing with laboratory-developed tests for which there is little information about analytical validity, and some patients are undergoing genetic testing with panels that include genes about which we know very little.

More information about cancer risk can be found in Research Advocacy Network's publication *Understanding Cancer Risk*, available at: http://researchadvocacy.org/general-resources.

EXAMPLES OF INHERITED MUTATIONS THAT INCREASE CANCER RISK

Name of Condition	Gene(s) Affected	Type of Cancer
Hereditary breast and ovarian cancer syndrome	BRCA1, BRCA2	Breast, ovarian
Cowden syndrome	PTEN	Breast, uterus, thyroid
Hereditary non-polyposis colorectal cancer syndrome (Lynch syndrome)	DNA mismatch repair genes (MLH1, MSH2, MSH6, PMS2)	Colorectal, endometrial
Familial adenomatous polyposis	APC	Colorectal
Li-Fraumeni syndrome	TP53	Soft tissue sarcomas (tumor in fat, muscle, nerve, joint, blood vessel, bone, or deep skin), breast, leukemia, lung, brain, adrenal

Table information from American Society of Clinical Oncology. Cancer.Net. Available at: http://www.cancer.net. National Cancer Institute. Understand Cancer Series. Available at: http://www.cancer.gov/about-cancer/causes-prevention/genetics/genetic-testing-fact-sheet. Accessed July 22, 2015.

Ethical Issues in Analyzing a Person's Genome

A person may elect to have his or her genome analyzed as part of a research project to develop a molecular diagnostic. For these tests, people provide tissue samples to be analyzed—tissue samples that contain all of their DNA. Many of us want to help research progress by providing samples that investigators can use to develop new molecular diagnostics and treatments. However, we also want to make sure that our tissue is not misused in any way. As a result, safeguards are essential to ensure that our tissue and genetic information is used only in the ways we approve.

> The ethical issues that arise with tissue samples provided for medical research purposes (e.g., to develop new molecular diagnostics) also apply to tissue samples provided for non-medical purposes, such as ancestry evaluation.

Ethical, Legal, and Social Implications (ELSI) Program

Given that molecular biomarkers are evaluated using molecular diagnostics, ethical issues related to human genome research also apply to molecular diagnostics. Many of these ethical considerations were anticipated by the Human Genome Project's program devoted to studying these issues: the ethical, legal, and social implications (ELSI) research program. Some of these questions are listed in the following table.

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? 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? How reliable and useful is fetal genetic testing? 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.) How do we prepare healthcare professionals for the new genetics? How do we prepare the public to make informed choices? 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? Should parents have the right to have their minor children tested for adult-onset diseases? 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? Can people always control their behavior? What is considered acceptable diversity? Where is the line between medical treatment and enhancement?
Health and environmental issues concerning genetically modified foods (GM) and microbes	Are GM foods and other products safe to humans and the environment? 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? 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.

Specific Considerations with Tissue Samples

When we provide tissue samples for genomic analysis—whether for research or other purposes—special ethical considerations apply. The following table outlines some of the specific issues related to tissues provided for genomic analysis. More information related to tissue samples for research can be found in Research Advocacy Network's publication *Pathology and Tissue Research*, available at: http://researchadvocacy.org/general-resources.

ETHICAL ISSUES RELATED TO HUMAN TISSUE SAMPLES PROVIDED FOR GENOMIC ANALYSIS

Issue	Discussion
Identifiability	Can the tissue sample be linked to the person who provided it? More information means higher value to researchers but also more concern over individual privacy and confidentiality.
Privacy and Confidentiality	Can third parties access the genomic information and use it against the person who provided the sample? Current standards ensure the protection of information in research, but it is important to remain vigilant about this issue.
Distribution and Access	Who should be allowed to use the tissue sample in research? Some patients favor broad access to help research progress, whereas others want more control.
Commercialization	Should researchers or businesses be allowed to purchase tissue samples? Some people may agree to have their tissue sample sold for further research, whereas others would not allow it. People may feel differently about having their tissue sample sold for non-research purposes (e.g., would we welcome advertisements about new nutritional supplements designed to improve memory if we have the Alzheimer disease risk genes?).
Ownership	Who owns the tissue sample? Can patients continue to determine the use of their tissue samples once they have provided them? Some patients may want this control, whereas others may not want to be bothered each time a researcher wants to do a study.

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Chapter 7: How Can Advocates Use This Information?

As advocates, we need to understand the basic features of molecular diagnostics because they represent the implementation of precision medicine in cancer. It is not enough to know that a particular DNA sequence is associated with cancer risk or response to treatment—we must be able to accurately and reliably detect that sequence in humans. The information in the preceding chapters can help us gain a working knowledge of molecular diagnostics and the underlying molecules that they detect.

Advocates may be interested in a number of issues related to molecular diagnostics and, importantly, may be in a position to influence them. Following is a description of several relevant issues.

Communication of Molecular Diagnostic Results

As noted in Chapter 1, molecular diagnostics may be applied in many use contexts. Often, the results of these tests are presented to patients so that they can participate in health-related decisions. In such cases, it is critically important that patients understand this information—an outcome that can depend on the way the information is presented. Some people have difficulty understanding numeric values and may benefit from a picture showing the amount of cancer risk accounted for by a given mutation. The same can be said for test results that help determine risk of cancer recurrence or monitoring—patients may not know whether the results put them in a high, medium, or low risk category.

Advocates can address this situation in many ways, such as:
- Help healthcare providers find ways to present the results of molecular diagnostics in ways that patients can understand. Often, simpler is better (see *Understanding Cancer Risk*: http://researchadvocacy.org/general-resources).
- Work with the developers of molecular diagnostics to ensure that the results are presented in an understandable format.
- Develop materials explaining different risk values and what they mean for patients.

Interpretation of Molecular Diagnostic Results

Interpretation of molecular diagnostic results is not always straightforward—for patients or physicians. For example, when a tumor tissue sample shows a germline variant of uncertain significance, as discussed in Chapter 4, physicians may not know what preventive measures to advise patients to take. Likewise, if a patient's tumor is tested for a panel of genetic or protein abnormalities, many of these may or may not have any clinical relevance or utility, yet the patient may be led to believe, or may believe him/herself, that his/her treatment should differ from standard of care.

Patients and their families may be distressed and confused. Even the best communication strategies do not make it easy to understand this type of result. The information in this tutorial can help advocates participate in the discussion regarding variants of uncertain significance (VUS) and other results of molecular diagnostics that are difficult to interpret. Advocates working with the Specialized Program of Research (SPORE) in breast cancer at the Mayo Clinic developed a brochure for patients to explain VUS (pdf available at: http://tbf.me/a/BazZ6o)

Actionable Findings with Molecular Diagnostics

Ideally, the results of molecular diagnostics would tell us what steps we should take to optimize our health. In reality, few tests give such clear-cut information. As we've seen in the preceding chapters, companion diagnostics provide information about whether a given

targeted therapy is likely to work for a given cancer. These findings are actionable; that is, they suggest a course of action because they confirm that the "target" of the targeted therapy is present.

For many molecular diagnostics, the results may or may not be actionable depending on whom you ask. Experts often disagree about whether a given molecular diagnostic results warrants any action and/or what that action should be. Physicians in different specialty areas have different perspectives and may disagree based on their experience. Moreover, patients may disagree based on personal beliefs, experiences, and a host of other factors. In Chapter 1, we discussed Angelina Jolie's choice to have her breasts and ovaries removed based on a positive BRCA1 test result. However, this choice may not be right for everyone. For example, many women may opt for frequent screening instead of surgery. Still others may make a decision based on whether they have already borne children.

Advocates can take a variety of actions to ensure that patients are aware of different preventive treatment options and different views about what actions should be taken when faced with the results of molecular diagnostics for cancer. It sometimes helps patients to know that there is no one right way and that, in consultation with their doctor, they should choose the path that seems best for them.

Incidental Findings with Molecular Diagnostics

Genomic knowledge and molecular diagnostic techniques have advanced rapidly over the past few decades. It is now possible to sequence a person's entire genome, and some experts believe that this practice will become affordable and commonplace in the foreseeable future. Today, it is possible for molecular diagnostics to evaluate biomarkers for multiple conditions at once, which has the potential to turn up unexpected findings. For example, a person undergoing a screening test for colon cancer-related mutations may be found to have a mutation related to Alzheimer's disease. This is called an incidental finding.

Incidental findings are not new in medicine—most people have probably heard at least one story about a patient who went in to the doctor for one medical problem and was found to have another, entirely unrelated problem. However, as whole genome screenings become more common, incidental findings will increase. Advocates can be an important part of the conversation about incidental findings, ensuring that patients receive all of the information they want and none of the information that they don't, and raising awareness of the need for genomic counseling.

Clinical Validation of Molecular Diagnostics

Molecular diagnostics cannot just be good at detecting specific biomarkers, they must also provide clinically relevant information; that is, molecular diagnostics must have clinical validity. Many advocates are interested in this issue and want to make sure that, if patients undergo molecular diagnostic tests, the information will be useful to them. Advocates can help ensure that the molecular diagnostics used in clinical practice are clinically valid by supporting and encouraging policies that require clinical validation studies.

Advocates may also help by encouraging patients to participate in clinical validation studies. In fact, only a small percentage of the patients who would be inclined to participate in cancer research studies actually do. According to the National Cancer Institute, one reason for this is because patients are not always informed about the studies by their healthcare providers. Advocates can help by encouraging physicians to tell their patients about research studies, providing educational materials, and elaborating the advocate point of view regarding the urgency of clinical validation research. Research advocates can also help ensure that the clinical trials are designed in such a way that patients understand them, are willing to do what is needed by the trial, and are willing to stay in the study. This typically involves working with the research team developing study protocols.

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. With the first biosimilar medicines being approved in the United State, it is important for advocates to understand the issues and possibilities these medicines represent for advancements in patient care. We hope that this information will be helpful to advocates and others interested in advancing the science and improving care for cancer patients.

Access to Molecular Diagnostics

Ideally, all people who could benefit from validated molecular diagnostic tests would be able to receive them. In reality, not all people do. Molecular diagnostic testing can be influenced by physician practice, insurance coverage, and other factors. Many advocates are interested in helping to make validated molecular diagnostics available to all who need them.

About Research Advocacy Network

Research Advocacy Network is committed to improving patient care through research. Our goals are to get results of research studies for new treatments and improved methods of detection of cancer 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, no organization has focused on advancing research through advocacy. RAN works with advocates and organizations to effectively integrate advocates into research activities. Please learn more about us at our website at www.researchadvocacy.org or contact us about our work by e-mailing us at info@researchadvocacy.org or FAX at 888-466-8803.

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