ARNA Genomics

ARNA BC - Product Overview
v.1.2
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Market review

What Is Cancer?

Cancer is a group of more than 100 different diseases. It can develop almost anywhere in the human body. It starts when cells grow out of control and crowd out normal cells. This makes it hard for the body to work the way it should.

In all types of cancer, some of the body’s cells begin to divide without stopping and spread into surrounding tissues.

Normally, human cells grow and divide to form new cells as the body needs them. When cells grow old or become damaged, they die, and new cells take their place.

When cancer develops, however, this orderly process breaks down. As cells become more and more abnormal, old or damaged cells survive when they should die, and new cells form when they are not needed. These extra cells can divide without stopping and may form growths called tumors.

Many cancers form solid tumors, which are masses of tissue. Cancers of the blood, such as leukemias, generally do not form solid tumors.

Cancerous tumors are malignant, which means they can spread into, or invade, nearby tissues. In addition, as these tumors grow, some cancer cells can break off and travel to distant places in the body through the blood or the lymph system and form new tumors far from the original tumor.

Unlike malignant tumors, benign tumors do not spread into, or invade, nearby tissues. Benign tumors can sometimes be quite large, however. When removed, they usually don’t grow back, whereas malignant tumors sometimes do. Unlike most benign tumors elsewhere in the body, benign brain tumors can be life threatening.

Some cancers grow and spread fast. Others grow more slowly. They also respond to treatment in different ways. Some types of cancer are best treated with surgery; others respond better to drugs called chemotherapy.

There are five main categories of cancer:

- **Carcinomas** begin in the skin or tissues that line the internal organs.
- **Sarcomas** develop in the bone, cartilage, fat, muscle or other connective tissues.
- **Leukemia** begins in the blood and bone marrow.
- **Lymphomas** start in the immune system.
- **Central nervous system cancers** develop in the brain and spinal cord.
We have collected from publicly available sources videos, that in our opinion describe cancer and will give you a basic understanding of how this disease works:

Table 1. Publicly available videos describing what is cancer.

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<thead>
<tr>
<th>Clip caption</th>
<th>Duration (mm:ss)</th>
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<tbody>
<tr>
<td>Animated Introduction to Cancer Biology (Full Documentary)</td>
<td>12:07</td>
<td><a href="https://youtu.be/46Xh7OFkkCE/">https://youtu.be/46Xh7OFkkCE/</a></td>
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<td>What is cancer?</td>
<td>5:04</td>
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<td>Metastasis and angiogenesis</td>
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<td><a href="https://youtu.be/eSwG5O_kiOQ/">https://youtu.be/eSwG5O_kiOQ/</a></td>
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<tr>
<td>How does cancer spread through the body?</td>
<td>4:43</td>
<td><a href="https://youtu.be/OeigJn8UJNQ/">https://youtu.be/OeigJn8UJNQ/</a></td>
</tr>
<tr>
<td>How do cancer cells behave differently from healthy ones?</td>
<td>3:50</td>
<td><a href="https://youtu.be/BmFEoCFDi-w/">https://youtu.be/BmFEoCFDi-w/</a></td>
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Normal cells may become cancer cells. Before cancer cells form in tissues of the body, the cells go through abnormal changes called hyperplasia and dysplasia. In hyperplasia, there is an increase in the number of cells in an organ or tissue that appear normal under a microscope. In dysplasia, the cells look abnormal under a microscope but are not cancer. Hyperplasia and dysplasia may or may not become cancer (source: Terese Winslow).
Cancer starts when gene changes make one cell or a few cells begin to grow and multiply too much. This may cause a growth called a tumor.

Cancer is a genetic disease — that is, it is caused by changes to genes that control the way our cells function, especially how they grow and divide.

Most cancers start due to gene changes that happen over a person’s lifetime. More rarely cancers start due to inherited faulty genes passed down in families. Cancer-causing environmental exposures include substances, such as the chemicals in tobacco smoke, and radiation, such as ultraviolet rays from the sun.

**DNA and Genes**

Inside almost every single cell in your body is a structure called the nucleus, which is the control center of the cell. Inside the nucleus are 23 pairs of chromosomes. These are long strings of DNA.
DNA stands for deoxyribonucleic acid. Each string of DNA is shaped like a twisted ladder, which is called a double helix.

You have more than two meters of DNA inside every cell, but it is very tightly coiled up so it all fits. DNA can be thought of as a code, containing all the instructions that tell a cell what to do. It is made up of genes. Humans have around 25,000 genes in total. You inherit half your DNA from your mother and half from your father, so you have two copies of every gene. Your genes carry all the information that makes you, you. They tell your body to have blonde hair, or brown skin, or green eyes for example. And they tell your cells what sort of cell to be, how to behave, when to grow and reproduce, and when to die. Some genes control how much each cell grows and divides.

Our genes pick up mistakes that occur when cells divide. These mistakes are called faults or mutations and happen throughout our lives. They are caused by the natural processes in our cells, and by various other factors. Sometimes people inherit certain faulty genes from their parents that mean they have an increased risk of cancer.

Oncogenes are genes that, under normal circumstances, play a role in telling cells to start multiplying and dividing. We can think of oncogenes as being a bit like the accelerator pedal in a car. When they are activated, they speed up a cell's growth rate. When one becomes damaged it is like the accelerator becoming stuck down. That cell, and all the cells that grow from it, are permanently instructed to divide. So a cancer develops.

The DNA in every cell in our body is constantly in danger of being damaged. But cells contain many different proteins whose job is to repair damaged DNA. Thanks to these, most DNA damage is repaired immediately, with no ill effects. But if the DNA damage occurs to a gene that makes a DNA repair protein, a cell has less ability to repair itself. So errors will build up in other genes over time and allow a cancer to form.

Scientists have found these genes to be damaged in some human cancers, including bowel cancer.
Apoptosis - a “natural” way of cell death
Some genes normally tell a cell to self destruct if it has become too old or damaged. This is called apoptosis or programmed cell death. It is a highly complex and very important process. Cells usually die whenever something goes wrong, to prevent a cancer forming. There are many different genes and proteins involved in apoptosis. If these genes get damaged, a faulty cell can survive rather than die and it becomes cancerous.

Necrosis - another way of cells dying.
Necrosis is caused by factors external to the cell or tissue, such as infection, toxins, or trauma which result in the unregulated digestion of cell components.
In contrast, apoptosis is a naturally occurring programmed and targeted cause of cellular death.
While apoptosis often provides beneficial effects to the organism, necrosis is almost always detrimental and can be fatal.

Picture 5. Structural changes of cells undergoing necrosis and apoptosis
It is axiomatic that all cells will die if a process necessary for their continued survival is blocked. In addition to being mortal, most animal cells can also be suicidal, meaning that they bear mechanisms whose physiological role is to cause their own death. One such physiological cell suicide process is termed apoptosis or programmed cell death. Cells that kill themselves by implementing this process typically exhibit a characteristic morphology. Unlike the worm, mammals have an additional cell death pathway that is controlled by certain members of the tumor necrosis factor (TNF) receptor family, often referred to as ‘death receptors’

Thirty years ago, pathologists described a pattern that they recognized in cells dying within tissues (Kerr et al., 1972). The condensed and fragmented nuclei and bodies of isolated cells are the hallmarks of apoptotic death and are associated with only rare inflammatory cells including largely white blood cells such as T-cells and macrophages. They could be distinguished from the blander necrotic cell bodies, often occurring in sheets of cells, and frequently associated with the cardinal sign of chronic inflammation, the activated white blood cell.

Necrosis is now defined by the absence of programmed cell death or by the release of individual molecules, which are metaphorically the biologic equivalent of a gunshot, or a baby crying, or the smelling of smoke — pattern recognition events which foretell “danger” and the need for attention by the host. During necrotic death, little is known about the release of mediators and how this might be regulated.

Cancer Is Primarily a Disorder of Cell Death, Not Cell Growth

Tumors arising in the setting of chronic inflammation, associated with rounds of cell death and reparative cell growth. The presence of chronic inflammation and immune cells actually promote the development of emergent cancer.

This results in the sense of “tumor take,” the ability to discern palpable masses beneath the skin or enumerate metastases within the viscera. What has not been so apparent to most, is that these cancers, for the most part, represent extraordinarily selected tumors derived from a subset of tumors, particularly the most common epithelial tumors — those of the breast, colon, lung and prostate.

As the latest researches science shows that from the earliest stage cancer cells starting dying from necrosis process in the absence of apoptosis process when the glucose metabolism is blocked. "This finding shows, for the first time, that cancer cells are unusually sensitive to dying by necrosis, when their ability to metabolize glucose is blocked," said Craig Thompson, MD

Until recently, necrosis, unlike apoptosis, was considered as passive and unregulated form of cell death. However, during the last decade a number of experimental data demonstrated that, except under extreme conditions, necrosis may be a well-regulated process activated by rather specific physiological and pathological stimuli. Mitochondrial collapse activates various proteases (e.g., calpains, cathepsin) and phospholipases, and eventually leads to plasma membrane destruction, a hallmark of necrotic cell death. Necrosis, in contrast to apoptosis, usually evokes powerful inflammatory response, which may participate in tumor regression during anticancer therapy. On the other hand, excessive spontaneous necrosis during tumor development may lead to more aggressive tumors due to stimulatory role of necrosis-induced inflammation on their growth.
Breast Cancer

Below we have provided a brief list of publicly available videos, that provide explanation on what exactly the breast cancer is:

<table>
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<tr>
<th>Clip caption</th>
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<tr>
<td>Introduction</td>
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</tr>
<tr>
<td>Breast Anatomy</td>
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<td><a href="https://youtu.be/IJPgVVY1h9-8">https://youtu.be/IJPgVVY1h9-8</a></td>
</tr>
<tr>
<td>Breast Cancer - What is Cancer?</td>
<td>2:00</td>
<td><a href="https://youtu.be/rEV_bc32HaY">https://youtu.be/rEV_bc32HaY</a></td>
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<td>Breast Cancer - Types of Tumors</td>
<td>2:34</td>
<td><a href="https://youtu.be/UAyYNjxIKoyA">https://youtu.be/UAyYNjxIKoyA</a></td>
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<td>Diagnosis - Why?</td>
<td>1:30</td>
<td><a href="https://youtu.be/DByVMajO7AI">https://youtu.be/DByVMajO7AI</a></td>
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<tr>
<td>Diagnosis - Diagnostic Method</td>
<td>3:50</td>
<td><a href="https://youtu.be/YrQW6GaK1e8">https://youtu.be/YrQW6GaK1e8</a></td>
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<tr>
<td>Diagnosis - Biopsy</td>
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<td><a href="https://youtu.be/h79vSF5f5g">https://youtu.be/h79vSF5f5g</a></td>
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<tr>
<td>Diagnosis - Lab Tests</td>
<td>2:11</td>
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<tr>
<td>Types &amp; Stages - Introduction</td>
<td>1:13</td>
<td><a href="https://youtu.be/qm7gd5bYKjQ">https://youtu.be/qm7gd5bYKjQ</a></td>
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<tr>
<td>Types &amp; Stages - Stage 0 &amp; 1</td>
<td>0:53</td>
<td><a href="https://youtu.be/vYa3nW8dgYU">https://youtu.be/vYa3nW8dgYU</a></td>
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<td>Types &amp; Stages - Stage 2 Breast Cancer</td>
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<td>Types &amp; Stages - Stage 4 Breast Cancer</td>
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<tr>
<td>Types &amp; Stages - Inflammatory Breast Cancer</td>
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<td><a href="https://youtu.be/vAo3aBf33s4">https://youtu.be/vAo3aBf33s4</a></td>
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Breast cancer is a group of diseases that affects breast tissue. Both women and men can get breast cancer, though it is much more common in women. Other than skin cancer, breast cancer is the most common cancer among women in the World. Some women are at higher
risk for breast cancer than others because of their personal or family medical history or because of certain changes in their genes.

*The term “breast cancer” refers to a malignant tumor that has developed from cells in the breast.*

Breast cancer is the most common cancer in women worldwide, with nearly 1.7 million new cases diagnosed in 2012 (second most common cancer overall). This represents about 12% of all new cancer cases and 25% of all cancers in women. It is the fifth most common cause of death from cancer in women.

Breast cancer risk doubles each decade until menopause, after which the increase slows. However, breast cancer is more common after menopause. Survival rates for breast cancer vary worldwide, but in general rates have improved. This is because breast cancer is diagnosed at an earlier and localised stage in nations where populations have access to medical care, and progressive improvement in treatment strategies. In many countries with advanced medical care, the five-year survival rate of early stage breast cancers is 80–90 per cent, falling to 24 per cent for breast cancers diagnosed at a more advanced stage.

Breast cancer occurs when cells in the breast divide and grow without their normal control. Tumors in the breast tend to grow slowly. By the time a lump is large enough to feel, it may have been growing for as long as 10 years. (Some tumors are aggressive and grow much faster.) Between 50-75 percent of breast cancers begin in the milk ducts, about 10-15 percent begin in the lobules and a few begin in other breast tissues.

Over time, cancer cells can invade nearby healthy breast tissue and make their way into the underarm lymph nodes, small organs that filter out foreign substances in the body. If cancer cells get into the lymph nodes, they then have a pathway into other parts of the body. The breast cancer’s stage refers to how far the cancer cells have spread beyond the original tumor.

Breast cancer is always caused by a genetic abnormality (a “mistake” in the genetic material). However, only 5-10% of cancers are due to an abnormality inherited from your mother or father. Instead, 85-90% of breast cancers are due to genetic abnormalities that happen as a result of the aging process and the “wear and tear” of life in general.
Breast cancer is typically detected either during a screening examination, before symptoms have developed, or after a woman notices a lump. Most masses seen on a mammogram and most breast lumps turn out to be benign; that is, they are not cancerous, do not grow uncontrollably or spread, and are not life-threatening. When cancer is suspected, microscopic analysis of breast tissue is necessary for a definitive diagnosis and to determine the extent of spread (in situ or invasive) and characterize the type of the disease. The tissue for microscopic analysis can be obtained via a needle or surgical biopsy. Selection of the type of biopsy is based on individual patient clinical factors, availability of particular biopsy devices, and resources.

**Non-invasive breast cancer - ductal carcinoma in situ (DCIS)**

Ductal carcinoma in situ (DCIS) occurs when abnormal cells grow inside the milk ducts, but have not spread to nearby tissue or beyond. The term "in situ" means "in place." With DCIS, the abnormal cells are still inside the ducts. DCIS is a non-invasive breast cancer. You may also hear the terms “pre-invasive” or “pre-cancerous” to describe DCIS.
Although DCIS is non-invasive, without treatment, it can develop into invasive breast cancer.

**Invasive breast cancer**

Invasive breast cancer occurs when abnormal cells from inside the milk ducts or lobules break out into nearby breast tissue. Cancer cells can travel from the breast to other parts of the body through the blood stream or the lymphatic system. They may travel early in the process when a tumor is small or later when a tumor is large. The lymph nodes in the underarm area (axillary lymph nodes) are the first place breast cancer is likely to spread.

Other less common types of breast cancer include medullary, mucinous, tubular, metaplastic, and papillary breast cancer, as well as other even rarer types. Inflammatory breast cancer is a faster growing type of cancer that accounts for about 1% to 5% of all breast cancers. At first it may be misdiagnosed as a breast infection because there is often swelling of the breast and redness of the breast skin that starts suddenly, and there is no breast mass or lump. Paget’s disease is a type of cancer that begins in the ducts of the nipple. The skin often appears scaly and may be itchy. Although it is usually in situ, it can also be an invasive cancer.

Most breast cancers are invasive, or infiltrating. These cancers have broken through the walls of the glands or ducts where they originated and grown into surrounding breast tissue.

The Surveillance, Epidemiology, and End Results (SEER) Summary Stage system is more simplified and is commonly used in reporting cancer registry data and for public health research and planning.

According to the SEER Summary Stage system:

- Local stage refers to cancers that are confined to the breast (corresponding to stage I and some stage II cancers in the TNM staging system).
- Regional stage refers to tumors that have spread to surrounding tissue or nearby lymph nodes (generally corresponding to stage II or III cancers, depending on size and lymph node involvement).
- Distant stage refers to cancers that have metastasized (spread) to distant organs or lymph nodes above the collarbone (corresponding to some stage IIIc and all stage IV cancers).

Although we generally refer to breast cancer as a single disease, it is important to note that it is distinguished by up to 21 distinct histological subtypes and at least four different molecular subtypes, which are biologically variable in presentation, response to treatment, and outcomes, and also associated with distinct risk factors. Gene expression profiling techniques have allowed us to better understand the genetic variability among tumors; however this is a costly and complicated process and is not currently standard practice. More convenient approximations of molecular subtypes have been identified using routinely evaluated biological markers, including the presence or absence of hormone (estrogen or progesterone) receptors (HR+/HR-) and excess levels of human epidermal growth factor receptor 2 (HER2+/HER2-), a growth-promoting protein.
The four main molecular subtypes

Luminal A (HR+/HER2-)
Most (74%) breast cancers express the estrogen receptor (ER+) and/or the progesterone receptor (PR+) but not HER2 (HER2-). These cancers tend to be slow-growing and less aggressive than other subtypes. Luminal A tumors are associated with the most favorable prognosis, particularly in the short term, in part because expression of hormone receptors is predictive of a favorable response to hormonal therapy.

Luminal B (HR+/HER2+).
Like luminal A breast cancers, luminal B breast cancers are ER+ and/or PR+ and are further defined by being highly positive for Ki67 (indicator of a large proportion of actively dividing cells) or HER2. About 10% of breast cancers are ER+ and/or PR+ and HER2+. Luminal B breast cancers tend to be higher grade and more aggressive than luminal A breast cancers.

HER2-enriched (HR-/HER2+).
About 4% of breast cancers produce excess HER2 and do not express hormone receptors. These cancers tend to grow and spread more aggressively than other breast cancers and are associated with poorer short-term prognosis compared to ER+ breast cancers. However, the recent widespread use of targeted therapies for HER2+ cancers has reversed much of the adverse prognostic impact of HER2 overexpression.

Breast cancer typically produces no symptoms when the tumor is small and most easily treated. Therefore, it is very important for women to follow recommended screening guidelines for detecting breast cancer at an early stage. When breast cancer has grown to a size that can be felt, the most common physical sign is a painless lump. Sometimes breast cancer can spread to underarm lymph nodes and cause a lump or swelling, even before the original breast tumor is large enough to be felt. Less common signs and symptoms include breast pain or heaviness; persistent changes to the breast, such as swelling, thickening, or redness of the breast’s skin; and nipple abnormalities such as spontaneous discharge (especially if bloody), erosion, or retraction. It is important to note that pain (or lack thereof) does not indicate the presence or the absence of breast cancer. Any persistent change in the breast should be evaluated by a physician as soon as possible.

Triple negative (HR-/HER2-).
Overall, about 12% of breast cancers are triple negative, so called because they are ER-, PR-, and HER2-; however, these cancers are nearly two times more common in black women than white women in the US. They are also more common in premenopausal women and those with a BRCA1 gene mutation. The majority (about 75%) of triple negative breast cancers fall into the basal-like subtype. Triple negative breast cancers have a poorer short-term prognosis than other breast cancer types, in part because there are currently no targeted therapies for these tumors.
Breast Cancer Screening

Screening is used to look for cancer before you have any symptoms or signs. Scientists have developed, and continue to develop, tests that can be used to screen a person for specific types of cancer. The overall goals of cancer screening are to:

- Lower the number of people who develop the disease
- Lower the number of people who die from the disease, or eliminate deaths from cancer altogether
- Identify people with a higher risk of a specific type of cancer who may need screening more often due to genetic mutations or diseases

Finding breast cancer early and getting state-of-the-art cancer treatment are the most important strategies to prevent deaths from breast cancer. Breast cancer that’s found early, when it’s small and has not spread, is easier to treat successfully. Getting regular screening tests is the most reliable way to find breast cancer early.

It is especially important that women are regularly screened to increase the chance that a breast cancer is detected early before it has spread. Recommended screening intervals are based on the duration of time a breast cancer is detectable before symptoms develop. Combined results from randomized controlled screening trials suggest that mammography reduces the risk of dying from breast cancer by about 20%, whereas studies of modern mammography screening programs in Europe and Canada found that the risk of breast cancer death among women exposed to screening was reduced by more than 40%. Early detection of breast cancer by mammography also leads to a greater range of treatment options, including less-extensive surgery (e.g., breast-conserving surgery like lumpectomy versus mastectomy) and the use of chemotherapy with fewer serious side effects, or even, in some cases, the option to forgo chemotherapy. However, mammography screening does have potential harms.

Main types of screening

Breast cancer screening means checking a woman’s breasts for cancer before she has any symptoms. A mammogram is an X-ray picture of the breast. Mammograms are the best way to find breast cancer early, when it is easier to treat and before it is big enough to feel or cause symptoms.

Mammography

Mammography is a low-dose x-ray procedure that allows visualization of the internal structure of the breast. There are three main types of mammography: film, digital, and digital breast tomosynthesis. Film mammography uses general-purpose x-ray equipment to record images of the breast, whereas digital mammography uses more specialized computerized equipment and delivers lower doses of radiation. Film mammography has been largely replaced by digital mammography, which appears to be even more accurate for women younger than 50 years of age and for those with dense breast tissue.

Please, watch the following videos, which explain mammography in details:

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Table 3. Publicly available videos describing what is mammography.
The most advanced image method is 3-dimensional mammography. In 2011, the FDA approved the use of digital breast tomosynthesis or 3-dimensional (3-D) mammography, which constructs a 3D image of the breast with multiple high-resolution x-rays, to be used in combination with a 2-D digital mammography image. The benefits and risks of tomosynthesis in community practice are still being assessed. A recent study indicated the addition of breast tomosynthesis to digital mammography may reduce false positives and detect slightly more invasive cancers compared to digital mammography alone. However, when the 2-D images are produced separately from the tomographic images, women receive about twice the radiation dose. Recently, the FDA approved the use of tomographic images to produce synthetic, conventional 2-D images, thus reducing the radiation dose to that similar to conventional digital mammography. This newer type of mammographic screening is not yet available in all communities or fully covered by health insurance.

Mammography sometimes leads to follow-up examinations, including biopsies, when there is no cancer; these are referred to as false-positive test results. A false-positive is most likely following a woman’s initial screening mammogram. Other factors that increase the likelihood of a false positive include the use of postmenopausal hormone therapy and having more dense breast tissue. On average, 10% of women will be recalled from each screening examination for further testing (most often additional mammographic views of areas of suspicion), but only 5% of these women will have cancer. According to one US study, over the course of 10 screening examinations, about one-half of women will experience a false-positive, and about 19% will undergo biopsy.

As with all screening tests, mammography is not 100% effective. Not all breast cancer will be detected by a mammogram, and some breast cancers that are screen-detected still have poor prognosis.

For example one of the latest research to detect the sensitivity of mammography and MRI show that sensitivity of mammography is 66% and MRI with gadolinium injection is 81%.

Despite these limitations, mammography is the single most effective method of early detection since it can often identify cancer several years before physical symptoms develop. It is the position of the American Cancer Society that the balance of benefits to possible harms strongly supports the value of regular breast cancer screening in women for whom it is recommended.
According to the National Health Interview Survey, the percentage of women 45 years of age and older who reported having had a mammogram within the past 2 years was 69% in 2013. Among women of all races combined 45 years of age and older, reported rates of mammography range from 66% in Wyoming to 87% in Massachusetts.

**Magnetic resonance imaging (MRI)**

MRI uses magnetic fields instead of x-rays to produce very detailed, cross-sectional images of the body. MRI exams for breast imaging use a contrast material (usually gadolinium DTPA) that is injected into a vein in the arm before or during the exam to improve the ability to capture detailed images of breast tissue. MRIs should supplement, but not replace, mammography screening.

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<tr>
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<tbody>
<tr>
<td>Breast MRI</td>
<td>Cedars-Sinai</td>
<td>2:10</td>
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<tr>
<td>How To Catch Breast Cancer Early: Stanford Doctors Explain Mammography Options</td>
<td>3:35</td>
<td><a href="https://youtu.be/ql11xKFMKg4">https://youtu.be/ql11xKFMKg4</a></td>
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<tr>
<td>Breast Cancer</td>
<td>Breast Biopsy</td>
<td>Nucleus Health</td>
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<td>Breast Cancer</td>
<td>Staging</td>
<td>Nucleus Health</td>
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<td>Breast Cancer Pathology</td>
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<td>Breast Cancer Pathogenesis</td>
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However, many hospitals and imaging centers do not have dedicated breast MRI equipment available. It is important that screening MRIs are done at facilities that are capable of performing an MRI-guided breast biopsy in case abnormalities are found. Otherwise, the scan must be repeated at another facility if a biopsy is necessary. Although MRI is more expensive than mammography, most major insurance companies will cover some portion of the costs if a woman can be shown to be at high risk.

Breast MRI is not a perfect tool. Although it is generally considered more sensitive for picking up breast cancer than mammography, it also can miss some cancers that would be detected by mammography. That is why breast MRI is recommended only in combination with other tests, such as mammogram or ultrasound.
Breast ultrasound
Breast ultrasound is sometimes used to evaluate abnormal findings from a screening or diagnostic mammogram or physical exam. Studies have shown that ultrasound detects more cancer than mammography alone when screening women with dense breast tissue; however, it also increases the likelihood of false-positive results. The use of ultrasound instead of mammograms for breast cancer screening is not recommended. An ultrasound can distinguish between a solid mass, which may be cancer, and a fluid-filled cyst, which is usually not cancer.

Clinical breast examination (CBE)
The American Cancer Society no longer recommends CBE for average-risk asymptomatic women based on lack of clear benefits for CBE in conjunction with screening mammography or alone. Compared to mammography alone, CBE plus mammography has been shown to detect only a small proportion of breast cancer tumors and increases the probability of false-positives. Given the time constraints of a clinical visit, the Society encourages clinicians to use this time to counsel women on the importance of being alert to breast changes and the potential benefits, harms, and limitations of screening mammography or to address other important aspects of preventive services.

Breast self-awareness
Although the American Cancer Society no longer recommends that all women perform monthly breast self-exams (BSE), all women should become familiar with both the appearance and feel of their breasts and report any changes promptly to their physician. Experts have concluded that self-awareness seems to be at least as effective for detecting breast cancer as structured BSE. Women who detect their own breast cancer usually find it outside of a structured breast self-exam while bathing or getting dressed. If symptoms develop, women should contact a doctor immediately, even after a recent normal mammogram. However, most lumps are not abnormal, and for women who are still menstruating, they can appear and disappear with the menstrual cycle. Most breast lumps are not cancerous.

Biopsy
The most common surgical test used to diagnose breast cancer is a biopsy. A biopsy is the removal of a small amount of tissue for examination under a microscope. Other tests can suggest that cancer is present, but only a biopsy can make a definite diagnosis. There are different types of biopsies, classified by the technique and/or size of the needle used to collect the tissue sample.

Fine needle aspiration biopsy
This type of biopsy uses a thin needle to remove a small sample of cells from a suspicious lump.

Core needle biopsy
This procedure uses a thicker needle to remove a larger sample of tissue. It is usually the preferred biopsy technique to find out whether an abnormality discovered during a physical examination or on an imaging test is cancer. A vacuum-assisted biopsy removes
several large cores of tissue. Medication to block the awareness of pain, called local anesthesia, is used to reduce a person’s discomfort during the procedure.

Image-guided biopsy

This test is done when a distinct lump can’t be felt, but an abnormality is seen with an imaging test, such as on a mammogram. An image-guided biopsy can be done using a fine needle, core needle, or vacuum-assisted needle, depending on the amount of tissue that needs to be removed. During the procedure, the needle is guided to the best location with the help of an imaging technique, such as mammography, ultrasound, or MRI. A stereotactic biopsy is done using mammography to help guide the needle. A small metal clip may be put into the breast to mark where the biopsy sample was taken in case the tissue is cancerous and more surgery is needed. This clip is usually titanium so it will not cause problems with future imaging tests, but check with your doctor before you have any additional tests or scans.

Surgical biopsy

This type of biopsy removes the largest amount of tissue. A surgical biopsy is either incisional if it removes part of the lump or excisional if it removes the entire lump. Because surgery is best done after a cancer diagnosis has been made, a surgical biopsy is usually not the recommended procedure for diagnosing breast cancer. Most often, non-surgical core biopsies are recommended to diagnose breast cancer. This means that only one surgical procedure is needed to remove the tumor and to take samples of the lymph nodes.

After a biopsy, a pathologist will look very closely at the tissue that was removed using a microscope. Based on this examination, the pathologist can tell which area of the breast the cancer started in (ductal or lobular), whether the tumor has spread outside this area (invasive or in situ), and how different the cancer cells look from healthy breast cells (the grade). If the tumor was removed, the healthy tissue around the edges of the tumor, called the margins, will also be examined to see if cancer cells are present and to measure their distance from the tumor, which is referred to as the margin width.

The most common surgical test used to diagnose breast cancer is a biopsy. A biopsy is the removal of a small amount of tissue for examination under a microscope. Other tests can suggest that cancer is present, but only a biopsy can make a definite diagnosis. There are different types of biopsies, classified by the technique and/or size of the needle used to collect the tissue sample.

A woman at average risk doesn’t have a personal history of breast cancer, a strong family history of breast cancer, or a genetic mutation known to increase risk of breast cancer (such as BRCA), and has not had chest radiation therapy before the age of 30. (See below for guidelines for women at higher than average risk.)

- **Women between 40 and 44** have the option to start screening with a mammogram every year.
- **Women 45 to 54** should get mammograms every year.
- **Women 55 and older** can switch to a mammogram every other year, or they can choose to continue yearly mammograms. Screening should continue as long as a woman is in good health and is expected to live 10 more years or longer.
- **All women** should understand what to expect when getting a mammogram for breast cancer screening – what the test can and cannot do.
American Cancer Society recommendations for the early detection of breast cancer vary depending on a woman’s age and include mammography, as well as magnetic resonance imaging (MRI) for women at high risk. In 2015, the Society updated its breast cancer screening guideline for average-risk women, and the most recent guideline for MRI use for high-risk women was released in 2007.

For the advanced countries like England 'Two-week wait' is the most common route to diagnosing breast cancer. Around half (51%) of female invasive breast cancer cases in England are diagnosed via the ‘two-week wait’ referral route. This proportion is high compared with the average across all cancer types. Round 8 in 10 (79%) of these cases with known stage are diagnosed early (stage I or II). Almost a third (31%) of female invasive breast cancer cases in England are detected by screening. This proportion is high compared with the average across all cancer types. More than 9 in 10 (94%) cases with known stage are diagnosed early (stage I or II).

**Statistics at a glance**

Breast cancer is the second most common cancer in the world and, by far, the most frequent cancer among women with an estimated 1.67 million new cancer cases diagnosed in 2012 (25% of all cancers). It is the most common cancer in women both in more and less developed regions with slightly more cases in less developed (883,000 cases) than in more developed (794,000) regions. Incidence rates vary nearly four-fold across the world regions, with rates ranging from 27 per 100,000 in Middle Africa and Eastern Asia to 92 in Northern America.

Breast cancer ranks as the fifth cause of death from cancer overall (522,000 deaths) and while it is the most frequent cause of cancer death in women in less developed regions (324,000 deaths, 14.3% of total), it is now the second cause of cancer death in more developed regions (198,000 deaths, 15.4%) after lung cancer. The range in mortality rates between world regions is less than that for incidence because of the more favorable survival of breast cancer in (high-incidence) developed regions, with rates ranging from 6 per 100,000 in Eastern Asia to 20 per 100,000 in Western Africa.

Breast cancer is the most commonly diagnosed cancer among women in 140 countries worldwide, and cervical cancer is the most common in 39 countries.

A few countries have other cancer types as the most commonly diagnosed in women, such as lung cancer in China and North Korea, liver cancer in Mongolia and Laos, and thyroid cancer in South Korea. There is more diversity in the most common cause of cancer death among women. Breast is the most common cause of cancer death in 103 countries, followed by cervix in 43 countries and lung in 27 countries. Other most-common causes of cancer death among women include stomach in Bhutan, Peru, El Salvador, Guatemala, and Tajikistan; liver in Laos, Mongolia, and The Gambia; colorectum in Japan and Slovakia; and esophagus in Turkmenistan.
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**Picture 7. Estimated number of incident cases, worldwide in 2012**

**Picture 8. Estimated age-standardised rates (World) per 100,000**
**Picture 9. Estimated number of breast cancer incident cases in 2012 worldwide, total 1.67 mln.**

**Picture 10. Estimated number of deaths from breast cancer worldwide in 2012, total 523 th. cases.**
The countries with the top 20 highest incidence of breast cancer in 2012 are given in the table below.

- Belgium had the highest rate of breast cancer, followed by Denmark and France.
- Slightly more cases of breast cancer were diagnosed in less developed countries (53%).
- The highest incidence of breast cancer was in Northern America and Oceania; and the lowest incidence in Asia and Africa.
Table 5. Top 20 age-standardized incidents of breast cancer by countries (for women only, 2012).

<table>
<thead>
<tr>
<th>Rank</th>
<th>Country</th>
<th>Age-Standardised Rate per 100,000 (World)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Belgium</td>
<td>111.9</td>
</tr>
<tr>
<td>2</td>
<td>Denmark</td>
<td>105.0</td>
</tr>
<tr>
<td>3</td>
<td>France (metropolitan)</td>
<td>104.5</td>
</tr>
<tr>
<td>4</td>
<td>The Netherlands</td>
<td>99.0</td>
</tr>
<tr>
<td>5</td>
<td>Bahamas</td>
<td>98.9</td>
</tr>
<tr>
<td>6</td>
<td>Iceland</td>
<td>96.3</td>
</tr>
<tr>
<td>7</td>
<td>United Kingdom</td>
<td>95.0</td>
</tr>
<tr>
<td>8</td>
<td>Barbados</td>
<td>94.7</td>
</tr>
<tr>
<td>9</td>
<td>United States of America</td>
<td>92.9</td>
</tr>
<tr>
<td>10</td>
<td>Ireland</td>
<td>92.3</td>
</tr>
<tr>
<td>11</td>
<td>French Polynesia</td>
<td>92.2</td>
</tr>
<tr>
<td>12</td>
<td>Germany</td>
<td>91.6</td>
</tr>
<tr>
<td>13</td>
<td>Italy</td>
<td>91.3</td>
</tr>
<tr>
<td>14</td>
<td>Finland</td>
<td>89.4</td>
</tr>
<tr>
<td>15</td>
<td>Luxembourg</td>
<td>89.1</td>
</tr>
<tr>
<td>16</td>
<td>New Caledonia</td>
<td>87.6</td>
</tr>
<tr>
<td>17</td>
<td>Australia</td>
<td>86.0</td>
</tr>
<tr>
<td>18</td>
<td>Malta</td>
<td>85.9</td>
</tr>
<tr>
<td>19</td>
<td>New Zealand</td>
<td>85.0</td>
</tr>
<tr>
<td>20</td>
<td>Switzerland</td>
<td>83.1</td>
</tr>
</tbody>
</table>
Global breast cancer burden incidence and mortality: 2015-2024

Most common cancer among women
- 19.7 million cases in next decade
- 10.6 million cases in less developed countries
- By 2020, over 1 million cases per year in LMCs

Most common cancer killer among women
- 5.8 million women will die in next decade
- 3.9 million deaths in less developed countries
- >1.5 million deaths premature and preventable

### Table 6. Estimated number of new cancer cases and deaths among females by world area (×1000), 2012, excludes nonmelanoma skin cancer.

<table>
<thead>
<tr>
<th>World area</th>
<th>Cases</th>
<th>Deaths</th>
</tr>
</thead>
<tbody>
<tr>
<td>Eastern Africa</td>
<td>171</td>
<td>116</td>
</tr>
<tr>
<td>Middle Africa</td>
<td>44</td>
<td>31</td>
</tr>
<tr>
<td>Northern Africa</td>
<td>115</td>
<td>67</td>
</tr>
<tr>
<td>Southern Africa</td>
<td>43</td>
<td>26</td>
</tr>
<tr>
<td>Western Africa</td>
<td>113</td>
<td>74</td>
</tr>
<tr>
<td>Eastern Asia</td>
<td>1,714</td>
<td>1,002</td>
</tr>
<tr>
<td>South-central Asia</td>
<td>802</td>
<td>490</td>
</tr>
<tr>
<td>South-eastern Asia</td>
<td>404</td>
<td>238</td>
</tr>
<tr>
<td>Western Asia</td>
<td>149</td>
<td>79</td>
</tr>
<tr>
<td>Caribbean</td>
<td>43</td>
<td>24</td>
</tr>
<tr>
<td>Central America</td>
<td>110</td>
<td>57</td>
</tr>
<tr>
<td>Northern America</td>
<td>866</td>
<td>329</td>
</tr>
<tr>
<td>South America</td>
<td>410</td>
<td>209</td>
</tr>
<tr>
<td>Central and Eastern Europe</td>
<td>523</td>
<td>287</td>
</tr>
<tr>
<td>Northern Europe</td>
<td>254</td>
<td>116</td>
</tr>
<tr>
<td>Southern Europe</td>
<td>339</td>
<td>163</td>
</tr>
</tbody>
</table>
Cancer is a leading cause of death worldwide among women in both high-income countries and middle-income countries. The cancer burden is also expanding in countries of all income levels due to the growth and aging of the population. This increasing burden is expected to be particularly pronounced in low- and middle-income countries (LMICs), where the average life expectancy is becoming longer due to public health advances such as the control of infectious diseases and reductions in maternal, infant, and childhood mortality. In addition to these increases stemming from population growth, the cancer burden is also growing in LMICs due to changes in the prevalence of cancer risk factors as countries experience economic transition. These risk factors include smoking, excess body weight, and physical inactivity. Changes in reproductive patterns which often accompany economic development, such as a later age at first childbirth and having fewer children, also affect the cancer burden in women. Due to these changes, cancers that were once common only in high-income countries are becoming more prevalent.

In addition to the burden of morbidity and mortality, cancer carries an economic burden. This includes direct costs, such as the costs of treatment, and indirect costs, such as the costs to family or society from loss of income or productivity due to illness or premature death. There are also other quantifiable costs of cancer, such as time spent by caregivers, transportation, and assistance in the home. The costs of cancer pose unique challenges in both high- and low-resource environments. In high-income countries, where the burden of cancer is already substantial, the costs of cancer and survivor care have skyrocketed. LMICs, on the other hand, are struggling to balance the growing demands on healthcare infrastructures with limited resources.

In addition to treatment, prevention and early detection interventions are needed in both high- and low-resource settings to avert cancer cases and deaths. Primary prevention is particularly important; about one-third to one-half of cancer cases could be averted based on current knowledge of risk factors.

A number of common cancers among females have known means of prevention and/or early detection which can be applied in resource-appropriate settings. As such, while the global burden of cancer among women is substantial, there is also significant potential to reduce suffering and loss of life, as well as to alleviate the economic burden to individuals, families, and societies.

According to estimates from the World Health Organization (WHO) International Agency for Research on Cancer (IARC), there were 6.7 million new cancer cases and 3.5 million deaths among females worldwide in 2012. Of these, 56% of cases and 64% of deaths were in less developed countries. These numbers are expected to increase to 9.9 million cases

<table>
<thead>
<tr>
<th>Western Europe</th>
<th>496</th>
<th>214</th>
</tr>
</thead>
<tbody>
<tr>
<td>Australia/New Zealand</td>
<td>62</td>
<td>23</td>
</tr>
<tr>
<td>Melanesia</td>
<td>6</td>
<td>4</td>
</tr>
<tr>
<td>Micronesia</td>
<td>0.4</td>
<td>0.1</td>
</tr>
<tr>
<td>Polynesia</td>
<td>0.6</td>
<td>0.3</td>
</tr>
</tbody>
</table>
and 5.5 million deaths among females annually by 2030 as a result of the growth and aging of the population.

The number of cancer cases and deaths is a function not only of cancer risk but also population size. The greatest numbers of cancer cases and deaths among females are in Eastern Asia, with 1.7 million cancer cases and 1 million deaths estimated in 2012. This figure is dominated by China, which constitutes about three-quarters of female cancer cases and deaths in the region. Following Eastern Asia, the greatest numbers of cancer cases and deaths are in North America and South-Central Asia. In North America, cancer cases and deaths in the US make up about 90% of the totals for the region, while cancer cases and deaths in India make up about 65% of the totals for South-Central Asia.

*Picture 13. Map of most commonly diagnosed cancers and deaths in 2012.*
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Burden

Breast cancer is the most frequently diagnosed cancer and the leading cause of cancer death among women worldwide, with an estimated 1.7 million cases and 521,900 deaths in 2012. It is also the most frequently diagnosed cancer in the majority (140 of 184) of countries and accounts for 25% of cancer cases and 15% of cancer deaths among women worldwide. Global breast cancer incidence patterns reflect both risk factors and the availability of screening. The highest breast cancer incidence rates are in North America, Australia/New Zealand, and Northern and Western Europe, while the lowest are in Africa and Asia. Mortality rates reflect the occurrence of the disease as well as the availability of early detection and treatment. Breast cancer mortality rates are higher in many LMICs, such as those in sub-Saharan Africa, despite their lower incidence because of late stage at diagnosis and limited access to treatment.

Trends

Breast cancer incidence rates increased in western countries between 1980 and the late 1990s. These increases are thought to be due to changes in reproductive factors, use of menopausal hormone therapy, and increased screening. Since around 2000, however, rates in several of these countries have stabilized or decreased, which is thought to be due to decreased use of menopausal hormone therapy or plateaus in screening participation. In many LMICs, however, incidence rates have continued to increase, possibly due to changing reproductive patterns, increased awareness, and/or screening.

In contrast to the historically rising incidence rates, mortality rates in many high-income countries have been decreasing since around 1990. These declines have been attributed to mammography screening and better treatments, although the relative contribution of each is debated. At the same time, however, mortality rates in countries with historically lower rates have increased. These increases are likely due to changes in risk factors in addition to limited access to early detection and treatment.

![Female breast cancer mortality trends, select countries, 1950-2013](image)

Screening

Mammography is an imaging method based on X-rays for the detection of breast cancer. By identifying tumors at earlier stages when treatment has a greater likelihood of success, screening with mammography reduces breast cancer mortality. A number of high-income countries have national programs for mammography screening, though the recommended age and frequency of screening varies across countries.

In high-income countries with organized or opportunistic mammography screening programs, other interventions such as clinical breast examination may not be recommended, because evidence for the effectiveness of those interventions is limited. Mammography screening needs high-quality equipment, skilled radiologists, and efficient infrastructure to communicate positive results and follow up with patients until they receive appropriate treatment or further diagnostic procedures. Due to limited resources, however, implementation of a mass-screening program based on mammography will not be a feasible cancer control intervention in most LMICs.

Currently, WHO recommends mammography screening in high resource settings for women aged 50–69 years if the health-care system and shared decision-making strategies meet certain conditions; the suggested screening interval is two years.

![Breast cancer survivors diagnosed in last five years](image)

*Picture 15, Breast cancer survivors diagnosed in last five years (through 2012 or latest available).*

The estimated global economic burden of cancer in 2009 was US$ 286 billion, which included medical costs (US$ 151 billion, 53%), non-medical costs (costs of transportation, caregiving, and so on; US$ 66 billion, 23%), and productivity loses (US$ 69 billion, 24%). Also, an additional sum of US$ 19 billion was spent on cancer research. The three cancer sites with the highest economic burden worldwide in 2009 in both sexes combined were lung (US$ billion 53), colorectal (US$ billion 33), and breast (US$ billion 24).

Approximately US$ 268 billion (or 94% of the total estimated global costs) was spent in high-income countries. The cost in other country income groups was US$ 1 billion in
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low-income, US$ 8 billion in lower middle-income, and US$ 9 billion in upper middle-income countries.

High-income countries spend more on early detection of cancer and cancer treatment and care. As a result, the cost of treatment per cancer in these countries is higher than in LMICs. For example, the average cost per treatment of breast cancer in 2009 was highest in the United States (approximately US$ 67,000) and lowest in Ethiopia (around US$ 110). Of 172 countries included in the analysis, the average cost of treatment per cancer was US$ 5,000 or less in 125 countries.

High costs of cancer treatment indicate the importance of cancer prevention measures in LMICs. It has been estimated that a US$ 11.4 billion investment in certain preventive interventions in LMICs can potentially save up to US$ 100 billion in cancer treatment costs.

The estimated economic burden of cancer due to loss of productivity in 30 European countries in 2008 was €75 billion (€49 billion in men; €26 billion in women). In the United States, YPLL from all cancers in 2006 was 4.5 million in men (15.4 per death) and 4.7 million in women (17.5 per death), corresponding to a productivity loss of US$ 94 billion in men and US$ 82 billion in women. The estimated productivity loss due to premature death from female breast cancer alone in 2008 was US$ 5.5 billion worldwide. In South Korea, the YPLL per death for both sexes combined in 2009 was 17.3, with an estimated total productivity loss of US$ 5.3 billion.

Cancer can also lead to economic loss to society by reducing productivity of family members of cancer patients and informal caregivers. Even in high-income countries, financial hardship after diagnosis of a cancer is common. For example, in a study in the United States, 32% of cancer survivors reported cancer-related financial problems. In many cases, a family member or another close person provides hands-on care for cancer patients. Caregivers may spend significant time providing care and support to patients,
especially during active treatment and end-of-life, and this usually has financial consequences for them and their families, in addition to the impact on their health (including a higher risk of anxiety and depression), lifestyles, and social relations.

It is most likely that cancer survivors and their families in LMICs face a greater financial hardship than those in high-income countries. The available evidence, although limited, indicates that many cancer patients in LMICs who manage to receive treatments will have problems with some of their basic needs, such as purchasing food or paying for their utility bills. This is despite the fact that many of those who afford cancer treatments in LMICs are of higher socioeconomic status.

**Cost effectiveness of screening**

Evidence on cost-effectiveness of breast cancer in LMICs is limited. However, in many LMICs mammography screening is not cost-effective and feasible because implementation of a national mammography screening is costly and needs advanced health care infrastructure. Currently, WHO recommends mammography screening for women aged 50–69 years in LMICs only when there is a relatively strong health system and the shared decision-making strategies for patients and healthcare providers meet certain conditions. In all limited resource countries (either with weak or relatively strong health systems), WHO recommends against screening in women in other age groups. Although clinical breast examination has been suggested as a promising method for breast cancer screening in low-resource settings, more research is needed before it or other potential screening methods can be recommended as a routine method for breast cancer screening at the population level in all LMICs.

![Map of countries with large-scale breast cancer screening programs, 2014](source: The Cancer Atlas, 2nd edition)

**Picture 17. Map of countries with large-scale breast cancer screening programs, 2014**

Few LMICs have a breast cancer screening program based on mammography, and many are unlikely to afford such a program in the near future.

An important factor to determine priorities and develop and assess the success of cancer control programs is the availability of reliable cancer surveillance data. This information comes from cancer registries and vital registration.
All cancer registries collect basic information on cancer incidence, such as patient age and cancer type. Some cancer registries also collect more detailed data, including data on stage at diagnosis, type of treatment received, and survival. There are variations in the quality of collected information and coverage of population-based cancer registries (nationwide versus regional coverage) across countries. No established cancer registries exist in several countries in sub-Saharan Africa and Central Asia.

Cancer death data come from a country’s vital registration system. Like cancer registries, there is wide variation globally in the quality and completeness of death certification. Many countries in the world, especially in Africa, the Middle East, and South-Central and South-Eastern Asia, lack vital registration.

Due to the limitations in quality and coverage of cancer registries described above, reported cancer rates in some countries may reflect the accuracy and the coverage of data collection rather than the true cancer incidence or mortality. This can affect the ability to draw comparisons between countries, as well as a country’s ability to rely on their data for priority-setting. These issues are particularly challenging for LMICs, which often lack high quality cancer and death registration to accurately assess changes in disease and death as the burden shifts from infectious to non-communicable diseases.

The burden of cancer among women is high in both HICs and LMICs, although the distribution of most common cancers differs. This burden is expected to increase as populations grow and age and as the prevalence of cancer risk factors increases in some countries, especially in LMICs. The costs of cancer are considerable and even catastrophic in HICs and LMICs alike. However, this burden of disease, loss of life, and economic hardship is not inevitable. All of the most common cancers among women worldwide, including lung, breast, cervix, liver, and colorectum, have known means of prevention.
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Table 7. Publicly available videos on cancer statistics.

<table>
<thead>
<tr>
<th>Clip caption</th>
<th>Duration (mm:ss)</th>
<th>Link</th>
</tr>
</thead>
<tbody>
<tr>
<td>Breast Cancer Awareness Infographic</td>
<td>1:52</td>
<td><a href="https://youtu.be/IJx0rsJuNuo">https://youtu.be/IJx0rsJuNuo</a></td>
</tr>
<tr>
<td>Breast Awareness &amp; Breast Cancer Prevention</td>
<td>9:28</td>
<td><a href="https://youtu.be/7D6OZmn0P5A">https://youtu.be/7D6OZmn0P5A</a></td>
</tr>
<tr>
<td>A Different Approach to Breast Cancer</td>
<td>1:38</td>
<td><a href="https://youtu.be/8XTUauWxfcA">https://youtu.be/8XTUauWxfcA</a></td>
</tr>
<tr>
<td>Breast Cancer Statistics</td>
<td>4:12</td>
<td><a href="https://youtu.be/PJK7r1v8t-k">https://youtu.be/PJK7r1v8t-k</a></td>
</tr>
<tr>
<td>Marjorie's story - Breast cancer</td>
<td>2:50</td>
<td><a href="https://youtu.be/7BSXbmNuO1l">https://youtu.be/7BSXbmNuO1l</a></td>
</tr>
<tr>
<td>Incidence Of Breast Cancer In India And Rest Of The World</td>
<td>3:50</td>
<td><a href="https://youtu.be/a30s5cKwaMfE">https://youtu.be/a30s5cKwaMfE</a></td>
</tr>
<tr>
<td>Cancer Awareness - World Cancer Statistics</td>
<td>1:03</td>
<td><a href="https://youtu.be/9LbsJ8zeX8">https://youtu.be/9LbsJ8zeX8</a></td>
</tr>
<tr>
<td>Breast cancer epidemic in the Philippines</td>
<td>5:08</td>
<td><a href="https://youtu.be/0B0ibpFD7fQ">https://youtu.be/0B0ibpFD7fQ</a></td>
</tr>
<tr>
<td>TOTAL</td>
<td>60:13</td>
<td></td>
</tr>
</tbody>
</table>

and/or early detection which can be applied to reduce incidence and mortality. Furthermore, lung cancer and cervical cancer, two of the top four cancers in women worldwide, have several proven prevention measures. These two cancers combined represent about 20% of all cancer deaths among women. Many of these deaths could be prevented through effective tobacco control, vaccination, and screening activities. There are a number of effective cancer control measures available to countries of all resource levels. Many of these measures are extremely cost-effective given the lives saved for the cost of the intervention, especially in the case of vaccination. To prevent cancer in the future, countries must prioritize policies to reduce known cancer risk factors and make prevention accessible to all.
Currently existing DNA technologies

DNA testing uses different technologies which are complementary in applications:

- Molecular (PCR):
  - Virology
  - Blood screening
  - Oncology
  - Infectious disease
- In situ hybridization (ISH):
  - Oncology
- Sequencing:
  - Whole genome sequencing and targeted sequencing
  - Mutation detection
  - Oncology
  - Pre-natal, newborn
  - Infectious disease

The new nucleic acid-based diagnostic tool has developed many names, e.g., molecular diagnostics, molecular pathology, molecular genetics, and molecular genetic pathology. Then, there are the inevitable subdivision names, such as molecular hematology-oncology, molecular oncology, molecular virology, and molecular microbiology. The list seems inexhaustible as we apply the tools of molecular biology to every section of the hospital diagnostic pathology department (molecular coagulation, molecular frozen section) and to general medicine (molecular cardiology).

For the purposes of this review we only briefly explain Molecular diagnostics, as our ARNA Breast product relates to this type of technology. Information on other technologies can be easily found on Internet.

**Molecular diagnostics**

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### Table 7. Publicly available videos on PCR, rtPCR and liquid biopsy.

<table>
<thead>
<tr>
<th>Clip caption</th>
<th>Duration (mm:ss)</th>
<th>Link</th>
</tr>
</thead>
<tbody>
<tr>
<td>rtPCR animation</td>
<td>3:54</td>
<td><a href="https://youtu.be/pRwoOBuk00c">https://youtu.be/pRwoOBuk00c</a></td>
</tr>
<tr>
<td>Webinar: Liquid Biopsy - Applications of CTCs and ctDNA as a Non-Invasive Tumor</td>
<td>53:01</td>
<td><a href="https://youtu.be/mJinudXpppE">https://youtu.be/mJinudXpppE</a></td>
</tr>
</tbody>
</table>
Molecular diagnostics is a collection of techniques used to analyze biological markers in the genome and proteome—the individual's genetic code and how their cells express their genes as proteins—by applying molecular biology to medical testing. The technique is used to diagnose and monitor disease, detect risk, and decide which therapies will work best for individual patients.

By analyzing the specifics of the patient and their disease, molecular diagnostics offers the prospect of personalized medicine.

These tests are useful in a range of medical specialisms, including infectious disease, oncology, human leucocyte antigen typing (which investigates and predicts immune function), coagulation, and pharmacogenomics—the genetic prediction of which drugs will work best.

Although the fundamental discoveries of the double helical structure of DNA and the flow of genetic information from DNA to RNA to proteins laid the foundation for clinical molecular diagnostics, it came into being only with development of a key technique called Southern blot hybridization analysis. This technique dominated molecular diagnostics in the 1980s and into the early 1990s. At that point, this labor-intensive, time-consuming technique limited molecular diagnostics to low volume esoteric testing performed in regional centers of excellence.

The development in biotechnology that revolutionized molecular diagnostics was the invention of polymerase chain reaction (PCR) in the 1980s. By the early 1990s, PCR became commonplace. Simply put, this in vitro nucleic acid amplification technique is a highly specific way to amplify targeted pieces of DNA (bacterial, viral, human) to obtain a much larger amount, so that DNA targets that were thought of as needles in a haystack in
the ’80s became haystacks full of specific needles in the ’90s. One could use any number of straightforward detection techniques to complete the laboratory test.

Today, PCR usually is performed by semiautomated instruments that couple amplification with simultaneous fluorescence-based detection — a combination known as “real-time PCR.” Front-end robotics may automate the entire process from specimen receipt to generation of laboratory test result, but only large specimen volumes tend to justify the high costs of robotics. Real-time PCR, coupled with small, low-throughput DNA/RNA purification instruments for low- to medium-volume tests complement high-volume “home run” tests.

In real estate, the mantra is “location, location, location.” In molecular diagnostics, the holy grail seems to be “content, content, content.” The marketplace is well served by the vendors that are supplying equipment and reagents to molecular diagnostics laboratories.

Modern medicine is based on remarkable discoveries of biomedical research. Medicine is conservative, however, reaching new levels of service incrementally and slowly. Only after years of research and trials does a new test or procedure become standard care. This is true in every specialty.

Much investigation remains in basic and translational research laboratories to establish the genes and gene expression profiles that are important in diagnosing and managing complex diseases. Many trials must be designed to determine whether test panels that are technically possible are useful clinically. Simply creating a panel of pathogens’ target nucleic acids may not mean physicians will embrace it as pivotal for managing their patients. The time is coming for molecular panels because progress in that endeavor is inevitable.

Modern molecular pathology is based on PCR (owned and marketed by Roche and licensed to several other companies that also market tests) and on PCR alternatives. The most popular alternatives are:

- Gen-Probe’s transcription-mediated amplification
- Becton Dickinson’s strand displacement amplification
- BioMerieux’s nucleic acid sequence-based amplification
- Branched DNA (bDNA) technology from Bayer and Chiron

These biochemical reactions performed on purified nucleic acids from patient specimens sensitively and specifically amplify DNA or RNA to facilitate the answering of diagnostic questions. Examples of these diagnostic questions include:

- Determine presence or absence of pathogenic DNA (e.g., virus, bacterium, parasite, fungus)
- Quantify viral load (e.g., HIV or cytomegalovirus) in infected patients to assess efficacy of antiviral therapy
- Detect minimal residual disease (usually cancer) post-therapy
- Identify mutations associated with genetic disease (e.g., leukemia, muscular dystrophy, and Alzheimer’s disease) to name but three of many molecular genetic (including oncology) targets
- Sequence-specific genes involved in hereditary cancers in an effort to apply interventional therapy rationally before disease strikes (e.g., sequencing of BRCA-1 and BRCA-2 in breast cancer patients and kindred)

In the case of molecular virology, “stat” molecular testing is now possible through rapid (~20 minutes) nucleic acid extraction from cerebrospinal fluid for detection of herpes simplex virus infection in about an hour. With positive identification, lifesaving antiviral
therapy can be rationally applied. Assessment of tumor gene “signatures,” also known as “gene rearrangement tests,” makes it possible not only to diagnose specific cancers, but also to monitor therapy and relapse. Advances in diagnostics have been made in many sections of the pathology department using the tools of molecular biology and extending to applications in identity testing, transplantation, anatomic pathology (especially fluorescent in situ hybridization, or FISH), and even biowarfare agent detection.

These examples of the strengths of molecular diagnostics demonstrate that the diagnostic pathology laboratory has fully embraced nucleic acid as an analyte. This type of testing has a market size equal or soon-to-be-equal to that of the protein tests for immunodiagnostics, serology, and other determinations. Clinical chemistry analysis of conventional and traditional analytes such as glucose or sodium still dominates the marketplace. Yet, the highly specific answers provided by molecular diagnostics coupled with their potential to provide physicians with valuable personalized information on complex diseases represent key strengths and growth potential.

In summary, pathology has entered the genomic era. As molecular diagnostics continues to mature, it is in an excellent position to grow as medicine moves into the post-genomic era with the elucidation of the role of more and more genes and gene products in complex diseases. While investigation of certain individual genes in some cancers is diagnostically advantageous, cancer and many other diseases, including hypertension, cardiovascular disease, obesity, and diabetes, are multigenic and complex. Molecular diagnostics is poised to take advantage of the additional information coming from sequencing of the human genome. The rate of growth of these diagnostic applications promises many more blockbuster applications for molecular diagnostics in this decade alone.

**World market volume for In Vitro Diagnostics (IVD).**

According to BCC Research’s *Blood Testing: Technology and Global Markets* (HLC184A), the global blood testing market is expected to reach $56.6 billion by 2019 and register a CAGR of 2.6% from 2014 through 2019.

The market for blood tests in cancer or oncology is the largest segment in terms of revenues. However, blood tests used for identification of serious infections, diabetes, and cardiovascular disease is expected to increase. The oncology blood testing market for 2013 was about $11.6 billion, which is expected to rise with a CAGR of 3.7% from 2014 to 2019.

The oncology, hematology, toxicology, inflammation, and heart profile blood testing markets have been positively impacted with the rise in diseases such as cancer, diabetes, heart disease, blood disease, and infectious diseases corresponding with an aging population. Therefore, the CAGRs of these markets are expected to increase between 2014 and 2019.

“The global market for blood testing is highly competitive and is driven mainly by advanced testing innovations, more reliable testing devices, and pricing,” says BCC Research healthcare analyst Shalini Shahani Dewan. “There are no specific guidelines for most tests. Further, many tests can be done in a lab, clinic, or healthcare setting depending on the need. This has resulted in availability of similar testing in the market with intense competition and price pressures.”
Molecular Diagnostics (MDx) has emerged as one of the largest and fastest growing segments in the $50 billion IVD industry. It now ranks 4th in overall market size behind the immunoassay, whole blood glucose and clinical chemistry segments in terms of overall manufacturer sales.

In the span of just over 20 years, molecular diagnostics has burgeoned from a practically non-existent market with approximately $10 million in manufacturer sales in 1990 to $4.2 billion worldwide in 2011. This represents a remarkable 33% annual growth rate over this time frame.

The first commercial molecular IVD tests, previously called “DNA probe or nucleic acid tests,” were for infectious diseases such as chlamydia/gonorrhea (CT/NG) and Legionnaire’s disease. These were introduced to the market by Gen-Probe, Inc., one of the early pioneers in molecular testing and who was recently acquired by Hologic, Inc. Through the 1990’s the market rapidly expanded with the introduction of PCR tests from Roche for CT/NG, HIV qualitative and HIV quantitative (viral load). Other companies soon followed suit with their own HIV, CT/NG and other tests. By 1998, the market for a relatively small menu of tests surpassed $500 million in sales.

As this growth phase was occurring, private reference laboratories and academic medical centers began to develop their own PCR based laboratory developed tests (LDTs) for rare genetic diseases, less common infectious diseases, and cancer mutations for which there were no FDA approved assays available. Thus, the menu of tests available to clinicians expanded rapidly.

The cancer testing segment includes tests for mutations and chromosome changes related to cancer predisposition, diagnosis, prognosis, monitoring and selection of therapy.

The cancer testing and genetic testing segments are similar in that the volume of molecular tests is relatively small for each test. This is due to the nature of the diseases where the incidence of each type of cancer is relatively small and the molecular tests are not used for broad based population screening. As a result, many of the cancer tests are performed using LDTs.

Even though IVD manufacturer sales of MDx tests for cancer are small today, this segment holds significant market potential because the clinical value of the tests is so high. For example, new companion diagnostic tests (CDx), which can command premium prices, are rapidly gaining adoption in clinical laboratories, a trend that is likely to continue given the numerous collaborations between pharma and diagnostic companies for CDx tests. Some of the key tests in this category include KRAS for colon cancer, EGFR for lung cancer, BRAF for melanoma and BCR-ABL for chronic myelogenous leukemia.

The other major segment within cancer testing is FISH testing. This segment has been growing at a double digit rate over the last decade or more. Perhaps, the most well-known FISH cancer tests are the HER2 test for breast cancer and the UroVysion® bladder cancer test from Abbott. There are literally dozens of other FISH probe reagents sold by Abbott and other vendors for use in cancer diagnosis and prognosis.

According to Roche which is the market leader in DNA testing the potential market for 2023 is more than $11 bn.
Some researchers believe the global market for such kits could be worth more than $10bn (£7.7bn) by 2022. According to other reports global DNA diagnostics market is expected to garner $19 billion by 2020, registering a CAGR of 9.8% during the forecast period 2014-2020.

There are diverse applications of DNA testing market out of which oncology diagnostics and histopathology is estimated to register lucrative growth over the forecast period. Factors attributing for considerable growth potential in this segment include identification of cancer causing genes & associated research, and facilitation in mutation detection.

North America dominated the market in 2014, with revenue estimated at over USD 2,600 million. Presence of sophisticated healthcare infrastructure, rising incidence of genetic abnormalities, favorable government initiatives with respect to genetic diagnosis and its application in in-vitro fertilization are major factors driving growth of this region.

Asia Pacific is anticipated to witness lucrative growth as a result of developments in health care infrastructure in this region coupled with presence of significant number of target population that is suffering with chronic as well as acute disorders. Government initiatives taken up herein are also attributive to influence sector growth to a great extent.


According to the seventh edition of Kalorama Information's report, the total market for clinical molecular testing products (includes molecular testing instruments, molecular reagents, and related supplies) is $7.3 billion. DNA-based diagnostics now represents more than one-tenth of the global IVD market with a growth trajectory secured through
platform innovation in lower-cost nucleic acid amplification tests (NAATs) and clinical next-generation sequencing (NGS).

“Molecular diagnostics industry is demonstrating revenue growth at more than twice the rest of the IVD market… While not matching its explosive market growth in years past, clinical molecular diagnostics has delivered on its promise in healthcare “ -Emil Salazar, IVD Industry Analyst and Author.

The future of the molecular diagnostics market lies with nucleic acid amplification tests and sequencing-based tests. Time to results for real-time PCR and isothermal amplification are crucial for several infectious disease testing applications including respiratory testing, hospital-acquired infections, bloodstream infections, and molecular point-of-care tests.

The development of real-time PCR profoundly expanded the role of molecular diagnostics in healthcare; the technology registers amplification-generated fluorescence or other optically detected changes in real time to determine positive results and the relative sample amount of analyte. Real-time PCR provides quantitative molecular results and has been integral to expanding molecular testing capabilities beyond high-complexity labs. The technology reduces “hands on” time or user requirements, improves test sensitivity, and speeds up the time to results. In clinical molecular diagnostics, nucleic acid amplification tests are most commonly performed using qPCR (and related qPCR from RNA analytes known as reverse transcriptase-PCR [qRT-PCR]) followed by isothermal amplification methods.

Microbiology is a key area to watch in molecular, though it has many applications. The infectious diagnostics market was traditionally dominated by virology or HIV and hepatitis testing including infection diagnosis, virus genotyping, and viral load testing. Together, HIV and hepatitis still represent roughly 40% of the entire molecular diagnostics market in microbiologys. Healthcare facility-acquired infections and bloodstream infections now represent the largest market segment due to the threat of antimicrobial resistance (AMR) in healthcare facilities and rising complexity of testing deployed in cases of inpatient infections. Molecular tests have been highly successful in healthcare facility markets due to their unique ability to identify the infection and characterize resistance within actionable timelines.

There is no surprise in terms of undetermined market share in the infectious disease diagnostics industry. Eight companies are estimated to account for over eighty-five percent of the global market. Roche Diagnostics has been an unmovable leader with a market share estimated at 21%.

Clinical molecular diagnostics refer to in vitro diagnostic (IVD) tests used in patient health care that detect, quantify or characterize nucleic acid analytes, whether pathogenic nucleic acid, patient DNA or patient RNA. As an IVD market, molecular diagnostics refer to the relevant test kits, other reagents and instruments that are used for clinical testing in settings that include reference laboratories, independent labs, healthcare facility labs, other centralized clinical labs and near-patient testing sites such as clinics and physician offices.

The primary technologies for clinical molecular diagnostics include nucleic acid amplification tests (NAATs) based on real-time PCR (qPCR) and other amplification-detection protocols; direct nucleic acid probe tests such as in situ hybridization (ISH) and its fluorescent ISH (FISH); next-generation sequencing (NGS); amplification followed by probe-based hybridization on arrays (or microarrays); and other related methods.
Brief facts about IVD are as follows:

- The IVD markets of China and India, two countries that account for roughly one-third of the world’s population, have demonstrated remarkable growth during the past 15 years.
- The IVD markets have seen steady growth rates:
  - 10-20% per year during the past 15 years
  - Growth in India is starting from a substantially lower point.
- Pace of growth for both countries has increased
  - China 25% growth
  - India 18% growth
- Increased prosperity and urbanization in both countries are allowing for a rapid increase of IVD use.
- The In Vitro diagnostic market is growing rapidly worldwide
- Rapid growth potential is certain virtually worldwide as developed countries readily use IVD testing
- As developing countries become westernized, the use of IVD testing will ensue as well.

**ARNA Breast market overview**

As we mentioned above the one of the key reason of women death around the world is breast cancer - with 1.7 mln new cases in 2012 and 522 th deaths in 2012 with a major diagnosed cancer in 140 of 184 countries for 25% of cancer cases and 15% of cancer deaths among women worldwide.

According to United Nations (https://esa.un.org/unpd/wpp/) the world population of women with age of 40+ and with age 40-74 in 2017:
Breast screening according to CDC (https://www.cdc.gov/cancer/breast/pdf/BreastCancerScreeningGuidelines.pdf) for women from 40 to 74 years old should be done annually.

As a conservative market size estimation we assume that the following percentage of women in countries ranged above will follow to globally accepted guidelines for screening, thus forming ARNA Breast potential market size:

- High-income countries - 25%
- Middle-income countries - 7%
- Low-income countries - 1%

This assumption gives us the 118.3 mln tests annually (25%*231mln + 7%*857mln + 1%*56mln).

According to Globoscan (http://globocan.iarc.fr/old/FactSheets/cancers/breast-new.asp) in 2012 in developed countries living 3.2 mln women with 5-years survival and 3 mln in less developed countries. And every year 0.8 mln new cancer incidents in high development countries and 0.9 mln in less developed countries.

Additionally according to American Association for Cancer Research only in USA in 2016, there are approximately 3.5 mln women living with a history of breast cancer in USA. (http://cebp.aacrjournals.org/content/26/6/809). An estimated 691,000 are alive in the UK after a diagnosis of breast cancer. This is predicted to rise to 840,000 in 2020. (https://www.breastcancercare.org.uk/about-us/media/press-pack-breast-cancer-awareness-month/facts-statistics).
From 1992 to 1994, the five-year survival rate for women under age 49 with newly diagnosed metastatic breast cancer was 18%. From 2005 to 2012, five-year survival had doubled to 36%.

Clearly, improvements in treatment are part of the explanation. But better imaging techniques have also resulted in earlier detection of metastatic disease and may help explain the increase in years of survival.

During the cancer treatment process to control the development of tumor there minimum of 5 tests. 40% women will be doing test for high developed countries and only 10% women for less developed countries.

To control the remission for the survived women for 5 years we need to do 4 tests a year. 40% women will be doing test for high developed countries and only 10% women for less developed countries.

So, the number of tests during treatment process 2 mln tests annually plus to control the remission process 6 mln tests annually.

The full conservative market for our test is around 125 mln tests per year.

Compared to mammography price which is roughly 120 USD per test ARNA Breast test will be 30 to 50 USD per test, thus our conservative prediction for the market size for ARNA Breast in case of 30 USD market price per test is 3,75 bln USD p.a. In case of neutral market size estimation we expect this amount to be up to 10 bln USD p.a.

Market power for ARNA breast test

According to American Cancer Society 5-year relative survival rates for breast cancer is tremendously higher for earlier stage, than for later stage.

The outlook for women with breast cancer varies by the stage (extent) of the cancer. In general, the survival rates are higher for women with earlier stage cancers. But remember, the outlook for each woman is specific to her circumstances.

- The 5-year relative survival rate for women with stage 0 or stage I breast cancer is close to 100%.
- For women with stage II breast cancer, the 5-year relative survival rate is about 93%.
- The 5-year relative survival rate for stage III breast cancers is about 72%. But often, women with these breast cancers can be successfully treated.
- Breast cancers that have spread to other parts of the body are more difficult to treat and tend to have a poorer outlook. Metastatic, or stage IV breast cancers, have a 5-year relative survival rate of about 22%. Still, there are often many treatment options available for women with this stage of breast cancer.

Finding breast cancer early and getting state-of-the-art cancer treatment are the most important strategies to prevent deaths from breast cancer. Breast cancer that’s found early, when it’s small and has not spread, is easier to treat successfully. Getting regular screening tests is the most reliable way to find breast cancer early.
According to San Diego State University research (https://qap.sdsu.edu/education/bcrl/Bcrl_bcinus/bcrl_bcinus_index.html) 80% of new, invasive breast cancer cases occurred in women 50 years and older. To break this down, 4.5% of new invasive cases in 2015 were in women under 40 years, 15.5% were in women ages 40-49, 23% in women ages 50-59 years, and 25.9% in women ages 60-69 years. After that, the incidence by decade declines to 18.3% in women ages 70-79 and 12.5% in women ages 80 and older.

Recent findings show that approximately:

- 74% of breast cancers are hormone receptor-positive/HER2-negative
- 12% are triple negative (i.e., hormone receptor-negative/HER2-negative)
- 10% are hormone receptor-positive/HER2-positive
- 4% are hormone receptor-negative/HER2-positive

### Table 8. Mortality Rates by Race/Ethnicity per 100,000

<table>
<thead>
<tr>
<th>Race/Ethnicity</th>
<th>Mortality Rates</th>
</tr>
</thead>
<tbody>
<tr>
<td>Black</td>
<td>29.6</td>
</tr>
<tr>
<td>White</td>
<td>21.0</td>
</tr>
<tr>
<td>American Indian/ Native Alaskan</td>
<td>14.7</td>
</tr>
<tr>
<td>Hispanic</td>
<td>14.5</td>
</tr>
<tr>
<td>Asian/Pacific Islander</td>
<td>11.2</td>
</tr>
</tbody>
</table>
5-Year Relative Survival Rate (2005-2009)

![Image of 5-Year Relative Survival Rate](image)


The difference in survival rates between black and other women is explained partially by differences in screening and treatment. Although overall screening rates between black and white women are now similar, black women with breast cancer are less likely to have undergone regular breast cancer screening.


Missing a mammogram, even in the year before a breast cancer diagnosis, increases the chance of a cancer diagnosis at a later stage. (Source: [http://www.ajronline.org/doi/abs/10.2214/AJR.13.10733/](http://www.ajronline.org/doi/abs/10.2214/AJR.13.10733/))

In patients in their 40s with or without a family history of breast cancer, no differences were detected in the proportion of invasive versus noninvasive cancers diagnosed, lymph node metastases, or mastectomy rates. Screening mammography should be performed in this age group regardless of family history (Source: [http://www.ajronline.org/doi/abs/10.2214/AJR.13.11194/](http://www.ajronline.org/doi/abs/10.2214/AJR.13.11194/)).

The diagnosis of breast cancer these days, very often, is done in its subclinical form, through routine mammography or population screening programs. In the US, mortality fell by 30% in women over 50 years old and 19% between 40 and 49 years on the account of
earlier diagnosis. The BI-RADS™ (Breast Imaging Reporting And Data System) was implemented as a screening method, and constitutes a report and terminology standardization system that ranks the abnormalities seen on imaging studies into categories, as recommended by the American College of Radiology

<table>
<thead>
<tr>
<th>Category</th>
<th>Definition</th>
<th>Risk of cancer (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>Inconclusive result</td>
<td>Additional imaging investigation required</td>
</tr>
<tr>
<td>I</td>
<td>Negative findings</td>
<td>0</td>
</tr>
<tr>
<td>II</td>
<td>Benign findings</td>
<td>0</td>
</tr>
<tr>
<td>III</td>
<td>Probably benign findings</td>
<td>&lt; 2</td>
</tr>
<tr>
<td>IV</td>
<td>Suspicious findings</td>
<td>3-94</td>
</tr>
<tr>
<td>V</td>
<td>Highly suspicious findings</td>
<td>≥ 95</td>
</tr>
<tr>
<td>VI</td>
<td>Known biopsy-proven malignant lesion</td>
<td>100</td>
</tr>
</tbody>
</table>

Breast exam has moderate sensitivity and good specificity for the detection of cancer, and the predictive value varies according to age. Sensitivity is from 57 and 83% between 50 and 59 years, and 71% between 40 and 49 years. Specificity, in turn, is greater, 88 to 96% between 50 and 59 years and 71 to 84% among women aged 40 to 49 years. It is particularly useful in younger women, due to the limitation of mammography in this age group (dense breasts).

Mammography is the standard method for screening and diagnosis of breast cancer. Even in cases where the clinical picture is suggestive, a mammogram should be indicated in order to assess the extent of the tumor and to detect subclinical ipsilateral multicentric foci, as well as in the contralateral breast. Sensitivity is 46 to 80%, while specificity reaches 82 to 99%. Its predictive value depends on the size and location of the lesion, breast density, the quality of technical resources available and the examiner's ability. However, even using a technique of excellence, the false negative rate is 10 to 15%, reaching 40% in patients with dense breasts. Therefore, the clinical abnormality on palpation should not be neglected if the mammogram is normal, and the investigation should continue with other semiotic methods such as ultrasound, needle aspiration with thin or thick needle, or even conventional open biopsy.

Although ultrasound is a non-ionizing and more comfortable method, it presents a lower predictive value compared to mammogram for diagnosis of breast cancer. It does not display less marked calcifications and, therefore, is not effective for screening as a single examination method.

Magnetic resonance imaging is a new diagnostic method with increasingly important role in recent years. It is the imaging study with greater sensitivity for the detection of breast cancer, but must be requested with caution because its false positive rate is still not ideal, leading to unnecessary procedures. In addition, its cost is still high and its real impact in reducing mortality from breast cancer is unknown.

Recently, tomosynthesis or 3D mammogram was introduced, with the promise to detect more lesions than digital mammography. However, its use as a routine did not prove to decrease mortality.
In cases of suggestive lesions, percutaneous biopsies are preferred. The first is biopsy with fine needle (FNAB), an outpatient method with low cost and relatively simple. The false positive rate is very low (between 0 and 2%). However, 5-20% of cases are false negatives, mainly due to improper technique for slide preparation.

Core needle biopsy (CNB) is done with a large needle and an automated device that propels and retracts the needle that cuts the tissue. It is indicated for suggestive nodules larger than 1.0 cm or extensive calcifications. Under those conditions, sensitivity is 48 to 100% and specificity, 91 to 100%. On the other hand, it is contraindicated in case of deep image alterations (risk of pneumothorax and hemothorax), fibro-glandular distortion, in suggestive asymmetries, patients with hypomastia, or lesions adjacent to breast implant.

Mammotomy with core needle biopsy (CNB) is performed using a vacuum system with hollow cannula, which rotates at high speed, cutting through the tissue that is sucked out of the breast. It is also known as vacuum-assisted CNB. It is especially indicated for nodules smaller than 1.0 cm, unpronounced suspected calcifications, fibro-glandular distortion and asymmetric densities. Sensitivity reaches 93% and specificity, 98%. (Source: http://www.scielo.br/scielo.php?script=sci_arttext&pid=S0104-42302015000600543)

So, according to above state of the art diagnostic methods to make the screening of the breast cancer and especially diagnosis, a patient needs to do many unpleasant procedures like:

- Mammography (feels like the elephant stepped on the breast) with the median sensitivity of 70% for normal breast and less than 50% for dense breast (young women, Asian women and etc.) with a price from 120 USD
- MRI is very expensive (> 500 USD) with median sensitivity of 80% but very bad for small tumors for first and second stages of cancer, and cannot be used for mass screening.
- Ultrasound is cheap, but very bad in sensitivity for reliable prognosis and used only as additional for another methods
- Biopsy depends from the method have sensitivity from 50% to 93% but mostly used after mammography to prove the diagnosis of breast cancer with specificity up to 100%. This method is expensive and very unpleasant for women, and of cause cannot be used for screening.

**The best possible solution cancer screening at early stage (in fact first stage of cancer can be easily treated for almost 100% of patients) is to receive information from the easiest to get substances, which are Blood, Saliva, Eye drop, etc.) (modern name is Liquid Biopsy) with sensitivity and specificity more than 90%, which can be easy to use and fast like blood test for HIV.**

The world is running to this goal, as fast as it possible, but in reality only few methods are ready to use like:

- Circulating Tumor Cells (CTCs), used to control the treatment of already metastatic cancers when cancer cells in blood vessels.
- Detection of biomarkers, parts of DNA, which are coding cancer, associated proteins. Can be used in screening on the earliest stage of cancer, but very difficult
to get good sensitivity and specificity as in cancer cell can be very big amount of different mutations. This method is very far to be commercialized.

- Detection of RNA in blood plasma is one of the best methods but RNA is very small and around us flying myriads of different RNAase, which can easy pollute the blood sample with the cancers RNAs. Also manipulations with cancers RNAs in blood sample is much more difficult compare to DNA, as RNA is much smaller and less stable. On the other hand nobody knows exact RNA biomarkers of cancer. This is science frontier and to commercialization of such technology - a long and thorny path. For example, to analyze ctDNA using sequencing and methylation technologies humanity spent as minimum 4 decades.

- CtDNA – circulating DNA in bloodstream after cells apoptosis or necrosis, this method also one of the best, but high difficulties to find right biomarkers (unique parts of DNA, responsible for cancer activities, which extracted to the blood vessel during decaying of cancer cells), gives a lot of uncertainty for the scientists. On the one hand to find the right biomarker this is tremendously difficult job. On the second hand ctDNA is very sensitive to preparation of plasma blood not to lose the right proportion of cancer ctDNA to the all DNAs in sample of patient blood. [https://youtu.be/ZJyvqhVWQyM](https://youtu.be/ZJyvqhVWQyM)

- Another type of DNA diagnostic is to analyze inheritable parts of DNA, failures in human DNA, responsible to develop cancer cells in human body due to crashing of apoptosis mechanism of sick cells dying, to predict the possibility of the future development of cancer and for targeted treatment. In Breast cancer the found genes for more than 3 decades are BRCA1, BRCA2, ATM, CDH1, CHEK2, MRE11A, NBN, TP53, PALB2, PTEN, RAD50, RECQL, RINT1 ([http://www.breastcancer.org/symptoms/diagnosis/other-gene-testing](http://www.breastcancer.org/symptoms/diagnosis/other-gene-testing)). But for the sake of diagnostic and screening it is not suitable as it is only prediction system for the future possibility of cancer incidents. Anyway the price for such test in USA starting from 300 USD, in Russia from 65 USD only for BRCA1 and BRCA2. ([http://www.breastcancer.org/symptoms/diagnosis/genomic_assays](http://www.breastcancer.org/symptoms/diagnosis/genomic_assays))

The companies developing innovative methods of cancer diagnostics have raised over 1,1 billion dollars within the last 5 years:

- Exact Sciences (ColoGuard test) – 200 million dollars.
- Guardant Health – 200 million dollars.
- Pathway Genomic – 130 million dollars.
- Grail (Illumina) – 100 million dollars. (investors – Bill Gates, Jeff Bezos and others)
- Foundation Medicine – 95 million dollars.
- Vermilion – 85 million dollars.
- Genomic Health - 60 million dollars.
- Epigenomics (Epi proColon test kit) – 50 million dollars.
- Inviata – 45 million dollars.
- Provista Diagnostics - 35 million dollars.
- TrovaGene - 25 million dollars.
Overview of cancer and related market of diagnostics

- MDxHealth – 25 million dollars.
- Personal Genome Diagnostics – 20 million dollars.

At this time there are no feasible market product in terms of Sensitivity, Specificity, Availability, Price affordability.

After more than 30 years of scientific and research experience in sphere of liquid biopsy using biomarkers of ctDNA we are claiming that we have found the Holy Grail technology to detect cancer in small amount of blood. This technology is easy to use and tremendously complicated. It is the collection of many modern market technics, author technics, know-how substances and unique set of biomarkers. Before this test our scientific leader developed the unique technology of searching exactly fine-tuned DNA biomarkers for the specific cancer, using our own “know-how” technology.

All tests are in development stage in liquid biopsy. Below is a list of breast cancer tests being developed currently in the world with their projected market price, sensitivities and specificities:

<table>
<thead>
<tr>
<th>Test name</th>
<th>Company</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>Status</th>
<th>Test market price</th>
</tr>
</thead>
<tbody>
<tr>
<td>ARNA-Breast</td>
<td>ARNA Genomics</td>
<td>&gt;95%</td>
<td>&gt;95%</td>
<td>Product developed</td>
<td>&lt;$50</td>
</tr>
<tr>
<td>BCtect</td>
<td>DiaGenic</td>
<td>69%</td>
<td>74%</td>
<td>Development</td>
<td>n/a</td>
</tr>
<tr>
<td>Videssa</td>
<td>Provista</td>
<td>86%</td>
<td>83%</td>
<td>Development</td>
<td>n/a</td>
</tr>
<tr>
<td>PanC-Dx</td>
<td>OncoCyte</td>
<td>85-93%</td>
<td>70-90%</td>
<td>Development</td>
<td>n/a</td>
</tr>
</tbody>
</table>

Let us briefly explain what are the major statistic parameters of medical tests are. Test results are usually presented in the form of the following table:

<table>
<thead>
<tr>
<th>Screening test</th>
<th>Sick</th>
<th>Healthy</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td>Truly Positive (a)</td>
<td>False Positive (b)</td>
<td>Positive tests (a + b)</td>
</tr>
<tr>
<td>Negative</td>
<td>False negative (c)</td>
<td>Truly Negative (d)</td>
<td>Negative tests (c + d)</td>
</tr>
</tbody>
</table>
There are 2 columns - one for actually patients known to be sick (having positive diagnosis), another - for patients known to be healthy using current “golden standard” diagnostic methods (they may be already sick, but these methods may not show this properly yet!). Also there are 2 rows - one is for positive results of the test under analysis, another - for negative results. Biostatistical parameters that is usually calculated in order to compare tests efficiency are:

- **Sensitivity**: probability that a test result will be positive when the disease is present (true positive rate) = \( a / (a+b) \).
- **Specificity**: probability that a test result will be negative when the disease is not present (true negative rate) = \( d / (c+d) \).
- **Positive likelihood ratio**: ratio between the probability of a positive test result given the presence of the disease and the probability of a positive test result given the absence of the disease, i.e. = True positive rate / False positive rate = Sensitivity / (1-Specificity).
- **Negative likelihood ratio**: ratio between the probability of a negative test result given the presence of the disease and the probability of a negative test result given the absence of the disease, i.e. = False negative rate / True negative rate = (1-Sensitivity) / Specificity.
- **Positive predictive value**: probability that the disease is present when the test is positive = \( a / (a+c) \).
- **Negative predictive value**: probability that the disease is not present when the test is negative = \( d / (b+d) \).

Our results in our laboratory after checking blood from 219 patients are astonishing:

<table>
<thead>
<tr>
<th>ARNA BC</th>
<th>Sick</th>
<th>Healthy</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td>153</td>
<td>3</td>
<td>156</td>
</tr>
<tr>
<td>Negative</td>
<td>6</td>
<td>57</td>
<td>63</td>
</tr>
<tr>
<td>Total</td>
<td>159</td>
<td>60</td>
<td>219</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Resulting ARNA BC parameter</th>
<th>Value</th>
<th>95% confidence interval</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sensitivity = ( a / (a+c) )</td>
<td>96,23%</td>
<td>91,97% - 98,60%</td>
</tr>
</tbody>
</table>
Overview of cancer and related market of diagnostics  ARNA Genomics  v. 1.2

<table>
<thead>
<tr>
<th></th>
<th>Specificity = d / (b + d)</th>
<th>Positive likelihood ratio</th>
<th>Negative likelihood ratio</th>
<th>Positive predictive value = a / (a + b)</th>
<th>Negative predictive value = d / (c + d)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>95,00%</td>
<td>90,48%</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>86,08% - 98,96%</td>
<td>81,22% - 95,43%</td>
</tr>
<tr>
<td></td>
<td></td>
<td>19,25</td>
<td>0,04</td>
<td>98,08%</td>
<td>94,42% - 99,35%</td>
</tr>
<tr>
<td></td>
<td></td>
<td>13,58 - 27,28</td>
<td>0,04 - 0,05</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The state of art technology is based on next principals:

- The unique method of amplification of ctDNA plasma in a test tube without extracting nucleic acids.
- Only an ultra-small amount of work material is needed (10 µl): a drop of blood is required to perform the test.
- The selection of genes for the biomarker is carried out on the whole genome.
- With the help of the set of ARNA-BC reagents we can analyze the index of DNA sequence of six genes by the real time PCR method.
- The amplification of the DNA fragments is carried out in real-time mode with the use of fluorescence-labelled DNA-probes, each of which contains fluorophore FAM at the 5-edge oligonucleotide and quencher BHQ at the 3-edge.
- The amplification is accompanied by the destruction of the DNA-probe by exonuclease activity of Taq-polymerase and the increase in the general fluorescence of the PCR mixture.
- The detection of the accumulation of the PCR product is carried out in every cycle in accordance with the accumulation of the fluorescence of fluorophore in the reaction that is recorded by the device detector.

The most complicated question from experts to our test efficiency:

- How can you claim that you have found specific genes for all the breast cancer nosologies, for they are all different diseases and there is over 35 and even 135 nosologies?
- The test didn't set out to look for differences in nosologies in all the subtypes of breast cancer, but we set out to find something “common” for all the types of breast cancer.
- Test works at the molecular level. ARNA-BC test kit is able to detect at early stages hormone-dependent and hormone-independent forms of breast cancer: Luminal A, Luminal B, Her2 Enriched, Tripple Negative/Basal.

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1 As sample sizes in the positive (disease present) and the negative (disease absent) groups do not reflect the real prevalence of the disease, both PPV and NPV should be ignored, they will be correctly evaluated during prospective trials.
In other words we do not need to create the new market - we need to disrupt existing market with new product, which is cheaper, easier to use, faster for more than 20 times, easy for the patients. The main goal is to get the Regulator approval and make this product available for patients as fast as possible - that is where our blockchain platform concept breaks in!

Rollout of our technology in real world is planned as a conception of laboratory “blocks” - fast and easy penetration into the market with reasonably small investments to have the possible fastest Return on Investment.

ARNA-BC product is more interested in the following markets, where the basis is women audience at the age of 40 to 74 years, and statistics on adoption of early diagnostics of breast cancer taking into account state screening program for 2017. The price of the test was calculated based on the cost of mammography for the purpose of early diagnostics:

- **USA**: The amount of the market for a total of about 30 mln tests annually with the number of women at the age of 40 to 74 years equal to 68 mln at expected market value 100 US dollars for one test will be about 3 bln US dollars annually. Market amount of mammography services in the USA in 2010 will be 7.8 bln US dollars with average cost of mammography for one patient - 260 US dollars. [https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4142190/](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4142190/)

- **Russia**: Market amount is about 10 mln tests annually with the number of women at the age of 40 to 74 years equal to 32 mln at expected market value 50 US dollars for one test will be about 500 mln US dollars annually. The average cost of mammography for one patient is 90 US dollars.

- **Europe**: Market amount is about 50 mln tests annually with the number of women at the age of 40 to 74 years equal to 125 mln at expected market value 100 US dollars for one test will be about 5 bln US dollars annually. Europe has a very high adoption of screening programs up to 95% of population and almost 125 mln women undergo programs of cancer detection at early stages regularly. The average cost of mammography for one patient is about 150 USD.

- **China**: Market amount is about 30 mln tests annually with the number of women at the age of 40 to 74 years equal to 300 mln at expected market value 40 US dollars for one test will be about 1.2 bln US dollars annually. The cost of mammography for one patient is about 60 US dollars. It is the most fast-growing market for diagnostics of cancer diseases using molecular diagnostic methods.

The “block” concept: one laboratory block is 50 m², 5 work units, 9 employees in two shifts, its capacity is around 29 000 tests per year.

The equipment configuration for the 1st laboratory block:
(initial CAPEX is about 100 thousand USD)
- Deep freezer, -80 °C;
- Two-compartment refrigerator /freezer (+4 / -20 degrees), with an ice compartment;
Overview of cancer and related market of diagnostics

ARA Genomics v. 1.2

- Amplifier with real-time fluorescent detection CFX96 Real-Time System (Bio-Rad, USA) or analog;
- Amplifier for PCR (2720 Thermal Cycler, Applied Biosystems, USA) or analog;
- Centrifuge type Beckman, 14 000 rpm, 10 g (Microfuge 18 Centrifuge, Beckman Coulter, USA) or analog;
- 8-hole minifugue for test strips (Bio-red, cat.C1301, USA) or analog;
- Fluorometer for DNA measurement (Fluorometer DyNA Quant 200, Hoefer, USA) or analog;
- Computer - blockchain platform node for automated results entry into blockchain.

Staff of the 1st laboratory block:
- 4 research associates who have received ARNA training and certification, shift 1,
- 4 research associates who have received ARNA training and certification, shift 2,
- 1 laboratory administrator,
- In total: 5 work positions, 9 employees in two shifts.