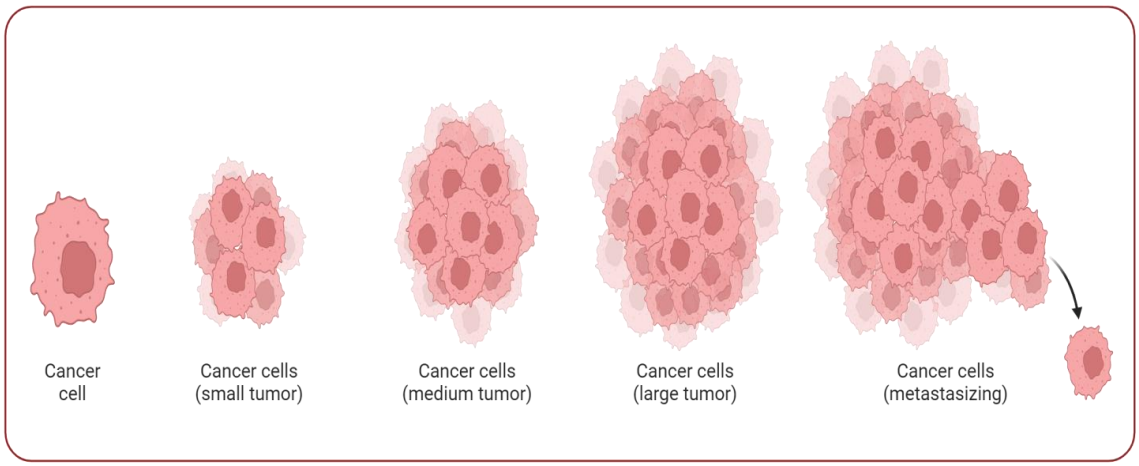
2

REVIEW OF LITERATURE

Cancer is a complex disease influenced by multiple factors, characterized by various levels of morphological abnormalities, uncontrolled cell proliferation, invasion, and potential metastasis. Uncontrolled cell division can disrupt the body's normal functions and, in severe cases, leads to death (Coughlin, 2019). The causes of cancer can stem from a range of sources, including external factors like tobacco use, exposure to infectious agents, chemical substances, and radiation, as well as internal factors such as hormonal imbalances, immune system disorders, and genetic mutations resulting from metabolic processes in various parts of the body. Each type of cancer exhibits its own unique growth rate, prognosis, and potential for treatment (Mattiuzzi and Lippi, 2019). The development of cancer cell is illustrated in **Figure 1**. The figure was retrieved from BioRender.

Figure 1: Development of cancer cell

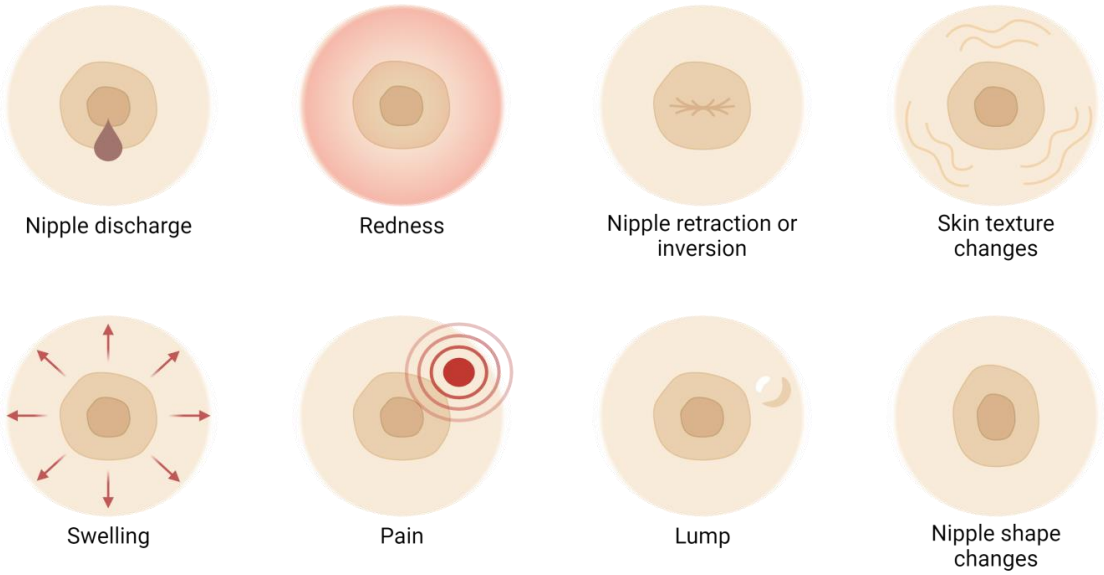


2.1 Breast cancer epidemiology

Breast cancer represents a significant global public health concern, ranking as the second most frequently diagnosed cancer and a leading cause of mortality among women worldwide (Suganya *et al*., 2022a). This disease has become alarmingly fatal, with

an ever-expanding list of risk factors. Breast cancer originates in the breast cells, often in the milk-carrying ducts or the milk-producing lobules. It exhibits a high tendency to invade and metastasize, spreading malignant cells to distant areas like the lungs, bones, liver, and brain. The conversion of normal breast cells into cancerous ones involves a complex interplay of genetic and epigenetic alterations. The incidence of breast cancer exhibits significant variation across regions and countries, reflecting differences in the racial and ethnic composition, healthcare resources, and lifestyle choices of the general population (Youn and Han, 2020). The symptoms of breast cancer are depicted in **Figure 2**. The figure was retrieved from BioRender.

Figure 2: Breast cancer symptoms



According to the World Health Organization (WHO), malignant neoplasms pose the most significant health burden for women worldwide, contributing to an estimated 107.8 million Disability-Adjusted Life Years (DALYs). Among these, breast cancer alone is responsible for 19.6 million DALYs. The incidence rates were highest in developed regions, Asia and Africa accounted for 63% of total deaths. Notably, women diagnosed with breast cancer in high-income countries tend to have better survival rates, while the opposite is often true for women in low-income and middle-income countries (Łukasiewicz *et al*., 2021). In low- and middle-income countries, breast cancer incidence

is expected to rise further due to the adoption of western lifestyles, including delayed pregnancies, reduced breastfeeding, sedentary habits, and poor diets (Francies *et al*., 2020).

Breast cancer is a growing concern in India, affecting both rural and urban areas. Startlingly, every four minutes, a woman in India is diagnosed with breast cancer. Unfortunately, cancer survival becomes increasingly challenging in the later stages of the disease, and more than 50% of Indian women are diagnosed with stage 3 and 4 breast cancer (Maurya and Brahmachari, 2021). Women can self-diagnose their condition and know of the presence of lumps or masses that suggest cancerous outgrowths. The very reason for the low breast cancer survival rate of women in India accounts for its lack of awareness and poor early screening and diagnosis rates. It's also more common in the younger age group. Almost 50% of all cases are in the age group of 25-50. More than 70% of the cases present in the advanced stage had poor survival and high mortality. Increasing awareness, promoting early detection, and improving access to healthcare services are essential steps in addressing the breast cancer challenge in India (Mehrotra and Yadav, 2022).

This global trend of increasing breast cancer cases is influenced by a multitude of external and internal factors that contribute to the development and progression of the disease, as depicted in **Figure 3**.

The number of risk factors of breast cancer is significant and includes both modifiable factors and non-modifiable factors.

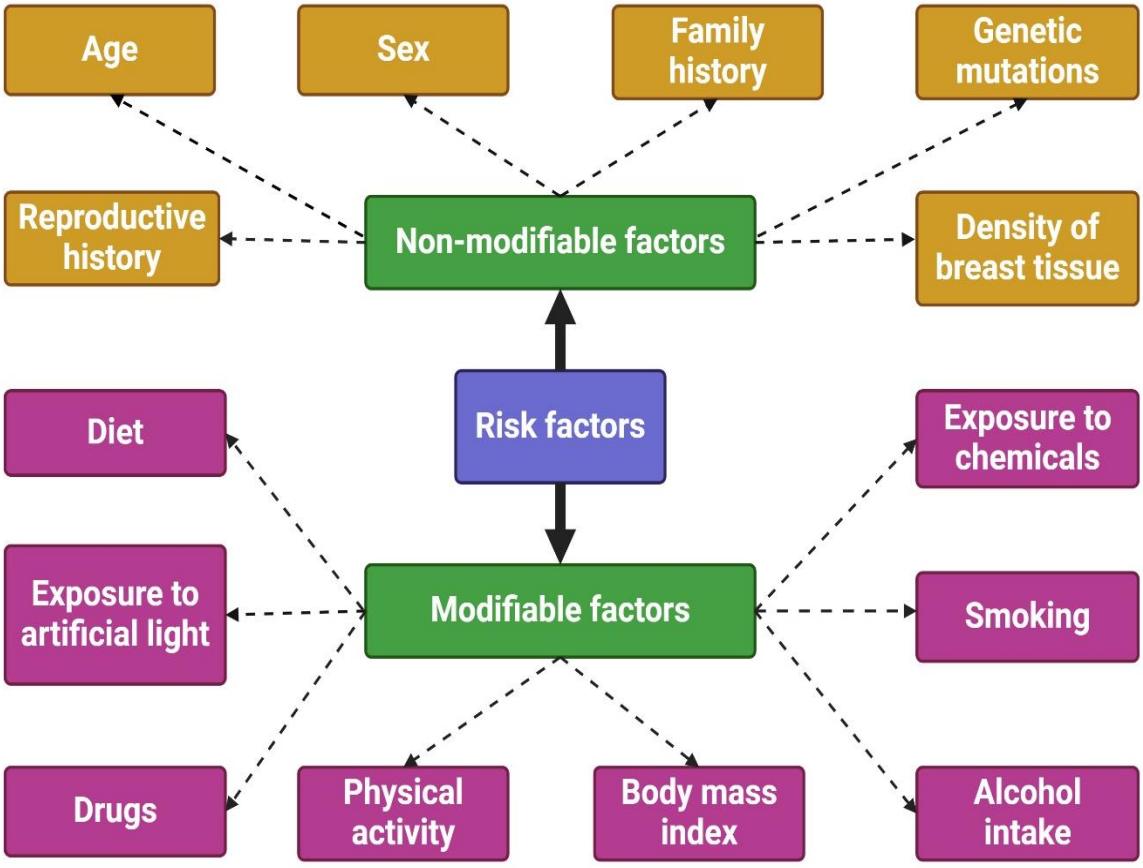
2.2 Non-modifiable factors

2.2.1 Age

Age is a pivotal risk factor for breast cancer, and the global surge in breast cancer incidence affects all age groups. Interestingly, the highest increase is observed among women under the age of 50. Breast cancer remains a significant clinical and social concern due to its aggressive nature (Momenimovahed and Salehiniya, 2019). Numerous studies have highlighted the distinctive characteristics of breast cancer in young women. It often exhibits greater histological malignancy, limited expression of steroid receptors, and frequent overexpression of the HER-2 receptor. In some cases, it falls into the

molecular biological subtype known as "basal-like" or "triple-negative" breast cancer. Furthermore, there has been a notable rise in the incidence of breast cancer among premenopausal women. Over 30 years, the incidence in this group has nearly doubled. These trends underscore the importance of understanding and addressing the unique challenges posed by breast cancer in younger women (McCarthy *et al*., 2021).

Figure 3: Multifactorial risk factors of breast cancer



2.2.2 Sex

Female sex constitutes one of the major factors associated with an increased risk of breast cancer primarily because of enhanced hormonal stimulation. Changes in the natural levels of endogenous sex hormones contribute to an increased risk of breast cancer in both premenopausal and postmenopausal women. These findings are supported by research from the Endogenous Hormones and Breast Cancer Collaborative Group (Tin Tin *et al*., 2021). Breast cancer in men is exceptionally rare, accounting for less

than 1% of all breast cancer cases. When it does occur, it tends to be diagnosed at a more advanced stage compared to women. Men are typically diagnosed with breast cancer at an average age of around 60. Several important risk factors for breast cancer in men include older age, mutations in the *BRCA2* or *BRCA1* genes, elevated estrogen levels, Klinefelter syndrome, a family history of breast cancer, and radiation exposure. These factors play a significant role in increasing the risk of breast cancer in men (Khan and Tirona, 2021).

2.2.3 Family history

A family history of breast cancer is a prominent factor strongly linked to an elevated risk of developing breast cancer. Approximately 13-19% of individuals diagnosed with breast cancer have a first-degree relative who has also been affected by the disease. Moreover, the risk of breast cancer rises significantly with an increasing number of first-degree relatives affected, and the risk is even greater when the affected relatives are under the age of 50 (Liu *et al*., 2021). The connection between a family history of breast cancer and an increased risk is influenced by a combination of epigenetic changes and environmental factors that can act as potential triggers. Additionally, a family history of ovarian cancer, particularly when associated with *BRCA1* and *BRCA2* mutations, can further heighten the risk of developing breast cancer (Mahdavi *et al*., 2019).

2.2.4 Genetic mutations

Numerous genetic mutations have been identified as strongly associated with an increased risk of breast cancer. Among the most notable are *BRCA1* and *BRCA2*, tumor suppressor genes located on chromosome 17 and chromosome 13, respectively. While they can be inherited autosomal dominant, sporadic mutations are also observed. *BRCA1* and *BRCA2* mutations disrupt the normal function of these genes in repairing damaged DNA, leading to genomic instability and an increased susceptibility to cancer development (Ellsworth *et al*., 2019).

Other genes, such as *TP53, CDH1, PTEN,* and *STK11,* have been identified in breast cancer risk. *TP53*, known as the "guardian of the genome," plays a vital role in cell cycle regulation and DNA repair. *CDH1* mutations are associated with hereditary

diffuse gastric cancer syndrome, which elevates the risk of lobular breast cancer. *PTEN* and *STK11* are both tumor suppressor genes involved in cell growth regulation, predisposing individuals to breast cancer and other malignancies (Wendt and Margolin, 2019).

Furthermore, various DNA repair genes have been implicated in breast cancer development, including *ATM, PALB2, BRIP1,* and *CHEK2*. These genes interact with *BRCA1* and *BRCA2* in the homologous recombination pathway, which is crucial for repairing double-stranded DNA breaks. Mutations in *ATM, PALB2, BRIP1*, and *CHEK2* can compromise this DNA repair mechanism, leading to genomic instability and an increased risk of breast cancer. While these mutations typically have lower penetrance compared to *BRCA1* or *BRCA2*, they still significantly contribute to the overall genetic predisposition to breast cancer (Angeli *et al*., 2020).

2.2.5 Reproductive history

Numerous studies confirmed a strict relationship between exposure to endogenous hormones estrogen and progesterone has an excessive risk of breast cancer in females. Specific events such as pregnancy, breastfeeding, the onset of menstruation, menopause, and associated hormonal imbalances, play a crucial role in the potential initiation of breast cancer in the breast microenvironment (Satpathi *et al*., 2023). Early full-term pregnancies, especially in the early twenties, along with subsequent pregnancies, are associated with a reduced risk of breast cancer. Pregnancy itself offers protective effects against potential cancer, and no association between increased breast cancer risk and abortion has been established. Prolonged breastfeeding also lowers the risk of both estrogen receptor/progesterone receptor-positive and -negative breast cancers (Qiu *et al*., 2022). Early onset of menstruation is another risk factor for breast cancer and may be associated with tumor grade and lymph node involvement, potentially affecting overall prognosis. In contrast, early menopause, whether natural or surgically induced, reduces the risk of breast cancer (Łukasiewicz *et al*., 2021).

2.2.6 Density of breast tissue

Breast tissue density undergoes variations over a person's lifetime, and in clinical practice, it is categorized into low-density, high-density, and fatty breasts.

Women, especially those who are younger, have a lower BMI, pregnant, breastfeeding, or using hormonal replacement therapy, tend to exhibit higher breast tissue density. In general, increased breast tissue density is associated with a greater risk of breast cancer. This correlation holds for both premenopausal and postmenopausal women (Bodewes *et al*., 2022).

2.3 Modifiable factors

2.3.1 Drugs

The use of diethylstilbestrol during pregnancy is linked to an elevated risk of breast cancer, not only in mothers but also in their offspring. Multiple studies have indicated that women, who use hormonal replacement therapy (HRT), especially for durations exceeding 5 or 7 years, are at an elevated risk of breast cancer (Troisi *et al*., 2019). Hormonal contraceptives, which include oral formulations containing both estrogen and progestin, have been associated with a slight increase in breast cancer risk. However, this risk tends to diminish after discontinuation of the contraceptives (Burchardt *et al*., 2022). Aromatase inhibitors, utilized in the treatment of hormone receptor-positive breast cancer, may elevate the risk of breast cancer in postmenopausal women (Gnant *et al*., 2021). The long-term use of bisphosphonates, prescribed primarily for osteoporosis and bone metastases, has been linked to a higher risk of breast cancer (Jackson *et al*., 2021). Additionally, thiazide diuretics, commonly prescribed for hypertension, and certain antipsychotic medications have been implicated in a high risk of breast cancer. Moreover, growth hormone therapy and immunosuppressants, such as calcineurin inhibitors, have been associated with increased breast cancer risk, possibly due to their influence on hormonal and immune pathways (Mohammed *et al*., 2021).

Additionally, the intake of certain antidepressants, particularly paroxetine, tricyclic antidepressants, and selective serotonin reuptake inhibitors, may be linked to a higher risk of breast cancer (Li *et al*., 2020). Other drugs that might constitute potential risk factors for breast cancer include antibiotics, statins, antihypertensive medications (such as calcium channel blockers and angiotensin II-converting enzyme inhibitors), as well as NSAIDs (including aspirin and ibuprofen) (Xie *et al*., 2021).

2.3.2 Physical activity

Even though the mechanism remains undeciphered, regular physical activity is considered to be a protective factor of breast cancer incidence. Among females with a family history of breast cancer, physical activity was associated with a reduced risk of cancer. However, physical activity is beneficial not only in females with a family history of breast cancer but also in those without family history (Jurdana, 2021). There are several hypotheses aiming to explain the protective role of physical activity in terms of breast cancer incidence; physical activity might prevent cancer by reducing exposure to endogenous sex hormones and altering immune system responses or insulin-like growth factor-1 levels (Friedenreich *et al*., 2021).

2.3.3 Body mass index

Epidemiological evidence strongly suggests obesity with an increased risk of breast cancer. This association is particularly notable in postmenopausal women who are obese and have a higher likelihood of developing estrogen-receptor-positive breast cancer. Researchers have also observed that a higher body mass index (BMI) is linked to more aggressive tumor characteristics, including a greater incidence of lymph node metastasis, larger tumor size, and poorer clinical outcomes (Wang *et al*., 2020). Obesity may contribute to higher mortality rates and an elevated risk of cancer recurrence, especially in premenopausal women. Increased body fat can enhance inflammation and impact the levels of circulating hormones, which can facilitate pro-carcinogenic events. Poorer clinical outcomes are primarily seen in women with a BMI of $\geq$25 kg/m² (Cava *et al*., 2022).

2.3.4 Alcohol intake

Numerous pieces of evidence confirm that excessive alcohol consumption is a factor that might enhance the risk of malignancies within the gastrointestinal tract; however, it was proved that it is also linked to the risk of breast cancer. Interestingly, it's not the type of alcohol but the content of alcoholic beverages that primarily influences cancer risk. Alcohol intake leads to elevated estrogen levels, resulting in hormonal imbalances that affect the risk of carcinogenesis in female organs (Bala *et al*., 2021). Additionally, alcohol consumption often leads to excessive weight gain and higher BMI

levels, further increasing the risk of cancer. Alcohol consumption has been observed to specifically increase the risk of estrogen-positive breast cancers. When consumed before the first pregnancy, it significantly contributes to morphological alterations in breast tissue, making it more susceptible to further carcinogenic events (Song *et al*., 2022).

2.3.5 Smoking

Carcinogens found in tobacco are transported to the breast tissue increasing the plausibility of mutations within oncogenes and suppressor genes (*p53*). Thus, not only active but also passive smoking significantly contributes to the induction of carcinogenic events. Besides, a longer smoking history, as well as smoking before the first full-term pregnancy, is additionally pronounced in females with a family history of breast cancer (Gram *et al*., 2019).

2.3.6 Exposure to artificial light

Recent research has established a connection between artificial light at night (ALAN) and an elevated risk of breast cancer. The likely causative factor appears to be the disruption of the melatonin rhythm, leading to subsequent epigenetic changes. According to existing studies, higher exposure to ALAN is associated with a significantly increased risk of breast cancer when compared to individuals with lower ALAN exposure (Bożejko *et al*., 2022).

2.3.7 Intake of processed food/diet

According to the World Health Organization (WHO), highly processed meat is classified as a Group 1 carcinogen that might increase the risk of not only gastrointestinal malignancies but also breast cancer. Similar observations were made in terms of an excessive intake of saturated fats. Ultra-processed food is rich in sodium, fat, and sugar which subsequently predisposes to obesity recognized as another factor of breast cancer risk. It was observed that a 10% increase in ultra-processed food in the diet is associated with an 11% greater risk of breast cancer (Händel *et al*., 2021). A diet high in vegetables, fruits, legumes, whole grains, and lean protein is associated with a lowered risk of breast cancer. Generally, a diet that includes food containing high amounts of n-3 PUFA,

vitamin D, fiber, folate, and phytoestrogen might be beneficial in the prevention of breast cancer (Suganya and Sumathi, 2022a).

2.3.8 Exposure to chemicals

Prolonged exposure to chemicals can contribute to the development of breast cancer by impacting the tumor microenvironment and subsequently triggering epigenetic changes, as well as promoting pro-carcinogenic processes. Women who are chronically exposed to chemicals have a significantly higher likelihood of developing breast cancer (Lagoa *et al*., 2022). Numerous chemicals have been suggested as potential inducers of breast carcinogenesis. Notably, dichlorodiphenyltrichloroethane (DDT) and polychlorinated biphenyl (PCB) have been extensively investigated for breast cancer, particularly due to their disruptive effects on the development of mammary glands when exposed at an early age (Huang *et al*., 2019). Additionally, a potential connection has been observed with increased exposure to polycyclic aromatic hydrocarbons (PAH), synthetic fibers, organic solvents, oil mist, and insecticides (Sharma *et al*., 2023).

2.4 Tumorigenesis of breast cancer: a multi-stage process

In breast cancer, much like in many other cancers, the predominant genetic alterations are somatic. However, in a small percentage of breast cancers (around 4-10%), there is a germ-line mutation that predisposes individuals to the disease. This genetic mutation can contribute to the occurrence of specific somatic genetic changes that ultimately lead to the cancer phenotype (Biancolella *et al*., 2021). The genetic alterations seen during the development of malignancy are diverse which include chromosomal deletions, translocations, amplifications, rearrangements, duplications, whole chromosome losses (aneuploidy), and point mutations (Jain *et al*., 2019). These genetic alterations can lead to various outcomes, including:

- Activation of genes in a dominant manner, where a change in only one of the two copies of the gene. These genes are commonly referred to as oncogenes.
- Inactivation of genes leads to a loss of their normal function, which contributes to tumorigenesis. These genes are known as tumor suppressor genes.
- Activation or inactivation of genes whose products are involved in maintaining genome stability, such as genes that regulate mitosis and DNA repair enzymes.

Alterations in these genes can facilitate changes in other genes directly implicated in cancer, like oncogenes and tumor suppressor genes.

- Modification of gene expression may also result from changes in genes that impact chromatin remodeling or DNA methylation. These mechanisms are considered epigenetic, as they are potentially reversible, unlike mutations (Poornima *et al*., 2023a).

It's important to note that the activation or inhibition of a single gene is typically insufficient to transform a normal cell into a cancerous one. Multiple independent mutational events are required, and the accumulation of these genetic alterations, which interact and cooperate, is necessary to drive the transition to a tumor phenotype. At each stage of this multi-step process, each new genetic alteration acquired by the cell confers new properties, which can favor the selection of this particular clone and contribute to the development of the tumor phenotype (Dasari *et al*., 2021).

In cancer cells, 30,000-40,000 human genes exhibit altered expression. These altered genes are often not specific to a particular histological type of cancer (Finlay-Schultz *et al*., 2020). Understanding this complex process of carcinogenesis requires the identification of numerous genetic alterations and mutations, some of which play a crucial role in the initiation and progression of tumors. Ultimately, the identification of these key genetic alterations will have significant implications for diagnosis, prognosis, and treatment strategies in the field of cancer (Poornima *et al*., 2023b).

2.5 Molecular subtypes of breast cancer

The molecular subtypes are recognized mainly on the expression of estrogen receptor (ER), progesterone receptor (PR), and human epidermal growth factor receptor 2 (HER2). The different molecular subtypes are:

- Luminal A: ER-positive, PR-positive, HER2-negative
- Luminal B: ER-positive, PR-negative, HER2-positive
- HER2 type: ER-negative, PR-negative, HER2-positive
- Basal-like or triple-negative breast cancer (TNBC): ER-negative, PR-negative, HER2-negative (Sumathi *et al*., 2020).

2.6 Treatment strategies and side effects

2.6.1 Surgery

Surgery is the primary treatment option for patients with localized breast cancer, and it may also be considered for more advanced stages of the disease. The specific type of breast cancer surgery selected depends on the tumor's characteristics and its extent of spread, and it can be treated by radiotherapy or chemotherapy, either sequentially or in combination (Łukasiewicz *et al*., 2021). The main types of breast cancer surgery are as follows:

- **Lumpectomy:** In this procedure, small clusters of abnormal cells in the breast tissue are surgically removed, preserving the majority of the breast.
- **Mastectomy:** Mastectomy is a more extensive surgical procedure that involves the removal of the entire breast, including the breast tissue, lobules, nipple, areola, chest wall, ducts, fatty cells, and sometimes lymph nodes in the armpit, depending on the specific circumstances.
- **Sentinel node biopsy:** This surgery is performed to determine if cancer cells have spread to the nearby lymph nodes, and the affected sentinel nodes are removed.
- **Axillary lymph node dissection:** If cancer is detected in the sentinel nodes, this procedure involves the removal of many lymph nodes in the armpit to assess the extent of lymph node involvement (Tinterri *et al*., 2023).

Side effects

- Pain and discomfort at the surgical site
- Swelling in the arm on the side of surgery, known as lymphedema (for certain breast surgeries)
- Limited mobility in the arm and shoulder
- Changes in the appearance of the breast, including scarring
- Numbness or tingling sensations in the breast or chest wall (Trayes and Cokenakes, 2021).

2.6.2 Chemotherapy

Chemotherapy plays a crucial role in impeding the growth of tumors and can be administered through oral medications or injections. These medications enter the bloodstream and work to inhibit the growth of malignant cells (Behranvand *et al*., 2022). In breast cancer treatment, several drugs are commonly used in chemotherapy, including:

- **Doxorubicin (Adriamycin):** This anthracycline chemotherapy drug is effective in treating various types of breast cancer. It disrupts DNA replication and repair by intercalating with DNA and generates free radicals, causing further DNA damage.
- **Cyclophosphamide:** Often used in combination with doxorubicin, cyclophosphamide is an alkylating agent that hinders cell division by attaching alkyl groups to DNA strands.
- **Paclitaxel:** A taxane chemotherapy drug used for both early-stage and metastatic breast cancer. It stabilizes microtubules, preventing their disassembly during cell division, ultimately leading to cell death.
- **Docetaxel:** Another taxane drug used in breast cancer treatment, particularly for metastatic cases. It functions similarly to paclitaxel by disrupting microtubules and inhibiting cell division.
- **5-Fluorouracil (5-FU):** This antimetabolite drug interferes with DNA and RNA synthesis and is frequently used in combination regimens for breast cancer treatment.
- **Epirubicin:** Epirubicin, like doxorubicin, is an anthracycline drug used in combination chemotherapy for breast cancer. It is often combined with cyclophosphamide or taxanes.
- **Gemcitabine:** An inhibitor of DNA replication, gemcitabine is occasionally used in combination regimens for breast cancer treatment, especially in the metastatic setting.
- **Carboplatin:** A platinum-based chemotherapy drug that forms covalent bonds with DNA, leading to DNA cross-linking and inhibition of DNA replication. It is often used for triple-negative breast cancer.

- **Vinorelbine:** This plant alkaloid disrupts microtubules and cell division, making it especially valuable in the metastatic setting.
- **Vinblastine:** Another plant alkaloid that interferes with microtubules and cell division, used in breast cancer therapy.
- **Eribulin:** A microtubule inhibitor used for metastatic breast cancer, particularly in patients who have already undergone multiple lines of therapy.
- **Capecitabine:** An oral chemotherapy drug that is converted to 5-FU in the body. It is often used in metastatic breast cancer, particularly for patients who cannot tolerate intravenous chemotherapy.
- **Ixabepilone:** Ixabepilone stabilizes microtubules and is used to treat advanced or metastatic breast cancer.
- **Nab-Paclitaxel (Abraxane):** This is a nanoparticle formulation of paclitaxel often in metastatic settings (Claessens *et al*., 2020).

Side effects

- Nausea and vomiting
- Fatigue
- Hair loss (alopecia)
- Anaemia
- Cardiomyopathy
- Increased risk of infections (due to lowered white blood cell count)
- Easy bruising and bleeding (due to lowered platelet count)
- Neuropathy (numbness or tingling in the hands and feet)
- Changes in taste and appetite
- Cognitive changes often referred to as "chemo brain"
- Menstrual cycle changes or temporary infertility (Suganya and Sumathi, 2022b).

2.6.3 Radiotherapy

Radiation therapy is a common component of breast cancer treatment, often used in conjunction with surgery and chemotherapy to reduce the risk of cancer recurrence. It can be administered after surgery as adjuvant treatment or alongside chemotherapy before surgery as neoadjuvant therapy to shrink the tumor. In some cases, radiotherapy is used as a stand-alone treatment for patients with advanced metastatic breast cancer. Typically, radiotherapy involves 3 to 5 sessions per week for duration of 3 to 6 months (Haussmann *et al*., 2020).

Side effects

- Skin changes, such as redness, peeling, or darkening in the treated area
- Fatigue
- Cardiomyopathy
- Breast pain or tenderness
- Lymphedema in the arm
- Heart and lung complications (Janssen *et al*., 2018).

2.6.4 Hormonal therapy

Hormone therapy is a commonly employed approach in the treatment of hormone receptor-positive breast cancer. It involves the use of medications to block or inhibit the actions of hormones like estrogen and progesterone. Hormone therapy can be administered either before or after surgery or in combination with other treatments to reduce the risk of cancer recurrence. In cases where the cancer has already metastasized, hormone therapy can help shrink and control it (Drãgãnescu, and Carmocan, 2017). Various treatments used in hormone therapy include:

- Medications that prevent hormones from binding to cancer cells are known as selective estrogen receptor modulators.
- Medications that inhibit the production of estrogen in the body after menopause are known as aromatase inhibitors.

- Surgical procedures or medications to halt hormone production in the ovaries (Das *et al*., 2023).

Side effects

- Hot flashes
- Vaginal dryness or changes in sexual desire
- Mood changes and depression
- Joint and muscle pain
- Osteoporosis or bone thinning (Trayes and Cokenakes, 2021).

2.6.5 Targeted therapy

Targeted therapies, also known as biological therapies, represent a relatively recent approach to cancer treatment. They are designed to pinpoint specific biological processes that are crucial for tumor growth. Targeted therapy encompasses the use of monoclonal antibodies, vaccines, and gene therapies. These therapies precisely focus on cancer-specific processes, making them effective while causing less harm to healthy, non-cancerous cells (Suganya *et al*., 2022b).

Side effects

- Skin rash or irritation
- Diarrhea
- Hypertension
- Elevated liver enzymes
- Allergic reactions (Kroschinsky *et al*., 2017).

2.7 Pharmacogenomics and personalized treatment approach

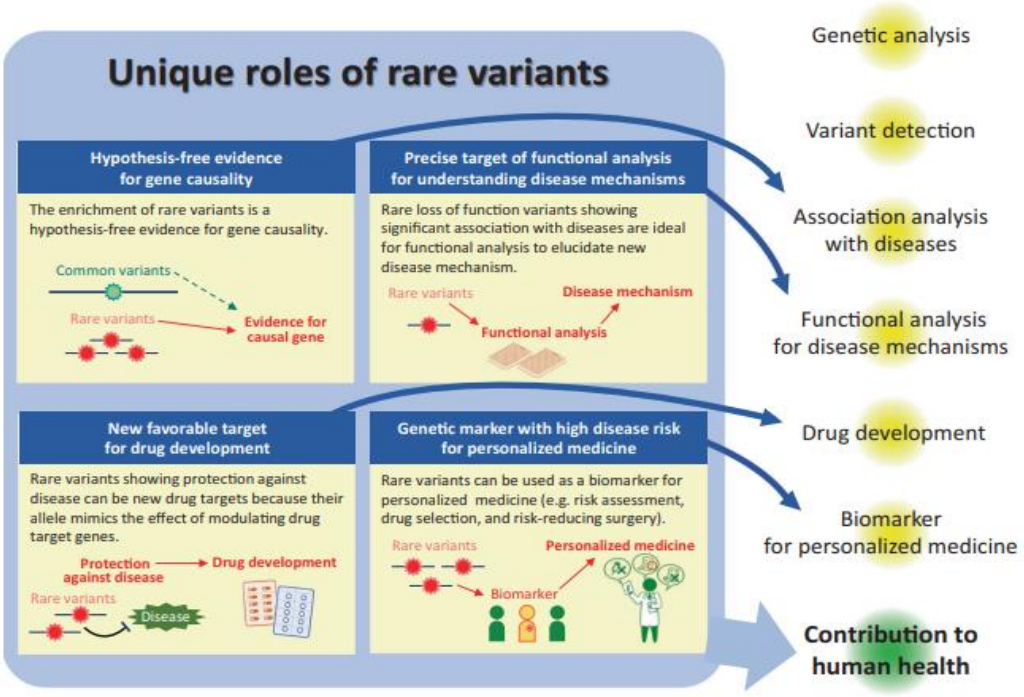
In personalized medicine for breast cancer, the primary objective is to deliver the most effective treatment tailored to each patient's unique characteristics. This approach involves categorizing cancer subtypes and determining the optimal treatment regimen based on patient characteristics, medical history, and response to therapy. Two pivotal strategies in the development of personalized therapies are: 1) optimization of drug

selection and dosage and 2) identification of genetic changes linked to cancer symptom occurrence and severity (Carr *et al*., 2021). Pharmacogenomics plays a crucial role in this process by assisting clinicians in selecting appropriate drugs and doses to minimize adverse effects and enhance efficacy. Understanding drug absorption, distribution, and metabolism is essential for treatment effectiveness, with genetic and epigenetic variations influencing these processes. Furthermore, multidrug resistance can be influenced by genetic polymorphisms, epigenetic changes, alterations in the microbiome, and demographic factors (Jeibouei *et al*., 2019). Pharmacogenomics focuses on how genetic variations impact drug metabolism and the integration of genome data can further elucidate drug responses. These approaches are extensively utilized in oncology to optimize the efficacy of targeted therapy and chemotherapy while minimizing toxicity. Additionally, pharmacogenomics is instrumental in developing new drugs based on genetic profiling and gene expression patterns, facilitated by innovative techniques and profiling instruments (Singh, 2020).

Recent studies have unveiled various genomic alterations, including single nucleotide polymorphisms (SNPs), gene overexpression, and metabolomic changes. These alterations can lead to variations in treatment efficacy and the impact on the severity of symptoms and quality of life in different individuals (Fernandez-Moya *et al*., 2020). Consequently, genetic biomarkers have emerged as valuable tools for determining the optimal drug choice and dosage in cancer treatment. This approach ensures that the treatment achieves the required efficacy without causing excessive discomfort to patients (Chang *et al*., 2020).

Genome-wide association studies have identified >10,000 genetic variants associated with various phenotypes and diseases. Although most are common variants, rare variants with minor frequency play a role in disease progression. Rare variants have unique roles that differ from common variants, characterized by lower impact on gene function and higher linkage disequilibrium with flanking variants and population specificity. The importance of rare variants is increasing, and they have great potential for use as biomarkers in personalized medicine (Momozawa and Mizukami, 2021). **Figure 4** shows the unique roles of rare variants that lead to improving human health.

Figure 4: Targeting rare variants for personalized treatment



(Momozawa and Mizukami, 2021)

Molecular profiling studies help to identify the rare genetic variants that can predict the risk of individuals developing common symptoms that are associated with cancer treatment. Studies on the metabolic changes that are associated with the occurrence and severity of certain cancer-associated symptoms have also helped to identify several molecular candidates on patients are at higher risk of increased severity (Krøigård *et al*., 2018). All of these would inform the development of novel strategies in planning for personalized therapies in symptom amelioration, thereby ensuring a better quality for patients undergoing cancer treatment (Moelans *et al*., 2019).

Despite recent advances in the identification of novel biomarkers such as rare variants that affect treatment efficacy and symptom severity, the molecular mechanisms of how they exert their effects are still not fully understood. Further studies on these issues are therefore warranted to enable the exploration and development of further strategies that can be utilized in optimizing cancer therapies for breast cancer patients, thereby augmenting the effectiveness of cancer treatment and improving the quality of life during the treatment process (Łukasiewicz *et al*., 2021).

2.8 Next generation sequencing (NGS)

The advancement of high-throughput DNA sequencing and bioinformatics analyses has opened up promising opportunities for personalized medicine. This approach has gained attraction in recent years, contributing to an overall improvement in the survival rates of cancer patients (Ben Kridis-Rejeb *et al*., 2020). Next-generation sequencing (NGS) techniques have revolutionized genomic understanding by offering diverse approaches for comprehensive DNA analysis in cancer studies. NGS techniques include:

- **Whole genome sequencing (WGS):** This method sequences the entire genome of cancer cells, providing a comprehensive view of genetic alterations, including mutations, structural variations, and copy number changes (Zhao *et al*., 2019).
- **Whole exome sequencing (WES):** WES targets and sequences only the cancer genome's protein-coding regions (exons), identifying somatic mutations that may drive tumorigenesis or influence treatment response (Zhang *et al*., 2021).
- **Targeted sequencing:** This technique focuses on specific cancer-related genes or pathways, enabling the detection of mutations, gene fusions, and other genomic alterations relevant to tumor development and treatment (Hayashi *et al*., 2020).
- **Liquid biopsy sequencing:** Liquid biopsy sequencing analyses circulating tumor DNA (ctDNA) or other tumor-derived materials in the blood, providing non-invasive detection of cancer-associated mutations, monitoring treatment response, and detecting minimal residual disease or recurrence (Chen and Zhao, 2019).
- **Chromatin immunoprecipitation sequencing (ChIP-Seq):** ChIP-Seq studies epigenetic alterations in cancer cells, such as histone modifications and transcription factor binding sites, contributing to gene expression dysregulation and tumor progression (Glont *et al*., 2019).
- **DNA methylation sequencing (Methyl-Seq):** Methyl-Seq profiles DNA methylation patterns in cancer genomes, identifying aberrant methylation patterns associated with tumor suppressor gene silencing, oncogene activation, and tumor heterogeneity (Pham *et al*., 2023).

- **Mitochondrial DNA (mtDNA) sequencing:** This method focuses on sequencing the mitochondrial genome, which contains valuable information for evolutionary studies and forensic analysis (Vega Avalos *et al*., 2022).
- **Nanopore sequencing:** Nanopore sequencing involves passing DNA molecules through nanometer-scale pores and measuring changes in electrical current to identify the sequence of individual bases (Helal *et al*., 2022).

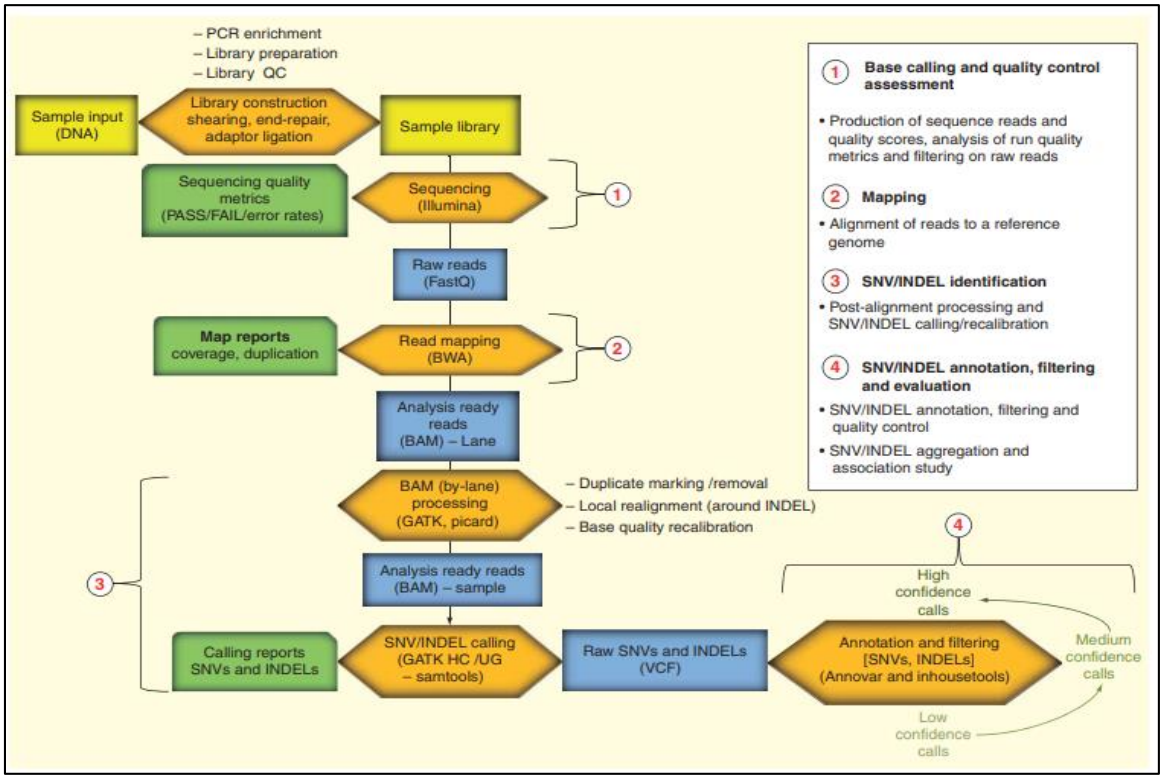
Several NGS techniques are employed in cancer studies, often utilizing different sequencing platforms for their specific advantages. Whole exome sequencing (WES) is widely used in clinical settings, which is crucial for understanding cancer driver mutations. WES represents a significant breakthrough in human genetics that enables the simultaneous analysis of the coding regions of over 20,000 genes, covering nearly all known coding exons in the human genome (Staaf *et al*., 2019). By comparing an individual's sequence with a standard reference, WES allows for the detection of rare variants that may be linked to diseases. WES has expedited the identification of disease-causing genes in research and is now rapidly making its way into clinical laboratories, transforming the landscape of clinical testing and diagnostics. Many laboratories already offer clinical WES tests to reduce the diagnostic challenges for patients (Zare *et al*., 2017).

Patients suffering from rare, clinically, and genetically diverse diseases with unclear or atypical presentations often face difficulties in diagnosis. Traditional diagnostic methods, like Sanger sequencing, require prior knowledge of the specific genes to sequence (Kumar *et al*., 2019). In contrast, WES offers a distinct advantage as a diagnostic tool. It doesn't require the pre-selection of genes and allows for the screening of genes suspected of being associated with the disorder. WES covers 1–1.5% of the human genome, yet this portion of the genome contains approximately 85% of the known disease-causing variants (Wittwer and Park, 2018).

The profound progress in understanding the cancer genome became possible with advancements in DNA sequencing technologies. WES has not only contributed to the identification of numerous new cancer susceptibility genes but has importantly enabled the detection of somatic driver mutations in sporadic cancers. By comparing germline and

tumor DNA, WES can catalog all somatic mutations in a given tumor (Bertucci *et al*., 2019). Subsequently, identifying genes that are recurrently mutated across a group of tumors helps discover novel driver genes and pathways without prior assumptions about the molecular mechanisms. The application of WES in this context has led to the discovery of unexpected oncogenic pathways (Lu *et al*., 2019).

Figure 5: Whole exome sequencing workflow



(Tetreault *et al*., 2015)

Research findings related to cancer genetics can significantly aid in patient diagnosis and be integrated into clinical practice. Some types of cancers exhibit genetic heterogeneity, making molecular diagnosis challenging. In these cases, multiple gene tests are often necessary to uncover the genetic underpinnings (Suganya and Sumathi, 2023). WES can potentially test all candidate genes in a single experiment, leading to a quicker diagnosis than single-gene testing. Screening at-risk individuals using this technique has the potential to increase early-stage cancer diagnoses. WES also plays a vital role in cancer prevention and early detection by enabling the screening of individuals (Wendt and Margolin, 2019). A typical WES analysis involves several

steps: base calling, quality control, mapping and variant identification, annotation, filtering, and prioritization are given in **Figure 5**.

Another essential application of WES in cancer care is in the realm of personalized medicine. Cancer patients, even with the same type or subtype of cancer, may respond differently to treatment, mainly due to genetic variants among patients. Pilot projects are currently investigating the ability of biomarkers to predict treatment responses in cancer patients (Van Hout *et al*., 2020). With NGS technologies like WES, it's possible to conduct a multiplex analysis of biomarkers and determine the tumor's mutational profile for each patient. This could eventually be used in clinical settings to identify the best therapy for individual patients. The use of WES in clinical practice offers numerous opportunities to enhance patient diagnosis and management (Qin, 2019).

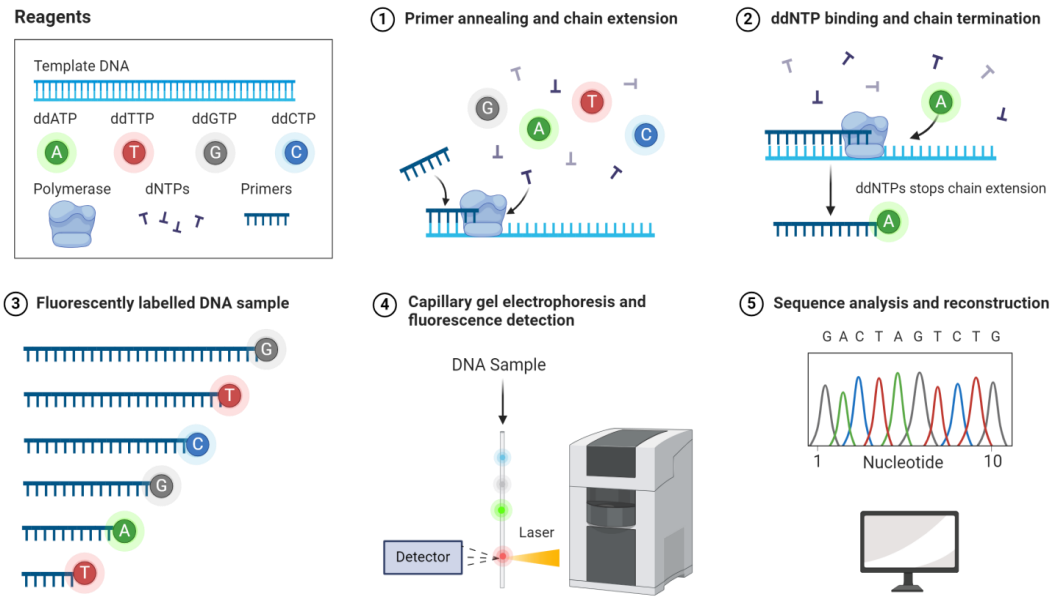
2.9 Sanger sequencing

However, it is still widely accepted that variants found using whole exome sequencing should be validated with the gold standard method, Sanger sequencing. WES still has certain limitations that can lead to inadequate coverage and sequencing inaccuracies (Hussein *et al*., 2020). Insufficient targeting by the exome capture kit, high guanine-cytosine (GC) content, and the presence of repetitive sequences can all impact WES coverage and sequencing alignment. WES base-calling inaccuracies can also result from allele dropout, which is the failure to amplify one or both alleles at a specific locus (Chang *et al*., 2020).

Sanger sequencing also known as dideoxy sequencing or chain termination sequencing is a foundational technique in molecular biology and genetics. Developed by Frederick Sanger in the 1970s, it has made a lasting impact on the field of genomics. One of its primary strengths is its precision and reliability (Borodinov *et al*., 2020). Sanger sequencing can generate highly accurate and error-free DNA sequences, especially for sequences up to 1,000 nucleotides in length. This precision makes it an invaluable tool for validating DNA sequences, confirming mutations, and verifying the quality of DNA constructs. Its versatility is another notable advantage, as it can be applied to a wide range of DNA samples, such as PCR products, and genomic DNA (Cheng *et al*., 2023).

The steps involved in Sanger sequencing are depicted in **Figure 6**. The figure was retrieved from BioRender. Sanger sequencing excels in the identification of point mutations, insertions, and deletions, which involve alterations of individual nucleotides or small DNA segments. It is less suitable for detecting larger structural variations or complex mutations, which may be more effectively addressed by alternative sequencing technologies like next-generation sequencing (NGS) or long-read sequencing methods (Saini *et al*., 2023). However, Sanger sequencing maintains its status as the gold standard for validating mutations, particularly in clinical diagnostics and research applications. Additionally, Sanger sequencing has played a pivotal role in several ground-breaking discoveries, contributing significantly to the Human Genome Project, elucidating the genetic code, and identifying the underlying causes of various diseases (Crossley *et al*., 2020).

Figure 6: Steps of Sanger sequencing



2.10Artificial intelligence in breast cancer diagnosis and treatment

Artificial intelligence techniques are breaking into biomedical research and health care, including cancer research and oncology, with vast potential applications. These include detection and diagnosis of cancer, subtype classification, optimization of cancer treatment, and identification of new therapeutic targets in drug discovery (Elemento *et al*., 2021). Some of the key applications of AI include:

- **Early detection and diagnosis:** AI algorithms analyse mammograms and other medical imaging data to assist radiologists in detecting early signs of breast cancer, such as microcalcifications, masses, and architectural distortions. These algorithms can improve the accuracy and efficiency of screening programs, leading to earlier diagnosis and treatment. Example: ScreenPoint Medical has developed Transpara, AI-powered software for mammography analysis (Khanna, 2020).
- **Risk assessment and personalized screening:** AI models analyse patient data, including demographic information, family history, genetic markers, and lifestyle factors, to assess individual risk for developing breast cancer. This information can help tailor screening recommendations and preventive interventions for high-risk individuals. Example: CancerIQ offers a platform that uses AI algorithms to assess a patient's risk of developing breast cancer (Daowd *et al*., 2019).
- **Pathology and histopathology:** AI algorithms analyse histopathology slides of breast tissue samples to assist pathologists in diagnosing cancer and assessing tumor characteristics, such as histological subtype, tumor grade, and hormone receptor status. These algorithms can improve diagnostic accuracy and consistency, particularly in challenging cases (Ibrahim *et al*., 2020). AI-powered image analysis techniques, such as deep learning-based algorithms, can quantify biomarkers like the Ki-67 proliferation index and tumor-infiltrating lymphocytes, providing valuable prognostic information and guiding treatment decisions. Example: PathAI develops AI-powered pathology solutions to assist pathologists in diagnosing breast cancer and assessing tumor characteristics (Baxi *et al*., 2022).
- **Treatment planning and decision support:** AI-based decision support systems analyse clinical and molecular data to help oncologists develop personalized treatment plans for breast cancer patients. These systems consider tumor subtype, genetic mutations, biomarker expression, and treatment response prediction models to recommend the most effective therapies (Senthil Kumar *et al*., 2023). AI models can analyse medical literature, clinical trial data, and real-world patient outcomes to provide evidence-based recommendations for treatment selection,

dosage optimization, and therapeutic combinations. Example: Watson for Oncology by IBM (International Business Machines) is an AI-powered decision support system that analyses patient data and medical literature to assist oncologists in developing personalized treatment plans for breast cancer patients (Aikemu *et al*., 2021).

- **Monitoring and Prognosis:** AI-driven predictive models analyse longitudinal patient data, including imaging scans, laboratory results, and treatment response metrics, to monitor disease progression and predict patient outcomes. These models can identify early signs of recurrence or metastasis, enabling timely interventions and personalized follow-up care plans. Example: Tempus offers an AI-driven platform that analyses patient data to monitor disease progression and predict patient outcomes (Rajan and Paranthaman, 2022).

2.11 Cell proliferation assessment by mitosis detection using artificial intelligence

Accurate diagnosis of breast cancer is the fundamental process for improving treatment response and prognosis. Hematoxylin and eosin (H&E) are widely used for staining pathological slides. Histopathological images stained by H&E can visually show the cellular components and tissue structure. Pathologists grade breast cancer severity and then formulate the corresponding treatment plan (Syarti *et al*., 2020). The WHO stipulates that the Nottingham grading system (NGS) is the criterion of breast cancer grading in which pathologists comprehensively consider three factors: the number of normal glands formed (tubule formation), shape deformity of nuclei (atypia/pleomorphism); and the rate of tumor cells division (mitotic activity) (Jaroensri *et al*., 2022).

The number of mitotic cells is an important metric for grading and diagnosing breast cancer. It is used to evaluate the proliferation and aggressiveness of the tumor, which has important implications for accurate diagnosis, patient prognosis, and treatment. However, mitosis detection in breast cancer slides is performed manually (Ibrahim *et al.*, 2022). Pathologists usually observe pathological tissue slides under a microscope to identify the region of interest (ROI). On the other hand, mitosis detection is complex, laborious and time-consuming (Maroof *et al*., 2020).

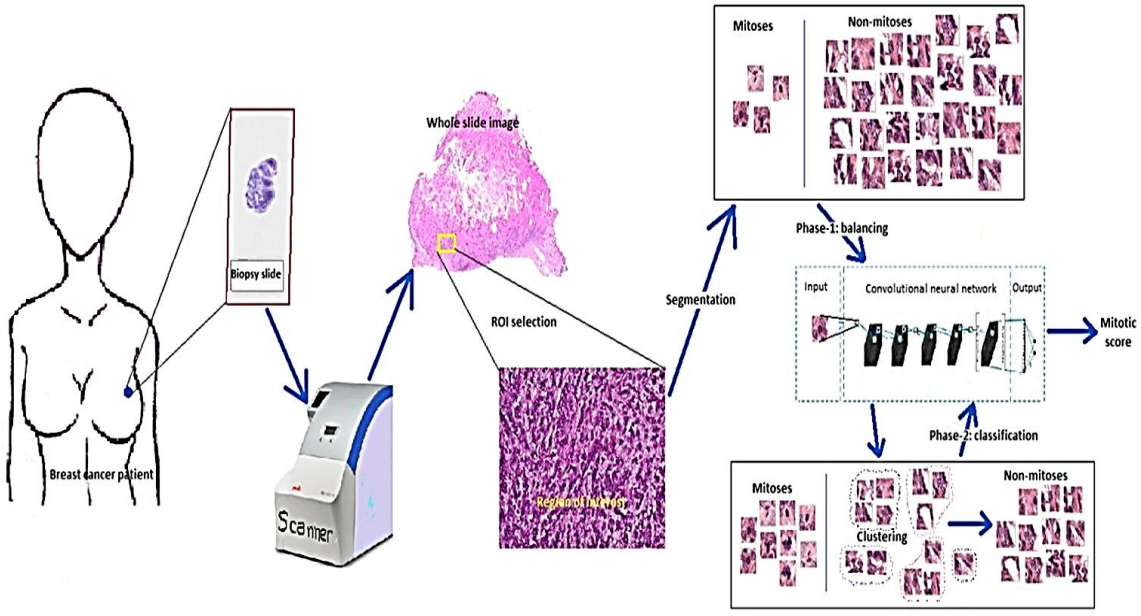
Computer-aided diagnosis (CAD) is performed to achieve auxiliary diagnosis using medical image processing technology and improves accuracy. Computer-aided mitosis automatic detection helps doctors screen, identify, and label mitotic cells (Alom *et al*., 2020). The development of artificial intelligence (AI) is becoming more rapid, and doctors can focus better on the critical problem region (Sigirci *et al*., 2022).

The main task of AI-mitosis detection is to construct a model based on digital pathological slides and then train a more robust recognition model. The model automatically detects which cells are mitotic and non-mitotic for newly imported unlabelled pathological slides. Researchers have proposed many automatic detection methods, but existing methods still need to achieve satisfactory results in clinical practice (Lei *et al*., 2020). The overview of mitotic detection using artificial intelligence is given in **Figure 7**.

With the development of deep learning and machine learning methods, a new generation of artificial intelligence technology has changed the medical field. The problem of mitosis detection has attracted increasing attention from technical teams. Investigators are more profoundly exploring candidate's segmentation, feature extraction, detection, and classification for mitosis detection in the pathological image of breast cancer, which effectively promotes the development of automatic mitosis detection in breast cancer (Rao, 2018).

Due to the extensive attention of many researchers, the results of artificial intelligence and mitotic diagnosis of breast cancer have also improved. However, the automatic detection of mitosis in breast cancer pathological images is a complex medical task. Although the detection results continue to improve, the existing breast cancer mitosis detection algorithm still cannot be used in clinical detection. In the future, detecting mitosis and treating cancer will become a meaningful direction. This pioneering work not only enhances the capabilities of medical professionals but also represents a significant advancement in medical imaging and healthcare (Mahmood *et al*., 2020).

Figure 7: AI-based mitotic detection



(Wahab *et al*., 2017)

Hence, the present study has been designed with the innovative goal of integrating socio-demographic profiles, genetic alterations, and disease progression within the context of breast cancer among the Tamil Nadu population. This approach holds the potential to significantly contribute to understanding the complexity of the disease and inform strategies for prevention and treatment tailored to specific populations.

The methodology adopted for the study is explained in the next chapter.