



A Review on Cancer Screening

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Abstract: Cancer (malignant tumor) is an abnormal growth and proliferation of cells. It is a frightful disease because the patient suffers pain, disfigurement and loss of many physiological processes¹. There are over 200 different known cancers that affect humans. In an analysis by Macmillan Cancer Support, it predicts that there will be a record 2.5 million people living with a cancer diagnosis in the UK in 2015. This article gives information about the various screening tests of cancer which helps in the diagnosis.

Keywords: Screening, Acute Toxicity Determination, Hollow Fiber Assay, Tumor Xenograft Models, Computed Tomography (CT) Scans, Pap and HPV Testing, Prostate- Specific Antigen (PSA).

Introduction:

Cancer is a group of diseases characterized by uncontrolled growth and spread of abnormal cells. Cancer is a complex genetic disease that is caused primarily by environmental factors. The cancer-causing agents (carcinogens) can be present in food and water, in the air, and in chemicals and sunlight that people are exposed to. Benign tumours can normally be removed by surgery. Malignant solid tumours will, if possible, be surgically resected, probably followed and even preceded by other treatment modalities. More diffuse tumours such as leukaemias with circulating tumour cells require systemic chemotherapy. There are many types of cancers but some of the main types of cancers are listed in the table².

S.No.	Types of Cancer	S. No.	Types of Cancer
1	AIDS Related Cancer	20	Lung Cancer
2	Anal Cancer	21	Lymphoma
3	Bladder Cancer	22	Melanoma
4	Bone Cancer	23	Mesothelioma
5	Brain Cancer	24	Multiple Myeloma
6	Breast Cancer	25	Oral Cancer
7	Cervical Cancer	26	Ovarian Cancer
8	Colon Cancer	27	Pancreatic Cancer
9	Endometrial Cancer	28	Prostate Cancer
10	Esophageal Cancer	29	Rectal Cancer
11	Ewing's Sarcoma	30	Renal Cell Cancer
12	Eye Cancer	31	Skin Cancer
13	Gastrointestinal Cancer	32	Stomach Cancer
14	Gynecologic Cancer	33	Testicular Cancer
15	Head and Neck Cancer	34	Throat Cancer
16	Kidney Cancer	35	Thyroid Cancer

17	Laryngeal Cancer	36	Uterine Cancer
18	Leukemia	37	Vaginal Cancer
19	Liver Cancer	38	Vulvar Cancer

Characteristics of Cancer:-

- Self-sufficiency in growth signalling
- Insensitivity to anti-growth signals
- Evasion of apoptosis
- Enabling of a limitless replicative potential
- Induction and sustainment of angiogenesis
- Activation of metastasis and invasion of tissue³.

Causes of the Cancer:-

The main causes of cancer are due to environmental factors and inherited genetics. Majority of the cases of about 90-95% of them are caused by the environmental factors. The environmental factors include tobacco smoking, diet and obesity, infections, radiation, stress, lack of physical activity and environmental pollutants.

Cancer Screening:-

Screening is the systematic application of a test or inquiry, to identify individuals at sufficient risk of a specific disorder to benefit from further investigation or direct preventive action, among persons who have not sought medical attention on account of symptoms of that disorder.

Screening methods are routinely and extensively used to reduce cost and time of drug discovery. The traditional anticancer drug screening methods, include animal experiments and cell-based screening assays. Screening methods for the detection of anticancer activity are of importance in order to find solid tumor specific agents.

Types of Cancer Screening:-

1. *In Vitro* Testing:

- NCI-60 Tumor Cell Line Screen
- Sample Handling and Preparation

2. *In Vivo* Testing:

- Acute Toxicity Determination
- Hollow Fiber Assay
- Tumor Xenograft Models

3. Cell-Based Screening Assays:

- Conventional cellular screens
- Tailored Cellular Screens
- Biochemical Screening Assays
- Combination of Target and Cell Screen

According to National Cancer Institute, screening tests of cancer are of two types⁴. They are

Imaging Tests

- ❖ Mammograms
- ❖ Computed Tomography (CT) Scans

Laboratory Tests

- Understanding Laboratory Tests
- Blood chemistry test
- Cancer gene mutation testing
- Complete blood count (CBC)
- Cytogenetic analysis
- Immunophenotyping
- Sputum cytology (also called sputum culture)
- Tumor marker tests
- Urinalysis
- Urine cytology
- Pap and HPV Testing
- Prostate- Specific Antigen (PSA) Test

- **In Vitro Testing:**

NCI-60 Tumor Cell Line Screen

The In Vitro Cell Line Screening Project (IVCLSP) is a dedicated service providing direct support to the DTP anticancer drug discovery program. This project is designed to screen up to 3,000 compounds per year for potential anticancer activity. The operation of this screen utilizes 60 different human tumor cell lines, representing leukemia, melanoma and cancers of the lung, colon, brain, ovary, breast, prostate, and kidney. The aim is to prioritize for further evaluation, synthetic compounds or natural product samples showing selective growth inhibition or cell killing of particular tumor cell lines.

The screening is a two-stage process, beginning with the evaluation of all compounds against the 60 cell lines at a single dose of 10 μ M. The output from the single dose screen is reported as a mean graph and is available for analysis by the COMPARE program. Compounds which exhibit significant growth inhibition are evaluated against the 60 cell panel at five concentration levels.

The cell lines have been characterized using ~120,000 SNP arrays

1. By oligonucleotide-base HLA typing
2. Mutations in a number of cancer-relevant genes have been analyzed in these cell lines.
3. They have also been subjected to spectral karyotyping
4. The lines have also been characterized for variations in short tandem repeats (STR)
5. Although there are some technical issues in applying this technology, it was developed as a forensic tool to cancer cell lines.

Human cancer-derived cell lines are the most widely used models to study the biology of cancer and to test hypotheses to improve cancer treatment. The rise of “-omics” along with the development of new high throughput analytical methods allowed interrogation in depth of the clinical relevance of human cancer-derived cell lines. However, other studies have come to the opposite conclusion, suggesting the need for larger human cancer cell line panels, such as CMT1000 or the Cancer Cell Line⁵.

In Vivo Testing:

i. Acute Toxicity Determination

A toxicity assessment provides an estimate of how much of a substance causes what kind of harm. All quantitative toxicity assessments are based on the dose-response concept: as you increase the dose (exposure), the response (toxicity) also increases.

Scientists perform studies to determine exactly how high a dose causes what kind of a response, or effect. The smaller the dose needed to cause an effect, the more potent (toxic) the substance is. For carcinogens, it is often assumed that even the smallest dose can cause an effect. The carcinogenesis bioassay is a method of testing substances for carcinogenic effects that utilizes high-dose studies on laboratory animals. Long-term

carcinogenesis bioassays are the most valued and predictive means for identifying potential carcinogenic hazards of various agents to humans⁶. Scientists assess carcinogenic toxicity by following these steps:

- Test animals are administered different large doses of a substance daily over a lifetime (24-30 months in rats).
- At the end of the study, the animals are examined to see if cancer can be found.
- If cancer is found, scientists use available data and mathematical models to:
 - Estimate the cancer incidence at the lower doses more likely to occur in the environment.
 - Estimate the effect of the size and sensitivity differences between the test animals and human beings.

ii. Hollow Fiber Assay

The hollow fiber assay at full capacity allows screening of 50 or more compounds per 10-day assay. In addition to requiring less than two weeks completing, the assay requires at most only 450 mg of material, as opposed to the multigram quantities required for many xenograft studies. It was developed by Hollingshead et al. as a preliminary rapid screen for assessing novel putative chemotherapeutic compounds prior to their evaluation in the mouse xenograft model. The hollow fiber model has a shorter evaluation time and a reduced compound requirement compared to traditional xenograft models. The model allows for the effective pairing of a novel compound with the appropriate cell line by its capacity to utilize multiple cell lines⁷.

iii. Tumor Xenograft Models

National Cancer Institute (NCI) has conducted a retrospective review of the predictivity of their *In vitro* and *In vivo* screening efforts based on the 60 human cell line panel and xenograft testing in the 1990s. At the time of the review, the NCI procedures were mainly empirical and disease rather than target based⁸. Data were available on 39 agents with both xenograft data and phase II trial results. The analysts found that histology of a particular preclinical model showing *in vivo* activity did not correlate with activity in the same human cancer histology. The fact that none of the currently registered anticancer drugs was devoid of activity in preclinical tumor models, but showed activity in the clinic, led to the conclusion that activity in *in vivo* models of compounds demonstrating *in vitro* activity remains desirable.

• Cell-Based Screening Assays

The cell-based assay is not a mechanistic screen, but determining the mechanism of action of selectively toxic agents from this screen may identify new molecular targets (e.g., downstream effectors in a pathway that is derepressed by the deletion of a tumor suppressor gene) for subsequent target-based screening. From a pharmacologic perspective, this cell-based screen detects collateral sensitivity. Cell-based assays are also used to confirm the activity of agents discovered in target-based screening assays and to assess the drug's pharmacologic effects at the cellular level⁹.

i. Conventional Cellular Screens

Various procedures to determine cell growth are employed in screening laboratories. The earliest broadly used growth inhibition assays were developed by Mosmann and the NCI screening staff, namely, the methylthiazoldiphenyl tetrazolium (MTT) assay. The yellow MTT dye is reduced by mitochondria into a purple formazan, which can be read with ultraviolet/visible light scanners¹⁰. Currently employed in the NCI 60-cell-line screen is the sulforhodamine B (SRB) assay; SRB is a dye that stains protein. Most industrial-scale cellular screens prefer the use of fluorescence or luminescence detection systems. The use of one-dimensional or monolayer cultures to measure cell growth is the most convenient and frequently applied method.

ii. Tailored Cellular Screens

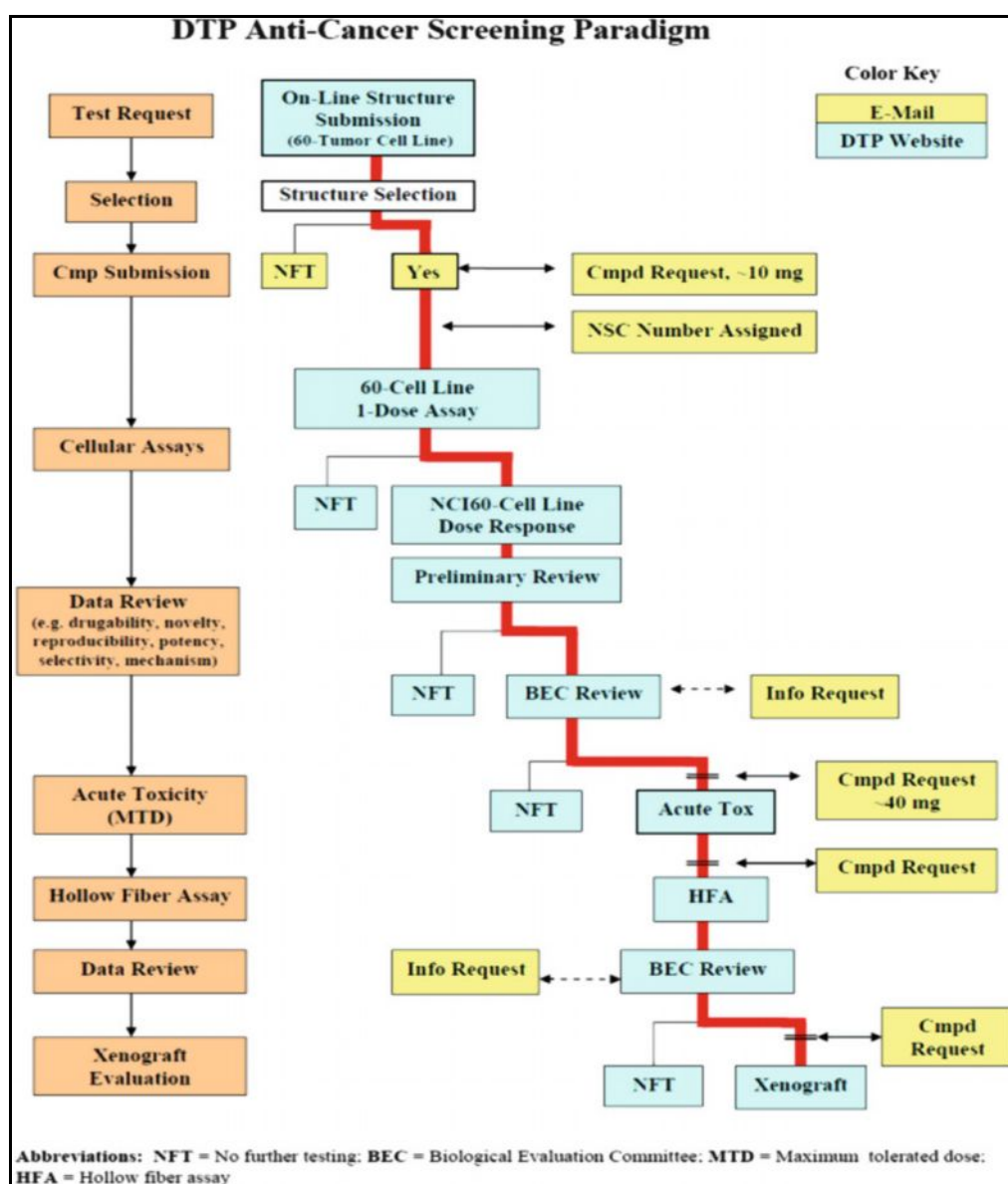
Cancer stem cells are a rare fraction of cells within a tumor which retain self-renewal properties. They also have self-protection mechanisms owing to the expression of high levels of drug efflux pumps¹¹. Tumor recurrence is usually associated with development of resistance to the agents to which the patient initially responded. Conventional cellular screens are not suitable to evaluate stem cell-targeted treatments because they are aimed to measure tumor cell inhibition or kill the bulk cell mass.

iii. Biochemical Screening Assays

Biochemical Screening Assays provide the means for evaluating high numbers of compounds. These screens are primarily employed in the pharmaceutical industry and institutions that harbor large compound libraries for systematic search of novel agents. An important advantage of biochemical screens is that they can be fully automated; thus, most steps can be performed by robot or computer systems such as dispensing of targets, addition of drugs and detection reagents, as well as compound library storage and management.

iv. Combination of Target and Cell Screens

The cell-based screening approaches will miss agents with certain defined modes of action when compared to target-based screening. For example, specific telomerase inhibitors owing to lack of cytotoxic potency in short-term assays. They might, on the other hand, identify compounds as active with previously unknown targets and hence allow for identification of novel mechanisms of action as well as the elucidation of their interplay in certain pathways. Another advantage of compounds identified in cellular screens are their proven cell permeable properties, which might be missing in cell-free systems. Most anticancer agents in current use were discovered either by chance (e.g., cisplatin and thenitrogen mustards) or through screening programs (e.g., vinblastine and paclitaxel [TaxolTM]).





Developmental Therapeutics Program (DTP) anticancer drug screening and decision-making process as of May 2008¹²

❖ Imaging Tests

▪ Mammograms

A mammogram is an x-ray picture of the breast. Screening mammograms are used to check for breast cancer in women who have no signs or symptoms of the disease. Diagnostic mammograms are used to check for breast cancer after a lump or other sign or symptom of the disease has been found.

Screening mammograms usually involve two x-ray pictures, or images, of each breast. The x-ray images make it possible to detect tumors that cannot be felt. Screening mammograms can also find microcalcifications (tiny deposits of calcium) that sometimes indicate the presence of breast cancer. Screening mammography can help reduce the number of deaths from breast cancer among women ages 40 to 74.

Efforts to improve conventional mammography include digital mammography, magnetic resonance imaging (MRI), positron emission tomography (PET) scanning, and diffuse optical tomography, which uses light instead of x-rays to create pictures of the breast.

Digital and conventional mammography both use x-rays to produce an image of the breast; however, in conventional mammography, the image is stored directly on film, whereas, in digital mammography, an electronic image of the breast is stored as a computer file. A subsequent analysis of women aged 40 through 79 who were undergoing screening in U.S. community-based imaging facilities also found that digital and film mammography had similar accuracy in most women. Digital screening had higher sensitivity in women with dense breasts¹³.



▪ Computed Tomography (CT) Scans

Computed tomography (CT) is an imaging procedure that uses special x-ray equipment to create a series of detailed pictures, or scans, of areas inside the body. It is also called computerized tomography and computerized axial tomography (CAT) scanning.

In cancer, CT may be used to help detect abnormal growths; to help diagnose tumors; to provide information about the extent, or stage, of disease; to help in guiding biopsy procedures or in planning treatment; to determine whether a cancer is responding to treatment; and to monitor for recurrence. The term *tomography* comes from the Greek words *tomos* (a cut, a slice, or a section) and *graphein* (to write or record). The newest CT scanners, called multislice CT or multidetector CT scanners, allow more slices to be imaged in a shorter period of time.

Colorectal cancer:-

CT colonography (also known as virtual colonoscopy) can be used to screen for both large colorectal polyps and colorectal tumors. CT colonography uses the same dose of radiation that is used in standard CT of the abdomen and pelvis, which is about 10 millisieverts (mSv)¹⁴.

Lung cancer:-

The NCI-sponsored National Lung Screening Trial (NLST) showed that people aged 55 to 74 years with a history of heavy smoking are 20 percent less likely to die from lung cancer if they are screened with low-dose helical CT than if they are screened with standard chest x-rays. The estimated amount of radiation in a low-dose helical CT procedure is 1.5 mSv.

❖ Laboratory Tests

▪ Understanding Laboratory tests

A laboratory test is a procedure in which a sample of blood, urine, other bodily fluid, or tissue is examined to get information about a person's health.

Although many laboratory tests are used to help diagnose cancer, a biopsy (the removal of cells or tissues for examination under a microscope by a pathologist) is usually needed to be certain that a person has cancer.

Laboratory tests are used in cancer medicine in many ways:

- To screen for cancer or precancerous conditions before a person has any symptoms of disease
- To help diagnose cancer
- To provide information about the stage of a cancer (that is, its severity); for malignant tumors, this includes the size and/or extent (reach) of the original (primary) tumor and whether or not the tumor has spread (metastasized) to other parts of the body
- To plan treatment
- To monitor a patient's general health during treatment and to check for potential side effects of the treatment
- To determine whether a cancer is responding to treatment
- To find out whether a cancer has recurred
- **Blood chemistry test**

The amounts of certain substances that are released into the blood by the organs and tissues of the body, such as metabolites, electrolytes, fats, and proteins, including enzymes. Blood chemistry tests usually include tests for blood urea nitrogen (BUN) and creatinine.

- **Cancer gene mutation testing**

The presence or absence of specific inherited mutations in genes that are known to play a role in cancer development. Examples include tests to look for *BRCA1* and *BRCA2* gene mutations, which play a role in development of breast, ovarian, and other cancers. It is used for the assessment of cancer risk.

- **Complete blood count (CBC)**

It is used to measure the number of different types of blood cells, including red blood cells, white blood cells, and platelets, in a sample of blood. This test also measures the amount of hemoglobin (the protein that carries oxygen) in the blood, the percentage of the total blood volume that is taken up by red blood cells (hematocrit), the size of the red blood cells, and the amount of hemoglobin in red blood cells. It is used in the diagnosis, particularly in leukemias, and monitoring during and after treatment.

- **Cytogenetic analysis**

It measures the changes in the number and structure of chromosomes in a patient's white blood cells or bone marrow cells. It is used in diagnosis, deciding on appropriate treatment.

- **Immunophenotyping**

It identifies the cells based on the types of antigens present on the cell surface. It is used in the diagnosis, staging, and monitoring of cancers of the blood system and other hematologic disorders, including leukaemias, lymphomas, myelodysplastic syndromes, and myeloproliferative disorders. It is most often done on blood or bone marrow samples, but it may also be done on other bodily fluids or biopsy tissue samples.

- **Sputum cytology**

It is also called sputum culture. The presence of abnormal cells in sputum (mucus and other matter brought up from the lungs by coughing) is measured. It is mainly used in the diagnosis of lung cancer.

- **Tumor marker tests**

Some measure the presence, levels, or activity of specific proteins or genes in tissue, blood, or other bodily fluids that may be signs of cancer or certain benign (noncancerous) conditions.

Some tumor marker tests analyze DNA to look for specific gene mutations that may be present in cancers but not normal tissues. Examples include *EGFR* gene mutation analysis to help determine treatment and assess prognosis in non-small cell lung cancer and *BRAF* gene mutation analysis to predict response to targeted therapies in melanoma and colorectal cancer.

Other tumor marker tests, called multigene tests (or multiparameter gene expression tests), analyze the expression of a specific group of genes in tumor samples. These tests are used for prognosis and treatment planning.

- **Urinalysis**

The color of urine and its contents, such as sugar, protein, red blood cells, and white blood cells are measured. It is used in the Detection and diagnosis of kidney cancer and urothelial cancers.

- **Urine cytology**

Presence of abnormal cells shed from the urinary tract into urine to detect disease is measured. It is used in the detection and diagnosis of bladder cancer and other urothelial cancers, monitoring patients for cancer recurrence.

- **Pap and HPV Testing**

A Pap test is a test of a sample of cells taken from a woman's cervix or vagina. Nearly all cases of cervical cancer are caused by infection with oncogenic, or high-risk, types of human papillomavirus, or HPV.

Cervical cancer screening includes two types of screening tests: cytology-based screening, known as the Pap test or Pap smear, and HPV testing. The main purpose of screening with the Pap test is to look for changes in the cells of the cervix and vagina that show cancer or conditions that may develop into cancer. The Pap test can also find noncancerous conditions, such as infections and inflammation. Current guidelines recommend that women should have a Pap test every 3 years beginning at age 21.

A doctor uses a device called a speculum to widen the opening of the vagina so that the cervix and vagina can be examined. It is the best tool to detect precancerous conditions and hidden, small tumors that may lead to cervical cancer. If detected early, cervical cancer can be cured

HPV testing is used to look for the presence of high-risk HPV types in cervical cells. These tests can detect HPV infections that cause cell abnormalities, sometimes even before cell abnormalities are evident. Testing for high risk HPV types is an important part of the management of women who have been treated for HSIL. Several studies have shown that these women are at increased risk of further high grade disease. After treatment, the vast majority of women will clear their oncogenic HPV infection within 24 months. The reliable negative predictive value of HPV testing allows these women to return to the routine Pap screening interval¹⁵.

• Prostate- Specific Antigen (PSA) Test

Prostate-specific antigen (PSA, also known as **kallikrein III, seminin, semenogelase, γ -seminal protein** and **P-30 antigen**) is a 34 kD glycoprotein produced almost exclusively by the prostate gland. It is a serine protease (EC 3.4.21.77) enzyme, the gene of which is located on the nineteenth chromosome (19q13) in humans¹⁶. The PSA test measures the blood level of PSA. The higher a man's PSA level, the more likely it is that he has prostate cancer. The PSA test has been widely used to screen men for prostate cancer. It is also used to monitor men who have been diagnosed with prostate cancer to see if their cancer has recurred (come back) after initial treatment or is responding to therapy. The results are usually reported as nanograms of PSA per milliliter (ng/mL) of blood.

In 1994, the FDA approved the use of the PSA test in conjunction with a digital rectal exam (DRE) to test asymptomatic men for prostate cancer. The most frequent benign prostate conditions that cause an elevation in PSA level are prostatitis (inflammation of the prostate) and benign prostatic hyperplasia (BPH) (enlargement of the prostate). There is no evidence that prostatitis or BPH leads to prostate cancer, but it is possible for a man to have one or both of these conditions and to develop prostate cancer as well.

Conclusion:

The various screening tests of the cancer have been discussed. Cancer is the second leading cause of death, where one in four deaths is due to cancer. Cancer screening aims to detect cancer before symptoms appear. Screening tests must be effective, safe, well-tolerated with acceptably low rates of false positive and false negative results. If signs of cancer are detected, more definitive and invasive follow-up tests are performed to reach a diagnosis. Screening for cancer can lead to cancer prevention and earlier diagnosis. Early diagnosis may lead to higher rates of successful treatment and extended life.

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