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Triple Negative Breast Cancer (TNBC): Cannabis sativa-Role of Phytocannabinoids

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Abstract

This review paper highlights the role of phytocannabinoids in controlling triple-negative breast cancer (TNBC). Triple-negative breast cancer (TNBC) is a specific subtype of breast cancer that does not express estrogen receptor (ER), progesterone receptor (PR), or human epidermal growth factor receptor 2 (HER-2), which has clinical features that include high invasiveness, high metastatic potential, proneness to relapse, and poor prognosis. TNBC is one of the most aggressive subtypes of breast cancer that is often associated with poor patient outcomes because of the development of metastases in secondary organisms like in the brain, bone, and lungs. Triple-negative breast cancer (TNBC) is a kind of breast cancer that does not have any of the receptors that are commonly found in breast cancer. The highest number of TNBC cases has been recorded in India. Currently, TNBC is highly prevalent among Indian women and develops approximately 20% to 43% of all patients with breast cancer. Triple-negative breast cancer (TNBC) is an aggressive malignancy that requires effective targeted drug therapy. Patients with TNBC develop metastasis and recurrence over time and have reduced survival compared to patients with other subtypes of breast cancer. Pre-clinical studies have demonstrated that phytocannabinoids exert important antitumor properties in the main breast cancer subtypes, particularly in TNBC, where different phytocannabinoids and synthetic cannabinoids have shown interesting therapeutic actions. Neither CBD nor THC are universally efficacious in reducing cancer cell viability. Focusing on *in vitro* studies, the effect of CBD on cancer cell viability ranges from no effect, to a modest reduction, and to significant cytotoxicity depending on concentrations, cancer cell lines, cell growth conditions, the performed assays, and the time of CBD exposure. However, it should also be noted that research into the efficacy, dosage and drug safety of cannabinoids in tumor therapy still has a long way to go, especially with regard to human clinical trials to be conducted, through which alone the benefits and advantages for cancer patients but also possible risks can be defined.

Keywords: Apotopsis; Breast cancer; Carcinoma; Cannabidol (CBD); Diagnosis; Phytocannabinoids; Triple-negative-breast-cancer (TNBC); Therapeutic target; Tumor

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1. Introduction

Triple Negative Breast Cancer (TNBC) is a heterogeneous disease that based on immunohistochemistry (IHC) is estrogen receptor (ER) negative, pro-gesterone receptor (PR) negative and human epidermal growth factor receptor 2 (HER2) negative [1-30]. TNBC is characterized by its unique molecular profile, aggressive nature, distinct metastatic patterns and lack of targeted therapies [1- 30-74]. This cancer is responsible for more than 15–20% of all breast cancers and is of particular research interest, as it is therapeutically challenging mainly because of its low response to therapeutics and highly invasive nature [1-40]. The non-availability of specific treatment options for TNBC is usually managed by conventional therapy, which often leads to relapse [1- 30]. TNBC is one of the most aggressive subtypes of cancer that is often associated with poor patient outcomes because of the development of metastases in secondary organisms like in the brain, bone, and lungs [1- 45]. Metastatic growth to these distant organs, represents a significant clinical challenge, as metastatic disease is currently incurable and is a primary death cause for the vast majority of TNBC patients [1- 38, 78]. Metastatic spread of cancer is a complex, poorly understood process, and involves multiple steps, such as angiogenesis acquisition of invasive properties through epigenetic and genetic alterations, intravasation through the basement membrane, extravasation of some cancer cells to distal tissues, and tumor-stroma interactions [1- 50]. Triple Negative Breast Cancer (TNBC) is typically observed in young African Americans (AA) women and Hispanic women who carry a mutation in the BRCA1 gene [1- 30]. The highest number of Triple Negative Breast Cancer (TNBC) cases among the youngest women in India has been recorded [38- 74, 78].

Many breast cancer cells have receptors for estrogen or progesterone. They may also have receptors for a protein called HER2 (also called ERBB2) [1- 38- 74, 78-85]. Triple-negative breast cancer means that the cancer cells do not have any of these receptors. Because it does not have any of these receptors, triple-negative breast cancer is considered a separate type of breast cancer with its own treatment options [38- 74, 78]. Most triple-negative breast cancers are invasive ductal carcinoma. Ductal carcinoma in situ (DCIS) may also be triple negative [1-38- 74, 78-85]. It is important to note that not all triple-negative breast cancers are basal-like, and not all basal-like breast cancers are triple negative [1- 38- 74, 78-90]. They are too similar, but distinct, subtypes of breast cancer. Scientists have not yet developed one internationally accepted definition of a basal-like breast cancer. But they know that it is different from other types of breast cancer. Women under the age of 40 and women of African or Asian ancestry have a higher risk of developing triple-negative breast cancer [1-38- 74, 78]. Basal-like breast cancers are more likely to be found in younger women and in women of African ancestry. Many triple-negative and basal-like breast cancers may be called interval cancers because they can develop between regularly scheduled screening mammography [1- 38- 74, 78-80]. Most triple-negative and basal-like breast cancers are high-grade, or aggressive, tumors. This means that they tend to grow and spread quickly. Many are diagnosed at a later stage when the cancer has already spread (metastasized) to lymph nodes or other organs [38- 74, 78]. These tumors tend to spread to the bloodstream, brain or lungs more often than breast cancers that are not triple negative. They do not spread to the lymph nodes or the bones as often as other types of breast cancer [1-38- 74, 78]. Triple-negative breast cancer usually responds to chemotherapy. However, it does have a higher risk of coming back (recurrence) within 5 years of treatment, compared to breast cancer that is hormone-receptor positive or HER-2 positive. After 5 years, this risk decreases [1-38- 74, 78].

Triple-negative breast cancer (TNBC) is an aggressive malignancy that requires effective targeted drug therapy. In 2020, female breast cancer emerged as the most commonly diagnosed cancer, with an estimated 2.3 million new cases worldwide [1- 74]. Notably, it also represents the leading cause of cancer incidence and mortality among women. Among the various types of breast cancer, triple-negative breast cancer (TNBC) accounts for approximately 10% to 15% of all diagnosed cases [1-78]. Targeted therapeutic strategies have been successfully employed for the treatment of ER-positive and HER2- positive subtypes of breast cancer. However, TNBCs do not respond to targeted therapies and are typically treated with non-selective chemotherapy drugs [1-38- 74]. TNBCs exhibit more aggressive clinical manifestations, higher rates of relapse, and the molecular mechanisms underlying relapse are not yet fully understood [1-38- 74]. Consequently, TNBCs represent the most malignant form of breast cancer, necessitating the urgent discovery of novel targeted therapies. Chemotherapy resistance in TNBC is a significant factor that negatively impacts patients' prognosis and overall survival rates [1-38- 74]. One of the main contributors to resistance in triple- negative breast cancer cells is the presence of breast cancer stem cells (BCSCs) within the tumor [1-38- 74]. Cancer stem cells (BCSCs) possess unique properties that enable them to self-renew and promote tumor cell growth cancer stem cells [1-38-74]. Furthermore, the increased invasiveness and metastatic potential of TNBC are influenced by the molecular pathways involving kinases present in cancer stem cells (BCSCs) [1-38- 74]. Among the kinases present in cancer stem cells (BCSCs), human casein kinase (CK2) plays a crucial role. CK2 is a serine/threonine protein kinase that is abundantly expressed and involved in various cellular functions, including cell growth, proliferation, and differentiation [1-38- 74].

2. Triple-Negative Breast Cancer (TNBC): Symptoms

TNBC differs from other types of invasive breast cancer, which tends to grow and spread faster, has fewer treatment options, and tends to have a worse prognosis. Once a breast cancer diagnosis has been made using imaging tests and a biopsy, the cancer cells will be checked for certain proteins [1-38- 76, 78]. If the cells do not have estrogen or progesterone receptors (ER or PR), and also do not make any or too much of the HER2 protein, the cancer is considered to be triple-negative breast cancer. Triple-negative breast cancer symptoms are the same as other more common breast cancers. Symptoms of triple- negative breast cancer includes: 1) Changes in breast shape, 2) Mass or suspicious finding on a mammogram, 3) Breast or nipple pain 4) Discharge from the nipple 5) Nipple inversion. 6) Swelling of the breast. 7) A lump, and thick tissue mass, 8) Thickening of the nipple skin, 9) A new lump or mass. 10) Swelling in all or part of breast, 11) Dimpled skin, 12) Breast or nipple pain, 13) Nipple retraction when nipple turns inward, 14) Nipple or breast skin dry, flaking, thickened or red, 15) Nipple discharge that is not the breast milk, 16) Swollen lymph nodes. This symptom happens when breast caner spreads to the lymph nodes under arm and near collarbone [38- 76].

3. Triple-Negative Breast Cancer (TNBC): Detection Tests

The first step might be a mammogram to evaluate a suspicious mass or lump in patient breast. Based on what they learn, healthcare providers might perform a biopsy to remove breast tissue [38- 76]. Medical pathologists determine subtype by studying cells under a microscope. Sometimes, providers use the following tests before treatment to determine tumor size and whether it has spread: Magnetic Resonance Imaging (MRI), ultrasound, Comptued tomography scan (CT) scan and Position emission tomography (PET) scan [38- 76, 220-225]. TNBC tends to grow quickly, is more likely to have spread at the time it is found, and is more likely to come back after treatment than other types of breast cancer. Because of this, the survival rates for TNBC are generally not quite as high as they are for other types of breast cancer [38- 76]. Survival rates can give an idea of what percentage of people with the same type and stage of cancer are still alive a certain amount of time (usually 5 years) after they were diagnosed [38- 76]. A **relative survival rate** compares women with the same type and stage of breast cancer to women in the overall population. For example, if the **5-year relative survival rate** for a specific stage of breast cancer is 90%, it means that women who have that cancer are, on average, about 90% as likely as women who do not have that cancer to live for at least 5 years after being diagnosed [38- 76, 220-225].

Triple-negative breast cancer has fewer treatment options than other types of invasive breast cancer. This is because the cancer cells do not have the estrogen or progesterone receptors or enough of the HER2 protein to make hormone therapy or targeted HER2 drugs [38- 76]. Because, hormone therapy and anti-HER2 drugs are not choices for women with triple-negative breast cancer, chemotherapy is often used. If the cancer has not spread to distant sites, surgery is an option. Chemotherapy might be given first to shrink a large tumor, followed by surgery. Chemotherapy is often recommended after surgery to reduce the chances of the cancer coming back. Radiation might also be an option depending on certain features of the tumor and the type of surgery [38- 76]. In cases, where the cancer has spread to other parts of the body (stage IV), platinum chemotherapy, targeted drugs like a PARP inhibitor or antibody-drug conjugate, or immunotherapy with chemotherapy might be considered [38- 76].

Some gene expression testing/profiling can also help to predict which women will most likely benefit from chemotherapy after breast surgery (adjuvant chemotherapy.) Hormone therapy is a standard treatment for hormone receptor-positive breast cancers, but it is not always clear when to use chemotherapy. These tests can help to guide that decision. Still, these tests cannot confirm any one woman for certain types of cancer, if her cancer will come back with or without chemotherapy [150]. The Oncotype DX, Mamma Print, and Prosigna are examples of tests that look at different sets of breast cancer genes to see if chemotherapy is needed help to reduce the risk of cancer coming back (recurrence) [150]. More tests are in development. The type of test that is used will depend on patient situation. Keep in mind that these tests are used for early-stage cancers, and testing is not needed in all cases. For example, if breast cancer is advanced, it might be clear that chemotherapy is needed, even without gene expression testing [150]. Oncotype DX: This test looks at a set of 21 genes in cancer cells from tumor biopsy or surgery samples to get a "recurrence score," which is a number between 0 and 100. The score reflects the risk of the breast cancer coming back (recurring) in the next 9 years. Furthermore, if patients are treated with hormone therapy alone and how likely patients are to be benefited from getting chemo after surgery.

Mamma Print: The Mamma Print test can be used to help to determine how likely breast cancers are to recur in a distant part of the body after treatment [150]. It can be used for any type of invasive breast cancer that is 5cm (about 2 inches) or smaller and has spread to no more than 3 lymph nodes. This test can be done regardless of a woman's age or the cancer's hormone or HER2 status. The test looks at 70 different genes to determine if the cancer is at low risk or high

risk of coming back (recurring) in the next 10 years. The test results come back as either "low risk" or "high risk." This test is also being studied as a way to determine whether certain women might benefit from chemotherapy. Prosigna: The Prosigna test can be used to predict the risk of recurrence in the next 10 years in women who have gone through menopause (postmenopausal) and whose invasive breast cancers are hormone receptor-positive and HER2-negative. It can be used to test early-stage cancers that have not spread to the lymph nodes, or early-stage cancers with no more than 3 positive lymph nodes. The test looks at 50 genes and classifies the results as low, intermediate, or high risk. Breast Cancer Index: The Breast Cancer Index test is done on patient tumor sample from when patients are first diagnosed. It can be used to predict the risk of recurrence in the 5 to 10 years after diagnosis in women whose invasive breast cancers are hormone receptor-positive and have not spread to nearby lymph nodes or have not spread to more than 3 lymph nodes**.** It can also help to predict who might benefit from hormone therapy for longer than 5 years. The test looks at 11 genes and classifies the results as low or high risk.

4. Triple-Negative Breast Cancer (TNBC): Diagnosis

Owing to a number of drawbacks associated with invasive cancer detection procedures, scientists and researchers worldwide are focusing on non-invasive cancer diagnosis through the use of cancer biomarkers[228]. A biomarker is a biological molecule that can be found in blood or other bodily fluids or tissues that can indicate an abnormal or normal process of a disease or condition like cancer, according to the National Cancer Institute. Numerous lines of evidence suggested that a range of techniques and biomarkers may be employed as diagnostic techniques for the identification and follow-up of breast cancer patients [228]. Mammography, MRI, SPECT, PET, CT, and other imaging modalities, as well as their development, may be used to diagnose and track patients with breast cancer. Even with continued advancements in imaging techniques, their use is hampered by a number of issues, including cost and sensitivity [228]. Therefore, it appears that finding new instruments is necessary for the diagnosis of patients with breast cancer. Research has demonstrated that the use of novel biomarkers, such as measuring the expression levels of different proteins (such as ER, Ki67, PR, and HER2) and molecules (such as exosomes and miRNAs), has created new avenues for the diagnosis and follow-up of breast cancer patients. One of the most crucial parts of treating breast cancer is early diagnosis of patients. Imaging methods are primary diagnosis approaches among a variety of diagnosis platforms that may yield important information about individuals with breast cancer. Numerous imaging modalities, including computed tomography (CT), positron-emission tomography (PET), mammography, magnetic resonance imaging (MRI), and single-photon emission computed tomography (SPECT), have been demonstrated to be useful for diagnosing and tracking patients with breast cancer at different stages of the disease. In addition to imaging methods, patients with breast cancer may benefit from the use of biochemical biomarkers such proteins, genes, DNA, mRNAs, and microRNAs as additional diagnostic and therapeutic tools. We reviewed a number of imaging modalities in this review, along with current conventional, cutting-edge, and prospective biomarkers that may be used to diagnose breast cancer in patients [228].

A two-step procedure typically employed to diagnose TNBC is imaging and immunohistochemistry (IHC) [228]. Imaging encompasses a mammogram, an ultrasound of the breast along with magnetic resonance imaging (MRI) [1-74, 149-220-225]. A mammogram requires a minimal dosage of radiation that does not easily penetrate the breast tissues. Breast cancer diagnosis via mammograms is determined by the presence of calcifications (white spots), growth, or tumor also known as masses [1-74, 149- 220-225]. The main challenge is the risk of a false-negative and positive results affecting the diagnosed patient's treatment outcome [1-74, 149- 220-228]. In addition, the side effects of radiation from mammograms may contribute to breast cancer development in high-risk individuals like BRCA gene carriers or family history. Finally, mammography effectiveness is operator-dependent, which may interfere with the results of imaging [1-74, 149- 220-225].

Diagnosis via ultrasound is performed when a lump or swelling is not detected in a mammogram but still can be felt and served as the primary approach to distinguish between breast cysts (fluid-filled sac) and tumors if sample collection is carried out in the right area and tested for cancer [1-74, 149- 220-225]. What differentiates breast cysts from a solid tumor is that breast cysts are most often benign, whereas a solid tumor requires further validation to characterize its malignancy [1-74, 149- 220]. Breast cancer diagnosis by MRI, on the other hand, is opted when a patient is categorized as high risk (family history/BRCA gene mutation) and to determine the severity of the carcinoma due to the efficiency of MRI to detect the early formation of breast cancer in comparison to breast ultrasound and mammogram [1-74, 149-220]. The main downside of MRI is that the imaging method cannot characterize the breast cancer types and can only confirm the presence of cancer in the breast [1-74, 149- 220].

Ideally, immunohistochemistry (IHC) is required for breast carcinoma typing performed by cell staining with biomarkers such as hormone receptor (progesterone receptor (PR) and estrogen receptor (ER)) as well as human epidermal growth factor receptor two (HER2) markers [1-74, 149- 220-225]. In order to enhance the efficacy and

accuracy of IHC testing for ER HER2 and PR, there are approximately 126 latest guidelines that have been provided by the American Society of Clinical Oncology (ASCO)/College of American Pathologists (CAP) [1-74, 149- 220]. The primary aim of these guidelines is to improve the reliability, reproducibility and to reduce the frequency of false-positive and false negative results from IHC testing [1-74, 149- 220]. Based on the recommendation, IHS testing for ER and PR is classified positive only if immunoreactive cancer cells' presence accounts for a minimum value of 1% [1-74, 149- 220]. Next, a second confirmation of HER2 should be conducted via fluorescent in situ (FISH) after initial IHC confirmation to obviate any potential false-positive/false-negative diagnosis that will affect the treatment's direction and effectiveness plan [1-74, 149- 220-225].

Blood-based liquid biopsy is a non-invasive diagnostic method that can be utilized for future TNBC diagnosis [1-74, 149-220]. Liquid biopsy captures the information of a tumor through blood specimen, which is analyzed for the presence of circulating tumor cells (CTCs), tumor-derived extracellular vesicles (exosomes), and circulating tumor nucleic acids (ctNAs), which include circulating tumor DNA (ctDNA) and microRNAs (miRNAs) [1-74, 149- 220-225].

Analysis of Circulating Tumor Nucleic Acids (ctNAs), ctNAs include circulating tumor DNA (ctDNA), microRNA (miRNA), and cell-free RNA (cfRNA) [1-74, 149- 220-225]. CtDNAs found in the bloodstream of a cancer patient is usually from the primary tumor. Furthermore, CTCs, apoptotic and necrotic cell deaths occur during cancer development and progression [1-74, 149- 220]. The volume of tumor ctDNAs in the bloodstream depends on the size of the tumor or metastases, and a study has concluded that the ctDNA concentration will increase the percentage of tumor burden [1-74, 149- 220]. Hence, it is difficult to detect ctDNA in an early stage of cancer as only a low concentration of ctDNA can be found [1-74, 149- 220]. This suggests that an ultrasensitive technology is urgently needed to detect the initial stage of cancer as the levels of ctDNA present are low [1-74, 149- 220]. One such technology is the droplet digital polymerase chain reaction (ddPCR), which was able to detect phosphatidy linositol-4,5-biphosphate 3-kinase catalytic subunit alpha (PIK3CA) mutations in the blood specimen of early-stage breast carcinoma patients [1-74, 149- 220-225]. However, further validation and development are necessary before ctDNA can be utilized as a biomarker for early breast carcinoma diagnosis [1-74, 149- 220]. On the other hand, assessing ctDNAs in the plasma can be used for real-time monitoring of the tumor burden and measure the effectiveness of treatment [220]. This is due to the fact that ctDNAs have a short half-life (15 min to several hours), allowing earlier observation of ctDNA level changes in the bloodstream than radiological images [220]. Moreover, ctDNA analysis can be an alternative to confirm the diagnosis of metastatic relapse, which was evident in a study. This has demonstrated that ctDNA analysis was capable of detecting early disease metastasis of a patient who underwent treatment for early breast carcinoma [220-225].

MicroRNAs (miRNAs) are short ribonucleic acids (RNAs) made up of approximately 22 nucleotides that regulate thousands of genes via binding to target messenger RNAs (mRNAs) [220-225]. miRNAs play various roles in many biological processes such as cell development, growth, differentiation, chromatic structure, cell death, metabolism, and morphogenesis [220]. In addition, miRNAs that also act as oncogenic miRNAs or tumor suppressors play an essential role in tumorigenesis [220]. Oncogenic miRNAs were used to demonstrate anti-apoptotic activity and were found to be overexpressed in cancer cells [220-225]. In contrast, tumor suppressor miRNAs usually display anti-proliferative, pro-apoptotic activity, and down regulated in cancer cells [220]. In contrast, the expression of miR-21 and miR-221 was down regulated in a study based in Hong Kong, highlighting the possibility of miRNA expression variation in distinct ethnic groups or the geographic location of the patients [220-225]. Moreover, several other non-TNBC specific studies showed different expression levels of miR-(21,221,195,145) and Let-7a in other types of breast carcinoma categories [220-225]. This suggests that the expression level of miRNA depends not just on the tumor type but also on the breast cancer stage and grading [220]. Thus, Frères et al. had developed a new screening tool for breast cancer by constructing a diagnostic test based on eight circulating miRNAs (miR-(16, 103, 107, 148a, 19b, 22) and let-7(d and i) [220-225].

The exosomes primarily involve transporting biomolecules, including DNA, RNA, proteins along with lipids to recipient cells [220]. In addition, exosomes also play a role in cell signaling and intercellular molecular communication [1-74, 149- 220]. During carcinogenesis, exosomes from the cancer cells were found to trigger cancer cell proliferation and stage immune defense escape ultimately promoting cancer progression and metastasis [1-74, 149- 220-225]. Several studies have shown that exosomal proteins can be used as diagnostic and prognostic markers [220]. Liquid biopsy provides real-time, reliable results, reduces the cost and diagnosis time, and allows patients to avoid the risk of surgery [220]. The nCounter® Breast Cancer 360™ (Seattle, WA, USA) Panel initiated in April 2018 is an analytical data tool comprising approximately 770 genes to aid in breast carcinoma classification based on molecular subtyping [1-74, 149-220]. In this diagnostic method, the patient's RNA sample is extracted and integrated overnight with the Breast Cancer 360TM panel assay before performing specimen and data analysis using the Nanostring nCouter® syst [1-74, 149- 220-225]. The system provides an in-depth understanding of the level of gene expression, immune defense mechanism towards the breast carcinoma, and tumor microenvironment along with breast cancer categorization formulated on

biological signatures such as prediction analysis of microarray 50 (PAM50) and tumor inflammation signature assay [1-74, 149- 220-228].

Digital Polymerase Chain Reaction (dPCR): Introduced by Vogelstein and Kinzler in 1999, digital PCR is a method that segregates the samples into multiple wells before the amplification process [1-74, 149- 220]. In general, digital PCR is utilized for circulating tumor DNA and miRNA identification in cancer patients [220-225]. The pros of dPCR compared to a conventional quantitative polymerase chain reaction (qPCR) are that there is no requirement for a standard curve for analysis. This method is able to tolerate any PCR inhibitors, able to analyze the presence of uncommon targets in large sample mixture, and capable of identifying minute fold changes [1-74, 149- 220-225-228].

A biosensor is a tool comprised of bioreceptor, detector, and the signal transducer, utilized for the identification and analysis of a wide range of biological specimen, including enzymes, immune components (antigen and antibodies), nucleic acid components (DNA, RNA, microRNAs, and ctDNA), and other biological components present in humans [220, 233-235]. In terms of TNBC cell detection, several nanobiosensors have been developed in the past [1-74, 149- 220-228, 233-235]. The zinc oxide (ZnO)-choline oxidase (ChOx) nanobiosensor generated in 2016 was able to identify the presence of choline in TNBC samples [1-74, 149- 220-225-228, 233-235]. Researchers from all over the world have begun to create and develop biosensors that might effectively detect cancer, unpaid for the non-destructive early diagnosis of the disease [233-235]. In essence, biosensors transform biological entities such as proteins, DNA, and RNA into detectable and studied electrical signals in order to identify and study a particular biological analyte [228, 233-235]. The term "Bios" enters the picture because the sensor detects biological materials [228, 233-235]. Nucleic acids, enzymes, antibodies, and microorganisms are a few examples of the biological stuff [228]. The contemporary glucose sensor is derived from the study of Prof. Leland C. Clark Jr., who is regarded as the "Father of Biosensors"[228, 233-235]. Three elements need to be taken into account when building a biosensor: a transducer to convert a biochemical response into a measurable signal, an immobilization matrix to immobilize a recognition biomolecule, and a bio-recognition element for the selective recognition of an analyte, also known as a bio receptor [228, 233-235].

Different types of biosensors used in cancer cell detection [228, 233-235].

- Calorimetric Biosensors
- Fluorescence Biosensors
- SERS – Based Biosensors
- SPR – Based Biosensors
- Electrochemical Biosensors
- Electro-magnetic meta materials-based Biosensors
- Crystal Optical Refractive Index Biosensors

Compared to conventional cancer diagnosis techniques, biosensors may offer a variety of benefits, including shorter test times, portability, high sensitivity and selectivity, simplicity, miniaturization, and adaptability [228, 233-235]. Biosensor-based diagnostics can help with cancer screening, increase early diagnosis rates, and lead to better prognoses [220-225, 233-235]. This technology can be especially helpful for improving healthcare delivery to the underprivileged and in public settings[233-235]. Biosensors may be used for automated testing, multi-target analysis, and economical testing [228, 233-235].

Immuno Positron Emission Tomography (PET): Positron emission tomography, also known as PET scan, is a medical imaging approach that utilizes a radioactive element/drug to analyze the organ and tissue functionality. This test is well-known for its capability to detect a particular disease even before detection by other imaging methods [1-74, 149-220-225]. In this approach, the radioactive element (tracer) is comprised of tightly linked radioactive atom-transport molecules (isotopes) that adhere to specific biomolecules (sugar, protein, etc.) in the human body and generate positrons that interacts with the surrounding electrons resulting in the formation of photons [1-74, 149- 220]. The PET scanner then detects the electrical signal emitted by the photons and utilizes the data obtained to generate the image of the organ/tissue/cell being investigated [1-74, 149- 220].

Triple-negative breast cancer (TNBC) is an aggressive type of cancer but lacks targeted therapy methods such as hormone therapy due to the low expression of three primary receptors (ER, PR, and HER2) [1-74, 149- 220]. Therefore, novel methods that can detect TNBC in real-time, accurate, and minimally invasive ways are urgently needed [220]. This ensures that proper treatment can be provided in the early stages of cancer, and the treatment's efficiency can be monitored [220-225]. In general, all three diagnostic methods discussed above are based on the presence and expression of specific genes by the cancer cells [1-74, 149- 220-225].

5. Triple-Negative Breast Cancer (TNBC): Biomarker Proteins

Triple-negative breast cancer (TNBC) represents a highly aggressive and heterogeneous subtype of breast cancer (BC), characterized by the absence of estrogen receptor (ER), progesterone receptor (PgR), and HER2 expression[1-74, 149-220-225-228]. The management of these patients poses significant challenges, primarily due to the scarcity of effective treatment choices and the development of resistance to chemotherapy. However, an avenue of hope lies in immunotherapy, harnessing the remarkable adaptability of the immune system, particularly in the context of TNBC, which exhibits the highest level of immunogenicity among breast-cancer subtypes[1-74, 149- 220-225]. In particular, immune-checkpoint inhibitors (ICIs) have broadened the treatment landscape of TNBC, both in the neo-adjuvant and adjuvant settings. This type of immunotherapy in metastatic TNBC (mTNBC) is biomarker-based. Accurate pathological testing holds utmost significance in the assessment of patients with these biomarkers. However, testing strategies may differ depending on the specific diagnostic scenario, encompassing sample availability and diagnostic assays[1-74, 149-220-225]. The key to improving the outcome of TNBC lies in the harmonization and complementation of current testing strategies for actionable biomarkers. Numerous immune-related biomarkers are currently approved in the clinical management of mTNBC, such as programmed death-ligand 1 (PD-L1), tumor-infiltrating lymphocytes (TILs), and mismatch repair (MMR) system[1-74, 149- 220-225]. Immune biomarkers in TNBC are now a subject of great interest due to the presence of tumor-infiltrating lymphocytes (TILs) in these tumors [219]. This characteristic often coincides with the presence of PD-L1 expression on both neo-plastic cells and immune cells within the tumor microenvironment [219]. Furthermore, a subset of TNBC harbor mismatch repair deficient (dMMR) TNBC, which is frequently accompanied by microsatellite instability (MSI) [219]. All of these immune biomarkers hold actionable potential for guiding patient selection in immunotherapy. To fully capitalize on these opportunities, the identification of additional or complementary biomarkers and the implementation of highly customized testing strategies are of paramount importance in TNBC [219]. The cancer secretome comprises factors secreted by tumors, including cytokines, growth factors, proteins from the extracellular matrix (ECM), proteases and protease inhibitors, membrane and extracellular vesicle proteins, peptide hormones, and metabolic proteins[1-38- 74, 218, 219]. Secreted proteins provide an avenue for communication with other tumor cells and stromal cells, and these in turn promote tumor growth and progression. Breast cancer is the most commonly diagnosed cancer in women in the US and worldwide [1-38- 74, 218, 228].

Triple-negative breast cancer (TNBC) is characterized by its aggressiveness and its lack of expression of the estrogen receptor (ER), progesterone receptor (PR), and HER2, making it unable to be treated with therapies targeting these protein markers, and leaving patients to rely on standard chemotherapy [1-38- 74, 218]. In order to develop more effective therapies against TNBC, researchers are searching for targetable molecules specific to TNBC [1-38- 74, 218]. Proteins in the TNBC secretome are involved in wide-ranging cancer-promoting processes, including tumor growth, angiogenesis, inflammation, the EMT, drug resistance, invasion, and development of the premetastatic niche[1-38- 74, 218]. Secreted factors released from primary tumors are able to alter the tumor microenvironment and, through both autocrine and paracrine mechanisms, the secretome of the tumor itself [1-38- 74, 218]. Collectively, these secreted factors (including proteins, RNAs, extracellular vesicles, etc.) make up the “secretome” [1-38- 74, 218]. The tumor cell secretome generally comprises cytokines, growth factors, proteins from the extracellular matrix (ECM), proteases and protease inhibitors, membrane and extracellular vesicle proteins, peptide hormones, and metabolic proteins[1-38- 74, 218]. This variety of secreted proteins renders the tumor cell secretome as an obvious mechanism by which tumor cells can promote chemoresistance, induce metastasis, and regulate the immunological response [1-38- 74, 218].

TNBC cells have an influence on surrounding cells as well, including a role in the activation of mesenchymal stromal cells and macrophages [1-38- 74, 218]. These changes lead to changes in the tumor microenvironment (TME) that encourage invasion and metastasis [1-38- 74, 218]. Furthermore, they may even affect the ability of TNBC cells to extravasate into various metastatic sites, including crossing the BBB. Proteins secreted from the TME outside of the TNBC cells themselves also play a role in tumor progression [1-38- 74, 218]. Secreted factors from adipocytes, fibroblasts, macrophages, and endothelial cells have been investigated. In addition to cytokines IL-6, IL-8, and CCL5, LCN2 was identified as a common cytokine factor secreted by stromal cells [1-38- 74, 218]. Some drugs and biologicals have already been created to target specific proteins secreted by TNBC [1-38- 74, 218]. Future treatments may require a multi-pronged approach that blocks several secreted proteins or cancer processes at once[1-38- 74, 218].

6. Triple-Negative Breast Cancer (TNBC):Therapeutic Treatment

Triple-negative breast cancer (TNBC) is an aggressive breast type of cancer with no expression of estrogen receptor (ER), progesterone receptor (PR), and human epidermal growth factor receptor-2 (HER2) [1-74, 149- 220-225].

It is a highly metastasized, heterogeneous disease that accounts for 10–15% of total breast cancer cases with a poor prognosis and high relapse rate within five years after treatment compared to non-TNBC cases. The diagnostic and subtyping of TNBC tumors are essential to determine the treatment alternatives and establish personalized, targeted medications for every TNBC individual. Currently, TNBC is diagnosed via a two-step procedure of imaging and immunohistochemistry (IHC), which are operator-dependent and potentially time-consuming[1-74, 149- 220-225]. Therefore, there is a crucial need for the development of rapid and advanced technologies to enhance the diagnostic efficiency of TNBC. TNBC is known as a heterogeneous type of cancer that is categorized into six subtypes. The subtypes are immunomodulatory (IM), luminal androgen receptor (LAR), basal-like 1 (BL-1), basal-like 2 (BL-2), mesenchymal (M), and mesenchymal stem-like (MSL). Subtyping TNBC tumors is vital in identifying the treatment alternatives and establishing personalized, targeted medications for every TNBC individual [1-74, 149- 220-225].

Breast cancer is a group of cancer cells (malignant tumors) that starts in the breast cells and grows out of control. All breast cancer tumor diagnosis starts with the detection of estrogen (ER), progesterone (PR), and human epidermal growth factor receptor-2 (HER2) receptors using immunohistochemistry (IHC) to differentiate the type of breast cancer. In general, breast cancers are classified into six different intrinsic subtypes including luminal A, luminal B, HER2 enriched, normal-like, basal-like, and claudin-low based on the presence or absence of the three primary markers (ER, PR, and HER2), basal marker (CK5/6, EGFR), and Ki-67 proliferation index [7–9]. Ki-67 protein is associated with cell proliferation, in which the increased expression of Ki-67 leads to a higher rate of cell division[1-74, 149- 220-225].

Cancer therapy is a rapidly evolving field, and one promising approach for the discovery of new anticancer agents is drug repurposing, which involves identifying new indications for existing and approved medications [1-38- 74]. Recently, a study proposed a set of approved drugs as new leads against breast cancer using a computational neural graph model. In the field of *in silico* drug design, molecular modelling serves as a valuable approach for structure-based drug design [1-38- 74]. It relies on the three-dimensional structures of proteins and encompasses various methodologies, including molecular docking, molecular dynamics simulations, structure-based pharmacophore modelling, and quantum mechanics calculations [1-38- 74]. Effective delivery of chemotherapeutic agents to target tumour sites remains a challenging task [1-38- 74]. Nanoparticle drug delivery systems (DDSs), such as gold nanoparticles, have emerged as promising strategies to enhance drug accumulation in tumours while minimizing adverse effects [1-38- 74]. Gold nanoparticles, in particular, serve as useful transport vehicles due to their ability to improve overall clinical outcomes and reduce side effects associated with chemotherapy [1-38-74]. Through molecular docking and MM/GBSA analysis, three drugs (sunitinib, bazedoxifene, and etravirine) were identified as having a higher binding affinity towards the ck2 alpha protein compared to the co-crystallized inhibitor[1-38- 74]. One of the study confirmed that a potential formulation of the drug as a gold nanoparticle for intravenous delivery in TNBC patients [1-38- 74]. The *in silico* results provided in this study indicated that etravirine could be repurposed in TNBC treatment regimens after further laboratory and consequent clinical assessments and trials [1-38- 74].

Triple-negative breast cancer (TNBC) is the most malignant subtype of breast cancer (BC) with a poor prognosis. Current treatment options are limited to surgery, adjuvant chemotherapy and radiotherapy[1-38- 74]. However, a proportion of patients have missed the surgical window at the time of diagnosis. TNBC is a highly heterogeneous cancer with specific mutations and aberrant activation of signalling pathways[1-38- 74]. Hence, targeted therapies, such as those targeting DNA repair pathways, androgen receptor signalling pathways, and kinases, represent promising treatment options against TNBC[1-38- 74]. In addition, immunotherapy has also been demonstrated to improve overall survival and response in TNBC. Patients with TNBC do not benefit from established endocrine or HER2-targeted drugs due to a lack of related receptor markers[1-38- 74]. Therefore, the standard of care for nonsurgical TNBC remains nonspecific chemotherapy[1-38- 74]. TNBC is the subtype with the best response to standard chemotherapy regimens, such as taxanes or anthracyclines [1-38- 74]. However, less than 30% of patients with TNBC achieved a complete response, and the recurrence and mortality rates remain higher than those of non- TNBC subtypes[1-38- 74]. Although TNBC is a clinical tumor entity, whole-genome sequencing studies have shown extensive inter-tumoral and intra-tumor molecular heterogeneity and have facilitated classifications of tumor subtypes[1-38- 74]. Previously, a few small molecule inhibitors, bromodomain and extra-terminal domain inhibitors, have demonstrated efficacy in TNBC. However, rapid resistance to these drugs develops via multiple mechanisms [1-38- 74]. Therefore, determining the molecular characteristics of TNBC, targeting specific changes in the internal and external tumor environment, and developing new treatment regimens represent demands in this field that must be urgently met[1-38- 74]. Considering the malignancy, heterogeneity, and drug resistance, multiple targeted therapeutic approaches and combinations of regimens are essential to improve the outcome of TNBC [1-38- 74].

Triple-negative breast cancer (TNBC) is a difficult and complex disease entity that is both confusing and frustrating for researchers, physicians and patients[1-74, 149- 220-225]. Adjuvant chemotherapy has been shown to not only prolong disease-free survival in patients but overall survival as a whole[1-38- 74]. However, TNBC lacks the typical targeted

receptors found in luminal or HER-2 disease and therefore, cannot be treated with hormonal agents, such as SERMS, aromatise inhibitors or HER2 antagonists [1-38- 74]. Although triple negative breast cancers are associated with a generally poor breast cancer specific outcome, most are not resistant to chemotherapy [1-38- 74]. These patients have an extremely poor prognosis and relapse and die quickly [1-38- 74]. Several therapies are being developed that target specific biomarkers of TNBC or basal-like subtype [1-38- 74]. To date there are multiple approaches attempting to improve care of triple negative breast cancer patients, including DNA damaging agents like platinum's, targeted EGFR and VEGF inhibitors, and, PARP inhibitors[1-38- 74]. However, none have been as clinically successful as anticipated and more targeted therapies need to be developed and explored.

The Wnt/b-Catenin, NOTCH and Hedgehog signaling pathways are being considered as novel therapeutic targets for TNBC [1-38- 74]. However, TNBC studies suggest that immunity in the patients is suppressed and they have the worst prognosis of all breast cancers. Therefore, it is imperative to understand the signalling phenomena in the tumor microenvironment and implement a multidisciplinary approach to diagnosis and therapy [1-38- 74]. Moreover, there are physical therapies such as hyperthermia, photothermal and photodynamic therapies that increase the delivery of drugs in the most drug-resistant zones [1-38- 74]. This multi-pronged marshaling mechanism helps in complete eradication of TNBC[1-38- 74]. The new discoveries in molecular biology, immunology, nanotechnology, and computer networks will allow the clinician to have more tools to make earlier and more accurate diagnoses to provide personalized treatment [1-38- 74]. Currently, efforts are focused on micro and nanofluidics to study the tumor microenvironment and understand more about the dynamic processes of cancer. Currently, nano-electromechanical systems (NEMS) and microelectromechanical systems (MEMS) are being used for tracing exosomes (cell vesicles with specific surface markers) that are not present in healthy pluripotential cells, but only in the tumour [1-38- 74].

From a chemotherapeutic perspective, TNBC is very sensitive, and treatments require extreme care. Common treatment involves the use of alkylating agents (such as cyclophosamide), anthracycline (doxorubicin topoisomerase blocker and DNA intercalating agents), anti-metabolite fluorouracil, and antimicrotubule agent (taxane) [1-38- 74]. For early diagnosis of TNBC, neoadjuvant chemotherapy and subsequent surgery are applied. No standard chemotherapy has been described for the treatment of relapsed TNBC [1-38- 74]. Treating advanced TNBC includes the following drugs: gemcitabine and capecitabine (antimetabolites), eribulin (non-taxane microtubule inhibitor), and platinum (DNA cross-linker) [1-38- 74]. For advanced TNBC, new therapies have been reported, particularly when surgery is not desired. Compared to other breast cancer subtypes, TNBCs showed greater immunogenicity[1-38- 74]. They have tumor-infiltrating lymphocytes in their microenvironment and express programmed cell death ligand (PD-L1) in high order[1-38- 74]. In 2018, talazoparib and olaparib were approved by the FDA for treating HER2 negative breast cancer. With the aim of improving TNBC treatment, several therapeutic strategies have been explored in clinical studies, including those that target or are immune specific for tumor stroma, intracellular or surface receptors, DNA damage response, and signaling pathways [1-38- 74]. So far, 399 studies have been shown on ClinicalTrials. gov for TNBC and are under phase III investigation [1-38- 74].

The concept of immune checkpoint inhibitors is to halt regulatory immune checkpoints and thus activate anti-tumor responses [1-38- 74]. This treatment strategy is considered a game changer in cancer therapy and involves molecules that can negatively alter the immune response. Immune checkpoints can be readily blocked by antibodies or modified by recombinant ligands[1-38- 74]. Research regarding the use of immune checkpoint inhibitor(s) either alone as a single agent or in combination therapy is ongoing[1-38- 74]. Neoadjuvant therapy has yielded mixed results. Promising anti-tumor activity and considerable safety were noted when neoadjuvant chemotherapy was used in combination with pembrolizumab in early stage TNBC [1-38- 74]. Immunotherapy involving targeting of the 2B receptor (A2bR) and adenosine 2A receptor (A2aR) is considered a promising approach for the reactivation of antitumor immunity and enhancement of cytotoxic T cell immune responses[1-38- 74]. Different types of poly (ADP-ribose) polymerase inhibitors have also been described. These inhibitors include niraparib, veliparib, olaparib, talazoparib, and rucaparib[1-38- 74].

The role of androgen receptor inhibitors in TNBC still needs to be explored, and more insights need to be explored. The first generation androgen receptor antagonist bicalutamide is a proof of concept for treating advanced TNBC, and the results showed a modest clinical benefit rate of 19% [1-38- 74]. Abiraterone, a second-generation anti-androgen inhibitor, shows promising targeting of androgen biosynthesis [1-38- 74]. Another second-generation androgen receptor inhibitor, enzalutamide, showed competitive binding to the androgen receptor ligand-binding domain and blocked its nuclear translocation, coactivator recruitment, and DNA binding [1-38- 74]. In addition to these therapeutic options, cell surface targets, such as tumor-associated carbohydrate antigens, have been explored as antigens for vaccine formulation [1-38- 74]. In particular, the Globo H antigen, which is expressed on the surface of different cancer types, can be explored for vaccine design[1-38- 74]. The antibody-drug conjugate remains stable in plasma, attacking antigens at the tumor cell surface with high specificity and affinity, followed by internalization, cleavage, and release of

the payload drug to exert anti-tumor activity[1-38- 74]. Ladiratuzumab vedotin or a short LV main target is a transmembrane protein (LIV-1). The protein has metalloproteinase and zinc transporter activity and is expressed in more than 90% of breast tumors [1-38- 74]. Ladiratuzumab vedotin comprises the microtubule-disrupting agent monomethyl auristatin E as payload[1-38- 74]. In addition to these therapeutic strategies against TNBCs, new platforms have been described. In this approach, EZH2 inhibitors were evaluated against the CDK2-EZH2 axis, thus reactivating ERα expression [1-38- 74]. In another new technique, the combination of histone deacetylase and DNA methyltransferase inhibitor results in ERα expression in breast cancer models. The different types of nanomedicines under experimental and clinical testing for TNBC [1-38- 74]. In another approach, targeting tumor Microenvironment for TNBC Therapy has been reported. The development of TNBC has strong association with the physiological state of tumor microenvironment (TME). TNBC has been characterized with unique tumor microenvironment (TME) and is different from other subtypes [1-38- 74]. The tumor microenvironment (TME) has strong association with induction of angiogenesis, proliferation, apoptosis inhibition, suppression of immune system and resistance to drugs [1-38- 74]. he exosomes function as promising nanovesicles that directs tumor microenvironment (TME) orchestration by communicating cells within TME milieu [1-38- 74]. The different components of TME particularly the soluble factors, transformed extracellular matrix, immune suppressive cells, reprogrammed fibroblasts and epigenetic modifications altogether helps in TNBC progression and metastasis. Hence, TME is regarded as a good therapeutic target [1-38- 74].

The research on novel therapeutic approaches in TNBC is currently focusing on immunotherapy-specific biomarkers, providing new treatment opportunities for numerous TNBC patients [219]. Although immunotherapy alone has shown limited success in a small subset of TNBC patients, combination strategies are emerging as potential ways to enhance immune responses against tumors. Currently, combining immunotherapy with conventional chemotherapy as a first-line treatment for PD-L1-positive mTNBC has demonstrated significant clinical benefits [219]. Nevertheless, the challenge of treatment resistance, whether inherent from the onset or acquired over time, persists. The tumor microenvironment, where tumor and immune cells interact, plays a crucial role in treatment outcomes [219]. The identification of response-associated biomarkers for ICI is crucial to identify patients who are more likely to have long-lasting responses with minimal side effects [219]. To achieve this, standardized and independently validated assays should be employed in larger prospective studies involving patients with TNBC who are receiving immunotherapy [219]. The identification of reliable prognostic and predictive biomarkers for treatment response is a priority in clinical practice to improve patient selection [219]. The improvement of biomarker predictivity will be facilitated by the utilization of advanced technologies capable of providing detailed information about the tumor microenvironment, such as spatial transcriptomics/proteomics and single-cell sequencing. It is probable that additional insights gained from studies on novel biomarkers in different cancer types will contribute to this advancement [219].

7. Triple Negative Breast Cancer (TNBC): Recurrence

TNBC to be more aggressive than other types of breast cancer. Studies have shown that triple-negative breast cancer is more likely to spread beyond the breast and more likely to recur (come back) after treatment [1-74, 226]. These risks appear to be greatest in the first few years after treatment. As years go by, the risks of the triple-negative breast cancer recurring become similar to those risk levels for other types of breast cancer [1-74, 226]. Five-year survival rates also tend to be lower for triple-negative breast cancer. A 2007 study of more than 50,000 women with all stages of breast cancer found that 77% of women with triple-negative breast cancer survived at least 5 years, versus 93% of women with other types of breast cancer [1-74, 226]. Another study of more than 1,600 women published in 2007 found that women with triple-negative breast cancer had a higher risk of death within 5 years of diagnosis, but not after that time period [1-74, 226]. The recurrence and survival figures in these and other studies are averages for all women with triple-negative breast cancer. Factors such as the grade and stage of the breast cancer will influence an individual woman's prognosis [1-74, 226].

Researchers are working to find the best approaches to treating triple-negative breast cancer. Some clinical trials are comparing the effectiveness of various older and newer chemotherapy medications, used in different combinations, for treating triple-negative breast cancer [1-74, 226]. Other clinical trials are trying to find out whether some targeted therapies are effective against triple-negative breast cancer. Anyone can get triple-negative breast cancer. However, researchers have found that it is more likely to affect younger people[1-74, 226]. Triple-negative breast cancer is more likely to occur before age 40 or 50, versus age 60 or older, which is more typical for other breast cancer types. Triple-negative breast cancer most commonly affects African-American women, followed by Hispanic women [1-74, 226]. Asian women and non-Hispanic white women are less likely to develop this type of cancer. A study found that black women were 3 times more likely to develop triple-negative breast cancer than white women and people with a BRCA1 mutation [1-74, 226]. When people with an inherited BRCA1 mutation develop breast cancer, especially before age 50, it is usually found to be triple-negative [1-74, 226]. Tends to be higher grade than other types of breast cancer. The higher the grade, the less the cancer cells resemble normal, healthy breast cells in their appearance and growth patterns.

On a scale of 1 to 3, triple-negative breast cancer often is grade 1 [1-74, 226]. Usually is a cell type called "basal-like." "Basal-like" means that the cells resemble the basal cells that line the breast ducts. This is a new subtype of breast cancer that researchers have identified using gene analysis technology [1-74, 226]. Like other types of breast cancer, basal-like cancers can be linked to family history, or they can happen without any apparent family link. Basal-like cancers tend to be more aggressive, higher grade cancers — just like triple-negative breast cancers [1-74, 226]. It is believed that most of the triple-negative breast cancers are of the basal-like cell type. **In 2017**, an estimated 10,100 women have been diagnosed with breast cancer in Canada. **In 2017**: An estimated 1,900 women have died of breast cancer in Canada [1-74, 226].

The Research Institute of the McGill University Health Centre, Canada (RI-MUHC) will host a new Phase 3 randomized clinical trial called TROPION-Breast04, as announced by the McPeak-Sirois Group, a provincial consortium of cancer centres across Quebec focused on breast cancer research [1-74, 226, 227]. This trial is intended for patients with triple-negative breast cancer (TNBC) or hormone-receptor (HR)-low/HER2-negative early stage breast cancer who have not yet received treatment [1-74, 226, 227]. The trial will evaluate the safety and efficacy of neo-adjuvant datopotamab deruxtecan (or Dato-DXd) in combination with durvalumab, followed by adjuvant durvalumab with or without chemotherapy compared to existing standard treatment for TNBC [1-74, 226, 227]. The research institute is proud to report that the first patient worldwide to be enrolled in this pivotal trial is a patient of the Cedars Cancer Centre of the McGill University Health Centre, Canada (MUHC) [1-74, 226, 227]. TNBC has a high recurrence rate and a high potential for metastasis. It shows resistance to conventional treatments, leading to poor prognosis and survival outcomes [1-74, 226, 227]. TNBC tends to be more common in women under 40 years of age. There is a need for novel and more effective treatment approaches. Hence the excitement for the TROPION-Breast04 clinical trial which has the potential to improve the lives of eligible patients in this setting [1-74, 226, 227]. Soon, in Quebec, Canada, and in many other countries, patients diagnosed with triple-negative or HR-low/HER2-negative breast cancer will have an exceptional opportunity to participate in a Phase 3 study exploring innovative drugs [1-74, 226, 227]. This study offers, a unique chance to improve the chances of recovery, reduce the risk of relapse and enhance quality of life [1-74, 226, 227].

In Brazil, data on the management of triple negative breast cancer (TNBC) as well as the burden of the disease in terms of health care resources utilization (HCRU) are scarce [236]. Three thousand and four patients were identified, of which 82.8% were diagnosed in early and locally advanced stages [236]. For early and locally advanced TNBC patients, 75.3% were treated in an adjuvant setting, mainly with anthracycline regimes [236]. For mTNBC patients, bevacizumab regimens were the main treatment prescribed. More than 48% of mTNBC patients were switched to a second line of treatment [236]. HCRU was higher for mTNBC patients when compared to early and locally advanced patients, with higher costs for metastatic disease management [236]. The treatment setting has little influence on the HCRU pattern or the cost of disease management [236]. The highest burden of disease was observed for metastatic management. In Brazil, TNBC is most detected in stage III, with lymphocytic infltration, multifocality, and tumor size>2 cm at diagnosis [236]. Compared to other BC subtypes, a higher proportion of patients with TNBC undergo radical surgery and chemotherapy [236]. Most patients treated with NAT receive anthracycline followed by taxane regimens [236]. Overall survival of Brazilian TNBC patients tends to be lower than that observed worldwide, mainly due to late diagnosis and difficult access to healthcare services [236]. However, data on the economic burden of TNBC remains scarce, especially in Brazil [236]. Expenditures for the private healthcare system are high, totaling 77% of all oncology expenditures in Brazil [236]. The lack of data on treatment patterns, HCRU and costs of TNBC treatment and management are limited, hinders the development of accurate pharmacoeconomic studies, policy planning and private system budget allocations[236]. One of the study demonstrated that anthracycline and taxane-based regimens given as adjuvant therapy were the mainstay treatments for early and locally advanced TNBC patients in the private healthcare setting in Brazil [236]. For metastatic patients, bevacizumab, gemcitabine, and capecitabine-based regimens were ofen prescribed. Although the proportion of patients receiving a subsequent systemic treatment decreased up to 12% in LOT3, in terms of overall costs treating mTNBC presented a higher HCRU compared to the curative setting [236]. This data presented reinforces the need to increase cure rates as a pathway to optimize resources allocation in breast cancer management in Brazil [236].

8. Triple Negative Breast Cancer (TNBC): Prognosis of TNBC

Poor prognosis has been observed in patients with TNBC. In contrast to other subtypes, TNBC development occurs more frequently in premenopausal women during early life [1-38- 74]. TNBC has a more aggressive expression profile (high p54 and Ki67 and low Bcl- 2 expression), large tumor size, and high nuclear mitotic grade [1-38- 74]. Many studies have demonstrated lower RFS in TNBC than in non-TNBC patients[1-38- 74]. The 4-year survival rate of TNBC patients was 85.5%, which is comparable to that of non- TNBC patients (94.2%)[1-38- 74]. In another study, relapse frequency was less frequently reported in TNBC [1-38- 74]. Tumor recurrence is 1.2 years which is shorter than that in non-TNBC patients [1-38- 74]. Similarly, TNBC has a worse prognosis in patients with recurrent breast cancers. The risk of tumor

recurrence and death is high in TNBC, in contrast to the other types. It has been reported that the hazard ratio (HR 4.2 for developing TNBC tumor recurrence when compared to other cancers [1-38- 74]. For triple-positive breast cancer, the 5 year survival is 91%, whereas for TNBC and HR-positive/ HER2-negative cancers, it is 81 and 94%, respectively [1-38- 74].

9. Triple Negative Breast Cancer (TNBC): Types

Scientifically, this cancer is categorized as a distant subgroup within a broad category of breast cancers. Majority of the triple negative breast cancer (TNBC) are the basal-like subtype, and many basal-like breast cancers are triple negative [1-38- 74]. They are not equivalent in terms of gene expression signatures and IHC analysis. It has been observed that a high percentage of BRCA1- associated hereditary and sporadic breast cancers are triple negative and express a high proportion of basal like cytokeratins (CK5,14,17), as well as P-Cadherin and HER1/EGFR [1-38- 74]. TNBC is well known for its aggressive behavior and is characterized by onset at a younger age, high mean tumor size, higher grade tumors and sometimes, a higher rate of node positivity [1-38- 74]. There are four transcriptional subtypes of TNBCs: two basal subtypes, which are grouped as BL1 and BL2, a mesenchymal subtype M, and a luminal androgen receptor subtype. Further, TNBC can be categorized into six different subgroups based on their molecular heterogeneity: immunomodulatory, luminal androgen receptor expression, mesenchymal stem-like, mesenchymal-like, basal-like, and unstable [1-38- 74]. TNBCs constitute 12–17% of all breast cancers and are naturally recurrent.

- Breast cancer includes molecular biomarkers include
    - ERα+ (estrogen receptor a-positive);
    - PR+ (progesterone receptor-positive);
    - HER-2 (human epidermal growth factor receptor-2);
    - EGFR (epidermal growth factor receptor) 45%–70% of Triple-negative breast cancer (TNBC) patients show this biomarker ;
    - CK5/6;
    - VEGF (vascular endothelial growth factor);
    - KI67[1-38- 74].

Currently, due to microarray technology, there is a better understanding of the molecular heterogeneity in tumors, aiding in the quantification of thousands of gene expression changes [1-38- 74].

- The classification of breast cancer cell types is considered as below:
    - Luminal A subtype, ERα+/PR+ or -/HER-2-;
    - Luminal B subtype, ER+/PR+/HER-2+;
    - HER-2 enriched subtype ER- and or/PR-/HER-2+;
    - Basal-like subtype ER- and/or PR-, HER2-, CK5/6+, CK14+, CK17+ and EGFR+;
    - Normal breast-like type (ER- and/or PR-, HER2-, CK5/6-, CK14-, CK17-, EGFR-)[1-38- 74].

Moreover, a subpopulation is described due to the Ki-67 index too. Therefore, molecular classifications are essential to provide personalized medicine and thus helps in selecting more specific drug according to the molecular signature markers of the tumour. The 4 TNBC subtypes are as follows [1-38- 74].

- Luminal androgen receptor-AR (LAR);
- Mesenchymal (MES);
- Basal-like immune-suppressed (BLIS);
- Basal-like immune-activated (BLIA) [1-38- 74].

The different subtypes of TNBC correlate well with the different chemotherapeutic responses as per retrospective studies. Unfortunately, these molecular classifications have not been shown to improve survival in hospital practice and current treatments.

- Basal-like namely, BL1 and BL2;
- MES;
- MES stem-like;
- immunomodulatory (IM);
- LAR subtype [1-38- 74].

10. Triple Negative Breast Cancer (TNBC): Clinical Behaviour

Breast cancer is the most common malignancy in women. Breast cancer is a highly heterogeneous disease[1-38- 74]. Clinical treatment and prognosis varies greatly between patients. Breast cancer is a pathological condition that occurs in the breast tissue [1-38- 74]. In most cases, emergence occurs from the milk duct, while other minor cases occur from lobules. The cancer of the ductile region is known as ductal carcinoma, while those involving mammary lobules are called lobular carcinomas [1-38- 74]. Gene expression profiling analysis often classifies TNBC as a subtype of basal-like breast cancer (BLBC). Metastatic cells outgrowth in a foreign tissue environment is considered as the rate-limiting step of breast cancer metastasis and in this stage, breast cancer cells are difficult to detect and show resistance to chemotherapy due to lack of proliferation [1-38- 74]. To date, this remains a clinical obstacle, since the patients considered as "survivors" can develop metastatic tumors years later. Disseminated tumor cells can enter a state of dormancy in the secondary organs by achieving a balanced state of proliferation and apoptosis [1-38- 74]. Triple-negative breast cancers or in short TNBCs are regarded as aggressive types of breast cancer and are the product of impaired expression of progesterone and estrogen receptors as well as human growth factor receptor 2 [1-38- 74].

The clinical behavior of TNBCs is relatively aggressive compared to that of other subtypes of breast cancer [1-38- 74]. This type of cancers have characteristic metastatic patterns and poor prognosis. TNBCs represent 24% of newly diagnosed breast cancers and a steady increase has been reported in their incidence [1-38- 74]. Approximately 56% of TNBC and BLBC gene expression profiles overlap [1-38- 74]. The overlap ratio can be as high as 60–90% between TNBC and BLBC, compared to only 11.5% between non-TNBC and BLBC [1-74, 149- 220-225]. Triple negative breast cancer (TNBC) is characterized by the absence of estrogen receptor (ER) and progesterone receptor (PR), as well as human epidermal growth factor receptor-2 (Her2) [1-38- 74]. Recently when referring to TNBC, the terms basal-like breast cancer (BC) and claudin-low BC should be mentioned. Gene expression profiling and molecular pathology have revealed that breast cancer naturally divides into luminal A and B, HER2-enriched, basal-like and claudin-low subtypes [1-38-74]. TNBC is characterized by a typically ductal histology, high grade, high proliferation and mitotic rates. It is associated with poor prognosis, a high risk of the local recurrence rate (LRR), poor disease-free survival (DFS) and cancer-specific survival (CSS) [1-38- 74]. As one special type of breast cancer, the pathogenesis of TNBC is at present yet to know[1-38-74]. Clinical data demonstrated that risk factors like race, age, premenopausal status, increased parity, hormonal contraceptive use, high histologic grade, and advanced disease were independently associated with TNBC [1-38- 74].

Triple-negative breast cancer (TNBC), a specific subtype of breast cancer that does not express estrogen receptor (ER), progesterone receptor (PR), or human epidermal growth factor receptor 2 (HER-2), has clinical features that include high invasiveness, high metastatic potential, proneness to relapse, and poor prognosis[1-38- 74]. Because TNBC tumors lack ER, PR, and HER2 expression, they are not sensitive to endocrine therapy or HER2 treatment, and standardized TNBC treatment regimens are still lacking [1-38- 74]. Therefore, development of new TNBC treatment strategies has become an urgent clinical need [1-38- 74]. As there are no first-line therapies specific for these patients at the moment, lots of researchers are working on this from different aspects, such as gene level, receptors, immunomodulatory, signaling pathway and others [1-38- 74]. Researchers also found some possible prognostic markers like EGFR, ALDH1 LOXL2, SNCG and LDHB[1-74, 149- 220-225]. However, most of these studies on TNBC were at the cellular level and subject to its limitation [1-38- 74]. Due to the low incidence of the disease, there are only a few clinical trials for TNBC patients so far. Therefore, more large scale clinical trials to be conducted in the future [1-38- 74].

11. Triple Negative Breast Cancer (TNBC): Epidemiology

Epidemiological data showed that TNBC mostly occurs in premenopausal young women under 40 years old, who account for approximately 15–20% of all breast cancer patients [1-38- 74]. Compared with other subtypes of breast cancer, the survival time of TNBC patients is shorter, and the mortality rate is 40% within the first 5 years after diagnosis [1-38- 74]. Triple-negative breast cancer (TNBC) is highly invasive, and approximately 46% of TNBC patients will have distant metastasis [1-38- 74]. The median survival time after metastasis is only 13.3 months, and the recurrence rate after surgery is as high as 25%[1-74, 149- 220-225]. The metastasis often involves the brain and visceral organs. Distant metastasis mostly occurs in the 3rd year after diagnosis. The average time to relapse in non-TNBC patients is 35–67 months, while that in Triple-negative breast cancer (TNBC) patients is only 19–40 months [1-38- 74]. The mortality rate of TNBC patients within 3 months after recurrence is as high as 75 [1-38- 74]. Due to its special molecular phenotype, TNBC is not sensitive to endocrine therapy or molecular targeted therapy[1-38- 74]. Therefore, chemotherapy is the main systemic treatment, but the efficacy of conventional postoperative adjuvant chemoradiotherapy is poor [1-38- 74]. The residual metastatic lesions eventually will lead to tumor recurrence. Bevacizumab has been used in combination with chemotherapeutic drugs to treat TNBC in some countries, but the survival time of patients did not increase significantly [1-38- 74]. Therefore, it is urgent to develop new treatment regimens and targets. Considering the

proposed subtypes and their molecular variations as defined by specific biomarkers and the current chemotherapy, immunotherapy, and targeted inhibitor combination options, great advances have been achieved in TNBC treatment [1-38- 74].

It has been reported that in 2018 about 2,088,849 cases of triple negative breast cancer (TNBC) were reported making it as a common cancer in women. The average survival rate from the disease is ~10.2 months in perspective on the currently available therapy, with a 65% 5 years survival rate in cases of regional tumors and 11% for those where the tumor is spread to distant organs [1-38- 74]. TNBC accounts for 15–25% of all breast cancers. The TNBC proportion in all age groups followed a similar trend. However, younger and older women have increased rates of BRCA and basal TNBC, apocrine and neuroendocrine TNBC [1-38- 74]. African American and Hispanic women are found to be at high risk of TNBC. African Americans have a worse prognosis compared to other groups [1-38- 74]. In a case study conducted in 2009, 187 Triple Negative Breast Cancer (TNBC) patients were reported to have a 2.5% higher risk for triple negative breast cancer (TNBC) who used oral contraceptives for more than 1 year. The risk is 4.2% among women aged less than 40 years. It was also noted that when the duration of oral contraceptive use increased, the risk increased. In the United States, TNBC is responsible for 12% of breast cancers, with a 5-years survival rate of 8–16% [1-38- 74].

12. Triple Negative Breast Cancer (TNBC): Risk Factors

Age of the women is the first important factor for TNBC. Approximately 80% of breast cancers (including TNBCs) are >50 years [1-38- 74]. The cancer risk increases with age: 1.5% risk at the age of 40 years, 3% at age 50, and more than 4% at age 70 years. In addition, a relationship exists between cancer subtype and age[1-38- 74]. This can be explained by TNBC, which is mostly diagnosed in the age group of <40 years, whereas in patients aged >70 years, luminal A subtype cancer is more common [1-38- 74]. The incidence of TNBC remains high among white non-Hispanic women. In addition, the mortality rate is significantly higher among black women, and black women are considered to have the lowest survival rates for malignancy [1-38- 74]. Another factor of TNBC is sex [1-38- 74]. Due to different sex hormonal stimulation, female sex is considered a higher risk for TNBC compared to male sex. Females have breast cells that are very susceptible to estrogen and progesterone hormones, as well as imbalances [1-38- 74]. Circulation of estrogens and androgens is associated with an increased risk of breast cancer. In case of pre-menopausal and post-menopausal women, physiological changes in endogenous sex hormones resulted in a higher risk of breast cancer [1-38- 74]. In men, the prevalence of breast cancer is 1%. The important factors which increase a mans risk of breast cancer are; older age, “BRCA2/BRCA1” mutations, and increased estrogen levels, genetic history in family, and highly exposure to radiation [1-38- 74].

Mutations in genes such as BRCA1 and BRCA2 were found to be strongly associated with TNBC. Mutations in TP53, CDH1, PTEN, and STK11 are also associated with breast cancer and TNBC incidence[1-38- 74]. Mutations in the XRCC2 gene are also associated with high risk of breast cancer. Further, it has been revealed that BRCA1-related tumors profile resembles the TNBC subtype, while the profile of the BRCA2-associated tumor correlates to luminal-like breast cancers, particularly the Luminal B subtype [1-38- 74]. Genetic history is one of the major risk factors associated with breast cancer (similar to TNBC). Approximately 13–19% of diagnosed breast cancer patients reported a first-degree breast cancer relative [1-38- 74]. Moreover, the risk is higher in family members of age <50 years. The genetic history of ovarian cancer in a family, particularly those with BRCA1 and BRCA2 mutations, has a greater risk [1-38- 74]. As per clinical practice, breast tissue density has been categorized as low-density breasts, fatty, and high-density breasts[1-38-74]. Women receiving hormone replacement therapy are reported to have denser breasts during early age, during pregnancy, and breastfeeding, even with lower BMI [1-38- 74]. In postmenopausal and premenopausal women, the density of the breast affects the risk of cancer, that is, the higher the density, the higher the chances [1-38- 74]. Breast tissue density screening could be a promising and quick approach for the rational surveillance [1-38- 74]. A history of radiotherapy can lead to the development of secondary tumors [1-38- 74]. This is mainly dependent on the patient’s state and age. Patients aged <30 years are considered at higher risk, and radiotherapy treatments, such as multiple-field IMRT (6F-IMRT) and double partial arc (VMAT) techniques can increase the chances of secondary tumors [1-38- 74].

Radiotherapy in patients with a family history of breast cancer is considered to be at a higher risk [1-38- 74]. The initial symptoms of cancer are cancerous lesions in the breast. Regarding the family history of disease, the other risk factors associated with breast cancer are; in-situ carcinoma, atypical hyperplasia, proliferative lesions and non-proliferative lesions [1-38- 74].

Breast cancer risks include a family history of breast cancer and benign lesions. Furthermore, diethylstilbestrol is a major cause of breast cancer during pregnancy[1-38- 74]. The breast cancer risk increases with an increase in diethylstilbestrol doses. Female age is another consideration, that is, the risk increases 1.9 times in women older than 40 years [1-38- 74]. Hormonal replacement therapy, when carried out for more than 5–7 years, increases the chances

of breast cancer. The continuous uptake of the selective antidepressants, paroxetine, tricyclic, and serotonin inhibitors, also increases the chances of breast cancer [1-38- 74]. Similarly, one of the study reported tetracycline can increase risk of breast cancer. Furthermore, the relationship between the risk of breast cancer and excessive use of hypertensive medications, anti-inflammatory non-steroidal drugs, and statins has also been studied. However, the research data in this regard are not efficient in supporting these data [1-38- 74]. According to several epidemiological studies, obesity is a potential risk factor for breast cancer [1-38- 74]. Epidemiologically, estrogen receptor-positive breast cancer develops in obese women in the postmenopausal period [1-38- 74].

However, women more than 50 years of age with greater body mass index (BMI) are at higher risk of breast cancer than those with low BMI [1-38- 74]. However, it has been reported that people with a higher body mass index (BMI) are at a high risk of tumors with a high percentage and size of lymph node metastasis. In premenopausal women, obesity is not only an evident cause of cancer, but also high mortality [1-38- 74].

Pro-carcinogenic events are facilitated by greater fat content in the body, which in turn enhances the circulation of hormones and inflammation. Females with a body mass index (BMI) greater than 25 kg/m^2 had poor clinical outcomes. Greater fat contents, although with the relevant body mass index (BMI) in post-menopausal women, have poor clinical outcomes [1-38- 74]. People with a family history of breast cancer are at a greater risk of breast cancer with greater body mass index (BMI) [1-38- 74]. The physical activity is considered the best action to be performed in order to prevent breast cancer. This is supported by one of the study that in women the breast cancer occurrence is reduced by physical activity during the postmenopausal period [1-38- 74]. The Physical activity reduces the exposure to endogenous sex hormones and can also alter insulin-like growth factor-1 levels and immune responses [1-38- 74]. Various studies reported alcohol consumption is a major cause of cancer in the gastrointestinal tract, along with breast cancer [1-38-74]. Alcohol and alcohol beverages can increase the risk of malignancy. The hormone balance is disturbed along with the enhanced production of estrogen, which in turn increases body weight [1-38- 74]. Alcohol and its beverages are considered to increase the risk of cancer growth. Alcohols are the major causative agents of estrogen positive breast cancers [1-38- 74]. Morphological alterations of the breast and its tissues have been reported with the consumption of alcohol before the 1st pregnancy [1-38- 74].

Vitamins are anti-cancer elements that can prevent breast malignancies. Research is underway to evaluate the risk of cancer with the consumption of vitamins, particularly vitamin B, C, and E folic acids and multivitamins [1-38- 74]. Vitamin D supplements, that is, high serum 25-hydroxyvitamin D, are thought to be potential cancer control agents in postmenopausal women and in the premenopausal period [1-38- 74]. Excessive expression of vitamin D receptors is associated with a lower mortality rate in patients with breast cancer [1-38- 74]. Artificial light exposure for a longer duration can increase the risk of breast malignancy. This occurs because of the activation of melatonin pigments and consequent epigenetic shifts [1-38- 74]. Females who have been exposed to dreadful carcinogenic chemicals are at higher risk of breast cancer and epigenetic alterations and mutations [1-38- 74]. Exposure and duration of exposure contribute to an increased risk of breast cancer mutagenesis. Exposure of mammary glands to polychlorinated biphenyl (PCB) and dichlorodiphenyltrichloroethane (DDT) chemicals increases the risk of breast cancer [1-38- 74]. Furthermore, continuous exposure to organic solvents, insecticides, pesticides and oil mist increases the risk of breast cancer. Antibiotics, statins, antidepressants, and antihypertensive drugs can increase the risk of breast cancer[1-38-74]. Similarly, NSAIDs that contain aspirin and ibuprofen are considered major risk factors for breast cancer. Tobacco causes mutations in oncogenes and p53 suppressor genes [1-38- 74]. Active smoking and passive smokers are at a risk of cancer. Smoking during pregnancy and chain smokers are at potential risk of malignancies [1-38- 74]. According to the WHO, processed foods, such as meat, are confirmed group-1 carcinogen for gastrointestinal cancer and breast malignancy[1-38- 74]. The excessive use of saturated fats is also considered a carcinogen. The obesity-causing ultra-processed diet plans that are enriched in elements such as sugar, sodium, and fats are thought to be carcinogenic and increase the risk by 11% [1-38- 74]. Diets that are rich in green vegetables, fresh fruits, protein-enriched grains, and legumes are anti-carcinogenic and therefore, reduce the risk of breast cancer [1-38- 74]. Similarly, diets rich in phyto-estrogen, folate elements, saturated fibers, n-3 PUFA, and vitamin D are regarded as anti-cancer agents [1-38- 74]. Hence, a low dose consumption of saturated fat and n-6 PUFA has been proposed. The antioxidants found in green tea have also shown anti-carcinogenic properties [1-38- 74]. Curcuminoids and sulforaphane (SFN) derived from turmeric are thought to be anti- carcinogens [1-38- 74].

13. Triple Negative Breast Cancer (TNBC): Signalling Pathways

The term Notch was first described by Thomas Hunt Morgan in 1917 and refers to transmembrane receptors and ligands [1-38- 74]. The Notch pathway has been identified in *Drosophila melanogaster*. The Notch pathway is a short-range cell-to-cell communication pathway that is critical for metazoan development. This signaling pathway is key in cell proliferation and differentiation most importantly, governs embryonic development and maintains tumor stemness

to TNBC tumor metastasis [1-38- 74]. During Wnt/β-Catenin signalling pathway different Wnt ligands, such as WNT3A, WNT11, and WNT5A, are reported to be pertinent in cancer migration and invasion. Wnt/β- catenin signaling pathway is activated in epithelial ovarian cancer and targets gene regulate cell proliferation and apoptosis thereby, mediating cancer initiation and progression [1-38- 74]. Furthermore, Wnt inhibitors can destroy drug-resistant cells and cancer stem cells [1-38-74]. During TGF-β Signaling Pathway, TGF-beta 1 is expressed exponentially in TGF-β1. TGF-β1 has been implicated its important role in breast cancer stem cells [1-38- 74]. Signaling Pathway of CSPG4 Protein: The CSPG4 protein (non-glial antigen) is expressed as a cell surface proteoglycan by basal breast carcinoma cells [1-38- 74]. Therapeutically, CSPG4 inhibition allows for efficient management of breast cancer. Monoclonal antibodies can block the CSPG4 protein, which hinders survival signaling pathways in tumor cells [1-38- 74]. In addition, controlling the over expression of CSPG4 by targeting therapeutically is seen in different TNBC cells [1-38- 74]. The Hedgehog signaling pathway is involved in cancer cell invasion, metastasis, drug resistance, and tumor recurrence [1-38- 74]. Over expression of this pathway results in poor prediction of breast cancer mortality, especially in TNBC patients [1-38- 74]. The Hedgehog signaling pathway is considered to initiate breast cancer malignancy [1-38- 74]. Thiostrepton is a novel experimental drug that suppresses TNBC CD44+/CD24− cancer stem cells. Furthermore, Rapamycin and paclitaxel drugs are used to inhibit the PI3K/ AKT/mTOR pathway and hence play a significant role in TNBC (PI3K/AKT/mTOR Pathway) [1-38- 74]. Despite these efforts on PI3K/AKT/mTOR pathway inhibitors, synthesis of novel inhibitors is needed to block the PI3K/Akt/mTOR pathway and act as therapeutic agents against TNBC [1-38- 74]. The Mammalian Target of Rapamycin (mTor) signaling pathway is responsible for poor prognosis due to the aggressive nature of the cancer and its good tissue invasion property. Moreover, high expression of a protein kinase enzyme (Akt) has been reported to be involved in tumor invasion and metastasis therefore, inhibiting the mTOR pathway can be an efficient anti-cancer strategy for several human malignancies [1-38- 74].

The epidermal growth factor receptor is reported in 89% of TNBC and is considered as an attractive therapeutic target, particularly in BL2 subtype tumors [1-38- 74]. The expression of this gene results in primary tumorigenesis and metastasis. The EGFR inhibitor gefitinib lowers the proliferation of cancer cells and increases carboplatin and docetaxel cytotoxicity [1-38- 74]. Several EGFR inhibitors, such as lapatinib and erlotinib, are currently being tested against TNBC, in addition to cetuximab and panitumumab (monoclonal antibodies) [1-38- 74]. The synergistic therapeutic approach of monoclonal antibodies and chemotherapeutics is considered to be more effective. This can be exemplified by the combined use of carboplatin, cetuximab, cisplatin and cetuximab proved to be more efficacious in patients with advanced TNBC [1-38- 74]. Additionally, tri-inhibitor therapy, including carboplatin, gefitinib, and docetaxel, enhances TNBC cytotoxicity [1-38- 74]. Cannabidiol inhibits breast cancer metastasis by interfering with the epidermal growth factor pathway [1-38- 74]. In approximately 70 and 23% of breast cancers, BRCA1 and BRCA2 mutations have been reported [1-38- 74]. For both of these mutations and TNBC, PARP inhibitors are regarded as the most vital drugs. The activation of PARP-1 and PARP-2 proteins is a consequence of DNA strand breaks. Veliparib and olaparib are both PARP inhibitors that have different catalytic inhibition mechanisms [1-38- 74-78].

The tumor microenvironment (TME) involves the surrounding blood vessels, fibroblasts, immune cells, signaling molecules and the extracellular matrix around the tumor[1-38- 74]. Tumor Infiltrating Lymphocytes (TILs) produce endogenous antitumor immune response for inhibiting tumor progression and improving free survival rate of TNBC patients[1-38- 74, 78]. Tumor associated macrophages are important for immunosuppressive role by secreting inhibitory cytokines, regulatory T cells infiltration promotion, and reactive oxygen species reduction [1-38- 74]. Cancer-Associated Fibroblasts lower anti-tumor immunity, favor tumor cell proliferation, invasion and reshape the extracellular matrix [1-38- 74]. Tumor associated neutrophils aid in lysing tumor cells and induce antitumor function [1-38- 74].

14. Triple Negative Breast Cancer (TNBC): India

Breast cancer is the most prevalent cancer among women worldwide. Breast cancer is a pathology that emerges from the breast tissue, especially milk duct (ductal carcinoma representing 80% of the cases) as well as the lobules [1-38-74]. The cancer emerging from the ductile region is known as ductal carcinoma while those emerging from the mammary lobules are known as lobular carcinomas [1- 37-74]. Among the different breast cancer subtypes, triple-negative breast cancer (TNBC), which is more prevalent among younger age women, and is the most aggressive form [1-38- 74]. Breast cancer is the most common cancer among women in urban India, with its incidence recently surpassing that of cervical cancer [37- 75]. Breast cancer is the most common cancer in India [37-74]. For example, in 2012, it is estimated that approximately 145,000 new patients were diagnosed with breast cancer in India, and nearly 70,000 women died of the disease [37-74]. Age-standardized 5-year breast cancer survival for Indian women diagnosed with breast cancer is 60% compared with 80% in Western countries [37-75]. TNBC accounts for approximately 12% to 17% of all invasive breast cancers in Western populations [1-37-75]. TNBC occurs more frequently in younger women and is associated with higher histologic grade and more advanced disease [1-37-74].

In India, breast cancer is diagnosed in approximately 100,000 women, with a case fatality ratio of 40% [37-75]. This shows that India has become a country of the highest breast cancer-related deaths worldwide [37-74]. Currently, TNBC is highly prevalent among Indian women and develops in approximately 20% to 43% of all patients with breast cancer. Breast cancer has been found to be the most common among urban Indian women and the second most common among the rural Indian women [37-74].

In India, breast cancer (BC) has emerged as the second most common type of malignancy within the past 25 years [37-74]. In terms of disability-adjusted life years (DALYs), where breast caner used to be less prevalent than the more prevalent stomach, cervical, and leukemia diagnoses, according to data accumulated up until 2016, breast cancer is surpassed only by cervical cancer [37-75]. Moreover, India has one of the highest rates of the most aggressive subtype of BC referred to as Triple-Negative Breast Cancer (TNBC) [37-75]. The rates of TNBC in India are almost double that of the United States (US) with estimates as high as 28% to one-third of all breast cancer in India compared to 12–15% in the US [37-75]. There was general agreement that breast cancer in India is more often seen in a younger population when compared to Western cohorts [37-75]. Data from Banaras Hindu University (BHU) revealed a mean age of 51, and data from the Sanjay Gandhi Postgraduate Institute of Medical Sciences (SGPGIMS) in Lucknow, India reports a mean age of 49.7 years [37-74]. Data from the Indian Council of Medical Research between 1982 and 2005 observed an annual percent change (APC) as high as 4.2% in Nagpur among women age 15–34 [37-74].

Another significant issue is the stage of presentation. There was no consensus on what screening modality would best serve the Indian population to downshift the stage of presentation[1- 37-74]. Given the known limitations of mammography in dense breast tissue and in young women. The higher rate of triple-negative breast cancer is observed in India in comparison to other countries. This may be partially explained by the detection methods in India, but more research and a National Breast Cancer Registry is needed [37-74]. A call for improved standardization of molecular subtyping across clinical centers in India as well as etiological studies of environmental exposures. Breast cancer within existing cohorts and case–control studies will be essential to understand the drivers of early age of onset and the etiologic behind increasing breast cancer incidence rates in India [37-74]. Prevalence of TNBC in India is considerably higher compared with that seen in Western populations [37-74]. As many as one in three women with breast cancer could have triple-negative disease. This finding has significant clinical relevance as it may contribute to poor outcomes in patients with breast cancer in India. Additional research is needed to understand the determinants of TNBC in India [37-74].

Numerous clinico-pathologic studies performed throughout the world strongly support the utterly poor prognoses and high recurrence rate of TNBC [37-74]. The data analysis on TNBC revealed that the proportion of TNBC ranges from 6.7% to 27.9% in different countries, with the highest reported percentage in India among all, followed by Indonesia, Algeria, and Pakistan [37-75]. Most of the other countries (Netherlands, Italy, London, Germany) had a TNBC incidence less than the mean level (ie, 15%). The high incidence of TNBC in the Indian population is associated with vivid risk factors, which primarily include lifestyle, deprivation status, obesity, family history, high mitotic indexes, and BRCA1 mutations [37-75].

Epidemiological studies have shown an association between breast cancer and smoking, alcohol consumption, a high-fat diet, reproductive factors, and socioeconomic status, which may explain its more frequent occurrence among women with a Western lifestyle [37-75]. However, the risk factors for specific pathological and molecular subtypes of breast cancer have not been accurately defined [1-74]. Therefore, the differential effect of risk factors on breast cancer subtypes, if any, remains unclear. A few studies have evaluated the risk factors for estrogen receptor negative breast cancer and suggested that higher parity and younger age at first child-birth may be associated with higher risk of developing this type [1-74]. The proportion of estrogen receptor (ER)-positive breast cancer in Indian women appears to be lower (about 45–60%) than that in their European and American counterparts [37-74]. Accordingly, the fraction of patients with triple-negative breast cancer has been reported to be higher (25–30%) in patients from India and other developing countries [37-74]. The differences in hormone receptor positivity between Indian and Caucasian patients could be a real ethnic variation or it could be a result of lower average age at diagnosis [37-74]. Furthermore, the results of one of the case–control analysis of the association of risk factors with breast cancer phenotypes suggests that lower waist-to-hip ratio, lower socio-economic status and possibly high parity could be differentially associated with triple-negative breast cancer compared with non-TNBC cancers. Although these associations were not statistically significant [37-75]. Most other reproductive and non-reproductive risk factors showed no significant association with breast cancer phenotypes [37-75]. Broad risk factor modification strategies are likely to be useful as population-level interventions [37-75].

Breast cancer is a heterogeneous disease with different biologic subtypes that are recognized by gene expression profiling studies [1-37-74]. Clinically, these subtypes are characterized on the basis of expression of estrogen receptor

(ER), progesterone receptor (PR), and human epidermal growth factor receptor 2 (HER2) [1-37-74]. Triple-negative breast cancer (TNBC) is an aggressive subtype that is defined by lack of expression of ER and PR as well as absence of over expressed or amplified HER2 [37-74]. Whereas breast cancer incidence seems to be increasing in the country, epidemiology of the disease is inadequately studied [1-37-74]. Prevalence of TNBC in India is reported to be higher than that observed in Western populations [37-74]. In everyday medical practice, breast cancer diagnosis relies on three different types of analysis: (A) clinical examination; (B) radiological/image examinations (that includes mammography, magnetic resonance imaging (MRI) ultrasonography etc.) and (C) immunohistopathological examinations. Employing all these tools, the clinical oncologist can stage the disease using TNM classification and reviewing the guidelines established in rigorous clinical trials. Although, they can also use genetic profiling tests such as MammaPrint and Oncotype DX to understand disease prognosis better [1-37-73].

The treatment of TNBC is greatly hampered due to the lack of targeted therapies. Hence, it requires earnest attention towards extensive research for the prevention and development of treatment modalities with high efficacy [1-37-74]. Existing therapies (eg, hormonal or trastuzumab-based therapy), which are based on targeting ER or HER2 oncogene cannot be expected to be helpful in case of TNBC, given that these tumors lack hormone receptor expression and HER2 protein over-expression and gene amplification [1-37-74]. Thus, chemotherapy and surgery, combined or individually, appear to be the only offered therapy. Therefore, TNBC definitely displays extremely severe outcome compared to all other subtypes of breast cancer [1-37-74].

There is an absence of specific treatment strategies for this tumor subgroup, and hence TNBC is managed with conventional therapeutics, often leading to systemic relapse[1-37-74]. The resistance of TNBC to conventional therapeutic agents has helped in the advancement of advanced TNBC therapeutic approaches including hyperthermia, photodynamic therapy, as well as nanomedicine-based targeted therapeutics of drugs, miRNA, siRNA, and aptamers. Artificial intelligence is another tool that is presented to enhance the diagnosis of TNBC [1-37-76]. To achieve better efficacy in the treatment of TNBC, the following aspects require continued research. First, more research is needed to improve the efficacy of existing drugs and to overcome drug resistance[1-37-74]. Second, in some clinical trials, combination therapy has shown better efficacy than single drugs. However, the sequence and timing of combination drugs still required further study [1-37-74]. Third, more research is needed to identify new targets, new biomarkers, and new drugs. We believe that with further advances in targeted therapeutic strategies for TNBC, patients with TNBC will have the opportunity to achieve better clinical outcomes [1-37-76].

15. Triple-Negative Breast Cancer (TNBC): Azerbaijan

Azerbaijan is a secular Eurasian country, with a predominantly Turkic population that gained its independence in 1991[77]. he cultural disposition underscores importance of privacy and family ties. In recent years, the practice of medicine is increasingly aligned with modern treatments [77]. The country has endorsed the importance of patients' rights legislation. The incidence of breast cancer reported by the State Statistical Committee in 2014 was 32.8 per 100 000 women [77]. The death rate from breast cancer is the leading cause of cancer-related deaths among women contributing 12% of all female cancer deaths. The mental well-being of Azerbaijani women undergoing surgical treatment for breast cancer has not been previously assessed [77]. To our knowledge, the relationship between insights, ie, degree of intrapersonal awareness, of cancer diagnosis and mental well-being has also not been previously studied [77].

Breast cancer is the most common cancer among women in developing countries and one of the main causes of decreased quality of life and high level of distress, especially due to body-debilitating surgeries and adverse effects of chemotherapy [77]. In some cultures, diagnosis of breast cancer has additional burden due to stigma associated with the word “cancer.” Information about having breast cancer may lead to severe negative reactions ranging from acute distress to depression, including risk of suicide [77]. For this reason, health providers avoid disclosure, ie, direct interpersonal communication of the diagnosis to the patient, or delegate disclosure with incomplete or no elucidation of diagnosis. Informed knowledge about diagnosis of cancer is a necessary step for a patient in her ability to fully participate and make informed decisions about treatment [77]. This study has a number of limitations. First, it reflects cross-sectional state anxiety and anxiety-depression symptoms prior to surgery. Second, the study sample size was small [77]. Results of the current study can form the basis for proposals for multicentered approaches with greater sample sizes to draw attention to the urgent training of clinical oncologists and allied specialists in these settings [77].

16. Triple-Negative Breast Cancer (TNBC): Role of Phytocannabinoids

Cannabis sativa L. belongs to the family *Cannabaceae* was used as a medicine before the Christian era in Asia**,** mainly in India**,** China, Bhutan, Nepal, Afghanistan, Pakistan, Azerbaijan, Iran, Africa, and Persians [75, 80-148]. It was found in various habitats ranging from sea level to the temperate and alpine foothills of the Indian Himalayan Region from where it was probably spread over the last 10,000 years. *Cannabis sativa* L. (hemp, or marijuana) produces male and female inflorescences on different plants (dioecious) [75,80-148, 229-232]. Therefore, the plants are obligatory out-crossers. In commercial production, medical Cannabis (marijuana type) plants are all genetically female; male plants are destroyed as seed formation reduces flower quality. Cannabis sativa is a dioecious cross pollinated plant with compound racemose type of inflorescences producing separate male and female plants [75, 80-148]. The female inflorescence is the main product of Medical Cannabis sativa (marijuana or drug type). *Cannabis sativa* produces a unique class of terpenophenolic compounds, called phytocannabinoids, as well as non-cannabinoid compounds [75, 80-148]. Δ9-tetrahydrocannabinol (THC) is the major psychoactive component and the toxicity of this metabolite of Cannabis sativa is the most studied. Many historians believed that Indian Himalayan Region was the centre of origin of *Cannabis sativa* L. and *Cannabis indica* L [75, 80-183-215, 229-232].

Cannabis sativa L., is classified into two types as Industrial *Cannabis sativa*, hemp or Medical *Cannabis sativa* L.(drug or marijuana) based on its THC (Λ9-tetrahydrocannabinol) content [75, 80-148]. Medical *Cannabis sativa* (drug or marijuana) contains very high levels of THC (Λ9-tetrahydrocannabinol) (above 0.3 to 38% of dry weight) [75, 80-183-215]. On the other hand, Industrial *Cannabis sativa* L. (Hemp) contains very low levels of THC (0 to 0.3% of dry weight). However, due to the presence of psychoactive molecules, Λ9-tetrahydrocannabinol (Λ9-THC) and Λ8-tetrahydrocannabinol (Λ8-THC), Cannabis cultivation and its use is restricted/regulated in many countries [75, 80-148-215, 229-232].

Cannabis sativa has been used as a medicine throughout history to treat a variety of diseases. Recently, the medicinal use of cannabis and cannabinoids has gained general acceptance [75, 80-148-183, 229-232]. However, the full therapeutic potential and efficacy of cannabis and compounds derived from it is still being elucidated for specific disease states. Phytocannabinoids are produced by the plant *Cannabis* and target both the endocannabinoid system and other biological pathways [75, 80-148-183]. This allows them to exert a wide array of effects both on the central nervous system and peripheral immune, cardiovascular, digestive, reproductive, and ocular systems [75, 80-148-183]. Both Medical Cannabis (Marijuana or drug type) and Industrial *Cannabis sativa* or hemp is used for controlling numerous diseases, such as cervical cancer, chronic pain, asthma, rheumatoid arthritis (RA), wound healing, constipation, multiple sclerosis (MS), cancer, inflammation, glaucome, neurodegenerative disorders (Epilepsy-seizure disorder, Alzheimer's disease, Parkinson's disease), dengue viral disease, Huntington's disease, Tourette's syndrome, dystonia, Lennox-Gastaut Syndrome (LGS), Dravet Syndrome (DS), obesity, weight loss, anorexia, emesis, osteoporosis, schizophrenia, cardiovascular disorders, sleep disorders, traumitic brain injury (TBI), post traumetic stress injury, drug addiction (Marijuana), AIDS, Wasting syndrome, amyotrophic lateral sclerosis (ALS), depression and anxiety, diabetes, migraine (headache disorder), Covid-19 (SARS-CoV-2), Leishmaniasis (Kala**-**Azar), and metabolic syndrome related disorders, to name just a few, are being treated or have the potential to be treated by Cannabinoid agonists/ antagonists/cannabinoid-related compounds [75, 80-148-215, 229-232].

Phytocannabinoids are cannabinoids that occur naturally in the cannabis plant. The classical cannabinoids are formed through decarboxylation of their respective 2-carboxylic acids (2-COOH), a process which is catalyzed by heat, light or alkaline conditions [75, 80-148]. These cannabinoids are abundant in the viscous resin that is produced by glandular structures in the cannabis plant called trichomes [75, 80-148]. This resin is also rich in terpenes, which are responsible for the characteristic smell of the cannabis plant. The two cannabinoids the most well known for their therapeutic properties are, Δ9-tetrahydrocannabinol (THC) and Cannabidiol (CBD) [75, 80-148]. THC and CBD are the neutral homologs of tetrahydrocannabinolic acid (THCA) and Cannabidiol acid (CBDA) respectively [149- 183, 229-232].

Medicinal plants have been used for decades for health benefits and to treat several different diseases [149- 183, 229-232]. The therapeutic potential of medicinal Cannabis was demonstrated in various medical conditions such as sleep disorders, nausea, anorexia, emesis, pain, inflammation, neurodegenerative diseases, epilepsy, and cancer [75, 80-148, 229-232]. Commercially available cannabinoids, such as dronabinol, nabilone, and others, are approved for the treatment of cancer-related side effects such as nausea and vomiting. Cannabinoids have also been shown to exhibit antitumorigenic properties in various preclinical cancer models [75, 80-183-215, 229-232]. Until now, more than 500 compounds from Cannabis sativa have been characterized, including cannabinoids, flavonoids, terpenes and fatty acids, present in the leaves and buds of the plant [75, 80-148]. About 100 of those compounds, accounting for about 24% of all products of the plant, are phytocannabinoids [149-183]. Currently, Cannabis sativa is also widely used for recreational purposes, being considered the number one illicit drug in several countries [75, 80-148]. Moreover, in

recent years, this plant has gained a significant clinical interest and its medical use is already regulated by law in several countries. Besides THC, cannabidiol (CBD) is another well-known phytocannabinoid that has gained a lot of attention in recent years because of its therapeutic potential [75, 80-148]. This is, in part, due to the absence of psychotropic activity. In the plant, THC and CBD are present as acidic precursors, Λ9--tetrahydrocannabinolic acid (THCA) and cannabidiolic acid (CBDA), respectively, being converted to THC and CBD, through decarboxylation induced by increased temperatures [75, 80-148, 229-232].

In addition to these two main phytocannabinoids, there are also other phytocannabinoids, designated as minor phytocannabinoids, that, despite being less studied, may present interesting pharmacological effects [75, 80-148-215]. Some of those compounds are cannabinol (CBN), the first cannabinoid isolated, cannabigerol (CBG) and cannabidivarin (CBDV) [75, 80-183]. Cannabinoids may also induce their actions through interaction with other receptors. One alternative receptor is the orphan G protein-coupled receptor 55 (GPR55), which, in humans, is mainly expressed in the brain and liver [75, 80-148]. GPR55 shares limited homology with CB1 (13%) and CB2 (14%), which is the reason why it was suggested as the third CB, CB3. However, there are still inconsistencies regarding its activation and modulation, representing a reason why more research is needed to classify it as a CB [75, 80-183-215].

Endocannabinoid system (ECS) is an ancient, evolutionary stable homeostasis system in human and animals [75, 80-170-215]. It consists of three components—ligands, including 2-arachidonoylglycerol (2-AG) and arachidonoyl ethanolamide (AEA or anandamide), receptors, such as cannabinoid receptor 1 (CB1) and cannabinoid receptor 2 (CB2), and the metabolizing enzymes—fatty acid amide hydrolase (FAAH) and monoacylglycerol lipase (MAGL) [75, 80-148-215]. The endocannabinoid system (ECS) is a complex cell-signaling system that helps to regulate our body functions, including sleep, mood, digestion, memory, reproduction, fertility, appetite and digestion, metabolism, chronic pain, inflammation and other immune system responses, motor control, cardiovascular system function, muscle formation, bone remodeling and growth, liver function, reproductive system function, stress, skin and nerve function [75, 80-148]. These functions all contribute to homeostasis, which refers to stability of our internal environment. For example, if an outside force, such as pain from an injury or a fever, throws off our body's homeostasis, then our endocannabinoid system (ECS) kicks in to help our body return to its ideal operation. The structure of the main psychoactive phytocannabinoid, tetrahydrocannabinol (Δ9-THC), was determined in Israel by Mechoulam and Gaoni in 1964 [75, 80-148-215]. This discovery opened the gate for many of the subsequent developments in the field of endocannabinoid system (ECS) research. Mechoulam's mile- stone discovery that Δ9 -THC (tetrahydrocannabinol) is the primary psychoactive principle, and the ensuing elucidation of the ECS, opened the gate for a new era in cannabis history [75, 80-148-215]. Both plant-derived Δ9-THC (tetrahydrocannabinol) and the first endocannabinoids were discovered in Israel by the laboratory led by Professor Raphael Mechoulam, clearly stood out as a giant of modern Cannabis science [75, 80-183-215, 229-232].

The concept of epigenetic reprogramming predicts long-term functional health effects. This reprogramming can be activated by exogenous or endogenous insults, leading to altered healthy and different disease states [75, 80-183-216]. The exogenous or endogenous changes that involve developing a roadmap of epigenetic networking, such as drug components on epigenetic imprinting and restoring epigenome patterns laid down during embryonic development, are paramount to establishing youthful cell type and health [75, 80-183-216]. This epigenetic landscape is considered as one of the hallmarks of cancer. The initiation and progression of cancer are considered to involve epigenetic abnormalities and genetic alterations. Cancer epigenetics have shown extensive reprogramming of every component of the epigenetic machinery in cancer development, including DNA methylation, histone modifications, nucleosome positioning, non-coding RNAs, and microRNA expression [75, 80-183-216]. Endocannabinoids are natural lipid molecules whose levels are regulated by specific biosynthetic and degradative enzymes. They bind to and activate two primary cannabinoid receptors, type 1 (CB1), type 2 (CB2), and together with their metabolizing enzymes, form the endocannabinoid system. The role of cannabinoid receptors CB1 and CB2 signaling in activating numerous receptor tyrosine kinases and Toll-like receptors in the induction of epigenetic landscape alterations in cancer cells, which might influence cancer metabolism and epigenetic reprogramming to a metastatic phenotype [75, 80-183-216]. Strategies applied from conception could represent an innovative epigenetic target for preventing and treating human cancer [75, 80-183-216]. The capability of GPCR agonists to positively regulate glycosylated receptor signaling in the absence of their ligands presents a role of cannabinoid receptors CB1 and CB2 signaling in activating numerous receptor tyrosine kinases and Toll-like receptors in the induction of altered epigenetic cancer cells. This process which might influence cancer metabolism and epigenetic reprogramming to a metastatic phenotype [75, 80-183-216].

Normal breast cells have receptors that respond to hormones such as estrogen and progesterone, which allows them to grow and regress in response to the hormone level [1-75]. Hormone receptors may or may not be present in breast cancer. About two-thirds of breast cancers are "positive" and contain these receptors like normal breast cells do. These are less aggressive cancers that are less likely to need chemo and are often treated with hormone therapy and surgery

[1-74]. Radiation may or may not be needed. HER2/neu (hormone epidermal growth factor receptor 2), is a protein molecule that has a role in cell proliferation in normal cells [1-74]. In some breast cancers, this protein is overly produced or "positive." For HER2-positive tumors, there a specific medication that targets this protein [1-74]. Triple-negative breast cancers are not positive for estrogen receptors, progesterone receptors or HER2 protein [1-74]. Since these targets (hormone receptors and HER2) are absent in triple-negative breast cancer, chemotherapy is needed [1-74]. Tripple-negative breast cancer is often very sensitive to chemotherapy, which, despite the side effects, is an effective treatment that can save lives. Because this is an aggressive cancer, and also treatment is aggressive[1-74].

Triple negative breast cancer (TNBC) represents an aggressive subtype of breast cancer, which is deficient in estrogen receptor (ER), progesterone receptor (PR), and human epidermal growth factor receptor 2 (HER2) expression [1-74]. Thus, TNBC cells are unable to respond to the conventional hormonal therapies, making chemotherapy the only therapeutic choice [1-74]. Patients with TNBC develop metastasis and recurrence over time and have reduced survival compared to patients with other subtypes of breast cancer [1-74]. Therefore, there is a need for innovative therapies. Data emerged from pre-clinical studies, highlighted various antitumor activities of plant-derived Cannabis sativa and synthetic cannabinoids (CBs), including Λ-9-tetrahydrocannabinol (THC) and non-psychoactive cannabidiol (CBD) [149-162-183-215]. On the contrary, some studies indicated that CBs might also promote tumor progression. At present, clinical studies are few on the effects of CBs from Cannabis sativa in cancer patients [149-170]. Among the various biological properties of phytocannabinoids, their ability to induce anti-proliferative effects in different human cancer cells raises the scientific interest in their therapeutic potential in the field of oncology. Concerning one of the most abundant non-psychoactive cannabinoids in Cannabis sativa, namely Cannabidiol (CBD), many *in vitro* and in vivo studies reported its effectiveness as an anti-cancer compound, although similar observations in humans are still missing [149-181-215]. Regarding cancer, cannabinoids were first introduced to manage chemotherapy-related side effects, though several studies demonstrated that they could modulate the proliferation and death of different cancer cells, as well as angiogenesis, making them attractive agents for cancer treatment. In relation to breast cancer, it has been suggested that estrogen receptor negative (ER-) cells are more sensitive to cannabinoids than estrogen receptor-positive (ER+) cells [149-182-215]. In fact, most of the studies regarding their effects on breast tumors have been conducted on triple-negative breast cancer (TNBC) [149-182]. Nonetheless, the number of studies on human epidermal growth factor receptor 2-positive (HER2+) and ER+ breast tumors has been rising in recent years. However, besides the optimistic results obtained thus far, there is still a long way to go to fully understand the role of these molecules [149-182]. Publications on growth inhibitory effects of cannabinoids have accumulated over the last two decades. The dominant cellular model used for this purpose in the 2000s was glioma cells. However, over time, a wide range of different tumor cell lines of various entities have been tested. Whereas early work focused on mechanisms leading to cannabinoid-induced apoptosis and cell cycle arrest of cancer cells, these investigations were complemented later by studies dealing with autophagy effects [149-183-215].

Triple-negative breast cancer (TNBC) is a heterogeneous subtype of breast cancer that displays highly aggressive with poor prognosis [1-74, 217]. Owing to the limited targets and drugs for TNBC clinical therapy, it is necessary to investigate the factors regulating cancer progression and develop novel therapies for cancer treatment [1-74, 217]. Ferroptosis, a non-apoptotic form of programmed cell death is characterized by accumulation of iron-dependent peroxidation of phospholipids, is regulated by cellular metabolism, redox homeostasis, and various cancer-related signaling pathways [1-74, 217]. Recently, considerable progress has been made in demonstrating the critical role of lipid metabolism in regulating ferroptosis, indicating potential combinational therapeutic strategies for cancer treatment [1-74, 217]. In this study, by drug combination screen of lipid metabolism compounds with ferroptosis inducers in decreasing TNBC cell viability, and found potent synergy of the CB1 antagonist rimonabant with erastin/(1 S, 3 R)-RSL3 (RSL3) in inhibiting TNBC cell growth. TNBC cell growth inhibition was observed by both *in vitro* and in vivo via promoting the levels of lipid peroxides, malondialdehyde (MDA), 4-hydroxynonenal (4-HNE) and cytosolic reactive oxygen species (ROS) production, enhancing intracellular glutathione (GSH) depletion and inducing G1 cell cycle arrest [1-74, 217]. This study identified that inhibition of CB1 promoted the effect of erastin/RSL3 on inducing ferroptosis and enhanced their inhibitory effect on tumor growth. Using RNA-Seq, fatty acid analyses and functional assays, this study found that CB1 regulated stearoyl- CoA desaturase 1 (SCD1)- and fatty acyl desaturase 2 (FADS2)-dependent fatty acid metabolism via phosphatidylinositol 3 kinase (PI3K)-AKT and mitogen-activated protein kinase (MAPK) signaling pathways to modulate ferroptosis sensitivity in TNBC cells [1-74, 217]. These data demonstrated that dual targeting of CB1 and ferroptosis could be a promising therapeutic strategy for TNBC [1-74, 217]. Ferroptosis, a non-apoptotic form of programmed cell death, is characterized by accumulation of iron-dependent peroxidation of phospholipids [1-74, 217]. Emerging evidence have manifested the pivotal role of ferroptosis in suppressing breast tumor growth by modulating various tumor properties. It has been reported that TNBC cells display a unique metabolic state of iron and GSH homeostasis, which can enhance their sensitivity to ferroptosis [1-74, 217]. Long-chain acyl-CoA synthetase 4 (ACSL4), a critical factor which links arachidonic acid (AA) and adrenic acid (AdA) to

phosphatidylethanolamine (PE) was shown to be highly expressed in basal type breast cancer cells and regulated ferroptosis sensitivity[1-74, 217].

During the last 20 years, intense research has been conducted in order to evaluate the clinical and pharmacological potential of cannabinoids, alone or in combination, for the treatment of different pathological conditions [149-183-215]. In fact, some cannabinoid-based medicines are already approved for clinical use in many countries [149-183]. Those treatments include nabiximols (Sativex®), a 1:1 mixture of THC and CBD used in multiple sclerosis, dronabinol (Marinol®) and nabilone (Cesamet®), two THC synthetic analogs used to relieve chemotherapy-related side effects, such as vomiting and nausea, and CBD oil (Epidiolex®) for the treatment of some pediatric epilepsy conditions, which was recently approved by the FDA [149-183-215]. However, there are conflicting data regarding the role in tumor development, since some studies suggest that it may be over activated, while others demonstrated that the activation of CBs reduces tumor growth, suggesting that the ECS may display tumor-suppressive actions [149-183-217]. This biphasic effect seems to be dose dependent, as low doses of cannabinoids promoted cell proliferation, while higher doses induced anti-proliferative actions [149-183]. The pioneering study of Munson et al. [163-164] and the pilot screening of Ligresti et al. [165], a detailed analysis of the data highlights controversial and divergent findings [165-166], suggesting that its therapeutic potential as an anti-cancer drug in humans is still unclear [149-183-215].

Focusing on *in vitro* studies, the effect of CBD on cancer cell viability ranges from no effect, to a modest reduction, and to significant cytotoxicity depending on concentrations, cancer cell lines, cell growth conditions, the performed assays, and the time of CBD exposure [149-183]. CBD concentrations below 3.0 μM induced a decreased cell viability in human primary lung carcinoma cells, but failed in epithelial colon adenocarcinoma cells, or myeloma cells [149-183-215]. At higher concentrations, a general reduction in cell viability was observed, although the anti-proliferative mechanisms vary from apoptosis to autophagy and cell-cycle arrest [149-162-183]. The anti-proliferative effects of phytocannabinoids were demonstrated for the first time in 1975, by Munson et al., [163-164] who showed that THC and CBN inhibit lung adenocarcinoma cell growth [149-166]. Over the years, other cannabinoids, including the endocannabinoids AEA and 2-AG and the synthetic cannabinoids WIN 55212-2 and HU-210, have also been highlighted for their antitumor actions *in vitro* and in vivo studies [149-183-215].

Analyzing the biological effects of CBD on MDA-MB-231, D'Aloia, et al., (2022)[162], have demonstrated that both CBD dosage and serum concentrations in the culture medium influence its outcomes [162]. Furthermore, light scattering studies demonstrated that serum impacts the CBD aggregation state by acting as a surfactant agent [162]. Pharmacological studies on CBD in combination with chemotherapeutic agents revealed that CBD possesses a protective action against the cytotoxic effect exerted by cisplatin on MDA-MB-231 grown in standard conditions [162]. Furthermore, in a low serum condition (0.5%), starting from a threshold concentration (5.0 μM), CBD forms aggregates, exerts cytostatic anti-proliferative outcomes, and promotes cell cycle arrest activating autophagy [162]. At doses above the threshold, CBD exerts a highly cytotoxic effect inducing bubbling cell death [162]. Finally, IGF-1 and EGF antagonize the anti-proliferative effect of CBD protecting cells from harmful consequences of CBD aggregates [162]. Therefore the study of D'Aloia, et al., (2022)[162] concluded that CBD effect is strongly associated with the physical state and concentration that reaches the treated cells, parameters not taken into account in most of the research papers [149-162]. At doses above the threshold (doses > 5 μM in a medium with 0.5% FBS), CBD exerts a highly cytotoxic effect, which determines the phenomenon of bubbling death [171-173]. This is an irreversible process of death previously described only in UV-irradiated or cold-shocked cells. In these cells, apoptosis stops, and an enlarging nuclear gas bubble containing nitric oxide begins to form from the nucleus and is released to the cell surface causing cell death[172], So, it is likely that, at these high concentrations CBD forms colloidal aggregates, such as those observed in the standard medium at a concentration of 12.5 μM by Dynamic Light Scattering from Nelson and colleagues [172], capable of perturbing the permeability and integrity of the plasma membrane, thereby determining the bubbling. Finally, apoptosis is not activated in these cells as CBD switches the cell death mechanisms to bubbling cell death [171-173-215].

The primary antitumor effects of cannabinoids rely on cell cycle arrest through the inhibition of the expression of growth factors and induction of apoptosis [149-181-215]. Additionally, they can also avoid angiogenesis and block invasion and metastasis by impairing the activation of the vascular endothelial growth factor (VEGF) pathway [149-162]. Until now, the actions of cannabinoids have been identified in different tumors, including gliomas, melanomas, lymphomas, breast cancer, skin cancer, lung carcinoma, liver cancer, pancreatic cancer, colon cancer and prostate cancer, indicating that the antitumor actions are not tumor type specific [149-167-183-215]. The assay conditions also affect CBD efficacy. Solinas et al. [168] reported that, in U87-MG and T98G glioma cells, the anti-proliferative effects of CBD were similar under hypoxic and normoxic conditions, while Macpherson et al. [169] reported that Caco-2 colon cancer cells were more sensitive to CBD under conditions of physiological oxygen in the colon than those routinely used in cell culture experiments. In the same Caco-2 cells, a serum-dependent CBD effect on cell viability was observed, with a decrease in 5% serum and an increase in 1% serum [169]. Furthermore, a recent study on HT-29 human colorectal

cancer cell line showed that CBD cytotoxic activity in cell culture media containing 10% serum is significantly reduced compared to a medium that contains only 0.5% serum, indicating an important influence of growth factors in CBD efficacy [170]. This wide pattern of results suggests that more mechanistic studies are required to clarify the therapeutic potential of CBD in cancer [149-181-215].

In fact, several clinical trials have already been conducted or are currently underway to evaluate the safety and effectiveness of cannabinoids or cannabinoid based preparations alone or combined with chemotherapy agents such as temozolomide, bortezomib, fluorouracil, oxaliplatin, leucovorin, bevacizumab, irinotecan, palonosetron and dexamethasone [149-183-215]. The results obtained thus far indicated that, generally, cannabinoids are well-tolerated compounds, without significant side effects and with a high potential for the modulation of pain and chemotherapy-related side effects [149-170]. In relation to breast cancer, data from pre-clinical studies suggest that cannabinoids may be beneficial for the treatment of the best-known breast cancer subtypes [149-170-215]. Taking into account the expression of specific molecular biomarkers, such as estrogen receptor (ER), progesterone receptor (PR) and human epidermal growth factor receptor 2 (HER2), breast cancer can be divided into four subtypes, luminal A, luminal B, human epidermal growth factor receptor 2 positive (HER2+) or triple negative (TNBC) [1-74, 149-181]. TNBC accounts for approximately 15–20% of all breast cancers, is typically high grade, presents a high proliferation index and is associated with a high rate of local and distant recurrence and, consequently, with a poor prognosis, usually being the most aggressive [1-74,149-170]. Moreover, it is characterized by the absence of ER, PR and HER2 expression. Around 80% of all TNBCs are basal-like, which means that these tumors are enriched in cells expressing characteristic genes of normal basal cells [1-74, 149-170]. Evidence obtained thus far has revealed that, as in other cancer types, the ECS is altered in breast cancer cases, being intimately associated with tumor aggressiveness [1-74149-170]. In fact, endocannabinoid concentrations and expression levels of CBs, and of the enzymes responsible endocannabinoid metabolism, are typically associated with cancer aggressiveness, reinforcing their involvement in cancer development [1-74,149-170]. It is known that CB2 is over expressed in breast cancer and that CB1 is present in significant lower quantities [149-170]. CB2 expression is mainly observed in HER2+ tumors, being detected in 90% of all $HER2^+$ tumors [149-170]. In this case, over expression of CB2 is linked to a poor prognosis [149-170]. In fact, a correlation has been established between CB2 expression and tumor aggressiveness, as mRNA CB2 levels were higher in ER-/PR- tumors than in ER+/PR+ tumors, as well as in HER2+ tumors than in $HER2^-$ tumors, and in high-grade histological tumors than in low-grade histological tumors [149-170]. On the other hand, CB2 expression on ER+ and ER-tumors is associated with a better prognosis [149-170]. In relation to endocannabinoids, in breast cancer cases, an increase in the levels of N-acylphosphatidylethanolamine, the AEA precursor, and in MAGL levels has been observed, mainly in ductal carcinomas [149-181-215].

The majority of the studies conducted on breast cancer were performed on TNBC models [149-170]. In fact, it has been established that the sensitivity of human breast cancer cell cultures to cannabinoids is correlated with their aggressiveness, being ER^- cell lines more sensitive than ER+ cells [149-170]. CBD is the most studied phytocannabinoid in TNBC [149-170-215]. It has been reported that this phytocannabinoid reduces the proliferation of MDA-MB-231 cells through the direct activation of TRPV1 receptors and possibly through other yet uncharacterized CBD targets [149-170]. However, it was also proposed that CBD induces apoptosis in this cell model through the involvement of CB1, CB2 and TRPV1 receptors [149-170-215]. This effect is mediated by endoplasmic reticulum stress and inhibition of the AKT/mTOR pathway, which ultimately culminates in autophagy and mitochondria-driven apoptosis [149-170]. In fact, this mechanism was already identified in other models, suggesting that this is a general mechanism of action. The induction of apoptosis by CBD in MDA-MB-231 cells was also recently verified by another study, where CBD inhibited cell survival and induced apoptosis, favored by an interplay among PPAR, mTOR and cyclin D1[149-170]. In addition, CBD also impairs the metastatic potential of MDA-MB-231 and 4T1 cells, probably by downregulation of Id-1, which is mediated by ERK[149-170-183-215].

In contrast, regarding the MDA-MB-231 cell line, a study showed that THC promotes the proliferation of these cells [149-181]. Furthermore, another study demonstrated anti-proliferative as well as anti-migratory and anti-invasive properties of CBD through the inhibition of EGF/EGFR signaling in several TNBC cell lines, including SUM159, 4T1.2 and SPC2 cells [149-181-215]. In addition, CBD effects on cell proliferation were also verified in xenografts generated from MDA-MB-231 cells in immunedeficient mice and in orthotopic xenografts generated from 4T1 cells in syngeneic BALB/c mice [149-181-215]. In both cases, CBD reduced tumor growth, but for the latter, acquired resistance to CBD was developed. In fact, ROS are known to play a key role in the breast cancer microenvironment, promoting the differentiation of neighboring cells, including fibroblasts that secrete growth factors, cytokines and metalloproteinases, leading to tumor development and growth [149-181-217]. Interestingly, the dependence on ROS levels seems to be particularly high in TNBC. Considering this, several antioxidant and mitochondria-targeted therapies have been suggested for breast cancer. The minor phytocannabinoids CBN and CBG also showed interesting results in TNBC. In

MCD-MB-231 and MDA-MB436 cells, both compounds reduced cell viability and cell migration through a process probably involving a decrease in Id-1 expression [149-181-183-215].

Combinations of minor and major cannabinoids have also been evaluated in TNBC. Besides the actions of the phytocannabinoids, synthetic cannabinoids have also demonstrated interesting effects on TNBC [149-181-215]. In two in vivo models, a xenograft-based and a PyMT genetically engineered model of TNBC, the synthetic cannabinoid JWH-133 provoked a significant reduction in tumor growth and inhibited angiogenesis [149-181]. In fact, anti-migration effects were induced by several synthetic cannabinoids, Met-F-AEA, WIN 55,212-2, JWH-133 and JWH-015, in MDA-MB-231 cells [149-181]. These effects were mediated by CB1, with inhibition of the focal adhesion kinase (FAK)/Src and RhoA-ROCK pathways, and by CB2, along with COX-2/PGE2 axis inhibition or through inhibition of the ERK pathway and cytoskeletal adhesion and stress fiber formation [149-181]. Met-F-AEA, WIN 55,212-2 and JWH-133 also induce cell cycle arrest. These studies suggest that the interaction of synthetic cannabinoids with CBs reduces the metastatic potential of TNBCs, a behavior already identified for the formation of lung metastasis [149-181-219]. Therefore, in vivo and *in vitro* studies have shown that cannabinoids exert anti-proliferative and anti-metastatic actions on TNBC models, mainly by the induction of apoptosis and autophagy via CB activation, as well as modulation of several signaling pathways involved in cell proliferation, such as AKT/mTOR and EGF/EGFR[149-181-215-219]. In relation to HER2+ breast cancer, over expression of CB2 has been verified, which is associated with a poor prognosis [149-181]. Moreover, in HER2+ breast cancer models, the antitumor effects are mediated by CB2 activation, which is the reason why CB2-directed therapy should be effective in growth inhibition of these tumors [149-181]. Therefore, cannabinoids represent an attractive potential therapy for this subtype of breast cancer [149-181]. Until now, the most prominent study regarding HER2+ breast cancer and cannabinoids was conducted in MMTV-neu mice, a good model for the study of HER2+ tumors [149-181]. In this study, it was revealed that THC impaired tumor growth, angiogenesis and the formation of metastasis through the induction of apoptotic cell death and inhibition of AKT, effects that were reproduced with JWH-133, a selective CB2 agonist [149-181-215].

A very recent in silico study demonstrated that THC analogs possess the ability to bind to ERβ and, probably, lead to its activation [149-181]. This may represent an important advantage for ER+ breast cancer treatment, since this receptor is associated with antitumor effects [149-181]. In fact, it has already been verified that THC prevents estradiol-induced proliferation in MCF-7 cells, in an ERα-independent manner [149-181-215]. Moreover, AEA and THC caused apoptotic cell death, while CBD induced autophagy to promote apoptosis [149-181]. Additionally, combinations of major and minor cannabinoids evaluated on MCF-7 cells revealed pro-apoptotic effects [149-181]. Therefore, in ER^+ breast cancer, cannabinoids also have the potential to inhibit cell growth and avoid metastatic development [149-181]. These effects are typically associated with cell cycle arrest, apoptosis and autophagy, through the involvement of CB1 and CB2 and the modulation of survival pathways, such as the mTOR and Raf-1/ERK/MAPK pathways [149-181-215].

Data from experimental studies highlight the antitumor effects of phytocannabinoids in TNBC through the regulation of different molecular pathways. TNBC cells are more aggressive and thus more sensitive to CBD treatment [149-181-215]. Most of these studies were conducted by using CBD. Ligresti *et al.* (55) and Bisogno *et al.* (56), demonstrated that CBD inhibited the proliferation of MDAMB- 231 cells, by inducing apoptosis *via* two possible mechanisms of action: i) the activation (direct or indirect) of vanilloid transient receptor potential vanilloid type-1 (TRPV1) and the involvement of CB2-R, ii) the increase in intracellular Ca2+ levels and ROS caused by unknown CBD targets [149-181]. Similar findings were described by Shrivastava *et al*. [149-181]. They demonstrated that CBD causes the death of TNBC cancer cells by inhibiting protein kinase B (Akt) and mechanistic target of rapamycin (mTOR) signaling, inducing endoplasmic reticulum (ER) stress, and promoting ROS generation [149-181]. Moreover, CBD mediates a balance between mitochondria-mediated apoptosis and autophagy in MDAMB- 231 breast cancer cells. Sultan *et al.* (58), demonstrated that CBD treatment stimulates an interaction between mTOR, PPARγ, and cyclin D1, thus promoting MDA-MB-231 cell apoptosis [149-183]. CBD modulates the tumor microenvironment (*e.g*., reduced proliferation, invasion, and impaired cell migration), by inhibiting the epidermal growth factor (EGF)/epidermal growth factor receptor (EGFR) signaling in several TNBC cell lines (4T1.2, SUM159, and SPC2) [149-181]. Specifically, CBD inhibits EGF-induced activation of nuclear factor kappa-lightchain- enhancer of activated B cells (NF-kB), that possesses a tumor pro-survival effect causing resistance to chemotherapy [149-181]. Furthermore, the authors demonstrated that CBD arrests matrix metalloproteinases (MMPs) secretion, thus inhibiting the effects of EGF induced on the cytoskeleton [149-181]. McAllister *et al*., in two distinct studies [149-181], highlighted an inhibitory role of CBD in TNBC proliferation by interfering with cell cycle progression and by promoting apoptosis [149-181]. Pre-clinical studies were also performed with minor phytocannabinoids, CBN, CBG, and CBC highlighting interesting results in breast cancer cells [149-181]. In MDA-MB-231 and MDA-MB-468 cells, CNB and CBG inhibited cell proliferation and reduced the invasiveness of cells, probably by reducing ID-1 expression [149-181]. Moreover, CBG and CBC inhibited MDA-MB-231 cell growth by activating CB2-R [149-181]. Altogether, the preclinical studies highlighted the antitumor effects of phytocannabinoids on TNBC models, by regulating several signaling pathways involved in cell proliferation and by enhancing apoptosis

and autophagy through the actions on CB-R. Further studies will be necessary to unravel the molecular mechanisms underlying the antitumor effects of phytocannabinoids [149-181-215].

Moreover, clinical studies demonstrated that dronabinol (Marinol®) and nabilone (Cesamet®), two THC synthetic analogs, are less effective in relieving cancer-related but overcome the side effects (*e.g*., nausea, vomit) induced by chemotherapy [149-181]. Important clinical aspects concerning chemotherapy have been reported on chemotherapy with paclitaxel and doxorubicin treatment in breast cancer and on the inhibition of paclitaxel-induced neuropathic pain by cannabidiol [149-181-215].

Pre-clinical studies proved shreds of evidence that CBD and THC, possess antitumor and anti-inflammatory activities on TNBC, by acting on specific molecular signaling pathways *via* action through CB-R (highly expressed in breast cancer cells) [149-181]. Therefore, phytocannabinoids can be viewed as promising agents for inhibiting TNBC progression, which has scarce therapeutic options and is featured by inauspicious prognosis and low survival rates [149-181]. Some preclinical studies, mainly conducted with THC in TNBC, showed that phytocannabinoids can also act as pro-tumoral agents [149-181-215]. This dual behavior depends on the CBs concentration tested, since lower concentrations promote cancer cell proliferation, while higher concentrations inhibit cancer cell growth thus enhancing cancer cell death [149-181]. Thus, this issue should be considered to develop therapies with satisfactory results. Despite the abundant pre-clinical studies, few clinical trials on the antitumor roles of CBs have been conducted in patients with cancer, probably due to the underlying molecular mechanism not yet known [149-181-215]. Some clinical studies have been performed to evaluate the safety and the effectiveness of CBs in several cancer types, including breast cancer, but more additional studies would be necessary to understand the potential of these molecules in TNBC and other breast cancer subtypes [149-181]. Overall, apart from the need for other studies aimed to dissect the molecular pathways underlying the antitumor CBs' properties, phytocannabinoids should be considered as potential agents for inhibiting TNBC progression [149-181-215].

Although phycannabinoids have been used for centuries for diverse pathological conditions, recently, their clinical interest and application have emerged due to their diverse pharmacological properties [149-181-215]. Indeed, it is well established that phycannabinoids exert important actions on multiple sclerosis, epilepsy and pain relief. Regarding cancer, phycannabinoids were first introduced to manage chemotherapy-related side effects, though several studies demonstrated that they could modulate the proliferation and death of different cancer cells, as well as angiogenesis, making them attractive agents for cancer treatment [149-181-215]. In relation to breast cancer, it has been suggested that estrogen receptor negative (ER^-) cells are more sensitive to cannabinoids than estrogen receptor-positive (ER+) cells [149-181]. In fact, most of the studies regarding their effects on breast tumors have been conducted on triple-negative breast cancer (TNBC) [149-181]. Nonetheless, the number of studies on human epidermal growth factor receptor 2-positive (HER2+) and ER+ breast tumors has been rising in recent years. However, besides the optimistic results obtained thus far, there is still a long way to go to fully understand the role of these molecules [149-181-215].

Cannabinoids exhibit anti-inflammatory and anti-tumorigenic properties. Contrary to most cannabinoids present in the *Cannabis* plant, some, such as O-1602 and abnormal cannabidiol, have no or only little affinity to the CB1 or CB2 cannabinoid receptors and instead exert their effects through other receptors [149- 183-215]. Several studies have shown the effects of cannabinoids on chemotherapy-sensitive breast cancer cell lines, but less is known about the anti-tumorigenic effects of cannabinoids in chemotherapy-resistant cell lines [149- 183]. Paclitaxel-resistant MDA-MB-231 and MCF-7 breast cancer cell lines were used to study the effect of O-1602 and abnormal cannabidiol on viability, apoptosis, and migration [149- 183-215]. The effects of O-1602 and abnormal cannabidiol on cell viability were completely blocked by the combination of GPR55 and GPR18-specific siRNAs [149- 183]. Both O-1602 and abnormal cannabidiol decreased viability in paclitaxel-resistant breast cancer cells in a concentration-dependent manner through induction of apoptosis [149- 183]. The effect of these cannabinoids on tumor growth *in vivo* was studied in a zebrafish xenograft model [149- 183]. In this model, treatment with O-1602 and abnormal cannabidiol (2 μM) significantly reduced tumor growth [149-183]. The experimental results of Tomko et al., (2019) [1183], suggested that a typical cannabinoids, like O-1602 and abnormal cannabidiol, exert anti-tumorigenic effects on paclitaxel-resistant breast cancer cells [149- 183-215]. Due to their lack of central sedation and psychoactive effects, these a typical cannabinoids could represent new leads for the development of additional anticancer treatments when resistance to conventional chemotherapy occurs during the treatment of breast and possibly other cancers [149- 183-215]. It is well documented that cannabinoids produce anti-tumorigenic responses in preclinical models of breast cancer. Cannabinoids such as Δ9-tetrahydrocannabinol can have an effect on cancer progression, cell proliferation, survival, angiogenesis, and metastasis [149-183]. While breast cancer mortality rates have declined because of improved therapies and early diagnosis, metastatic breast cancer (the primary cause of breast cancer mortality) is expected to develop in 20% to 30% of women with early breast cancer and remains incurable with a median 5-year survival of 25% [149- 183-215].

Although estrogen receptor (ER)– and progesterone receptor (PR)–positive (ER+/PR+) breast cancers are associated with a higher response rate to current therapies, innate and acquired resistance can occur, which represent a significant treatment challenge due to the likelihood of cancer recurrence and dissemination to other organs [149- 183-215]. Similarly, resistance can also be observed in other types of breast cancers, like the HER2+ subset, and triple-negative breast cancers. Triple-negative breast cancer shows the worst prognosis with aggressive proliferation, migration, and invasion abilities [149- 183]. Although some patients with this subtype respond well to chemotherapy, many others do not respond. While metastatic breast cancer often responds well to initial treatments, the eventual development of multidrug resistance is expected and presents a major challenge for effective long-term treatment [149- 183]. The endocannabinoid anandamide and other cannabinoids such as the isomer of cannabidiol (CBD) known as "abnormal" CBD, and a related compound, O-1602, have been shown to act as agonists of GPR18 and GPR55 receptors[149- 183]. It is possible that these drugs, just like other compounds activating these receptors, may mediate different effects. Increasing evidence demonstrated that ligands acting through these receptors play an important role in the progression of many cancer types [149- 183]. While it has been shown by other groups that different cannabinoids can reduce the viability of ER+/PR+ or triple-negative breast cancer cells, it has not been demonstrated that cannabinoids could be useful once these cells become resistant to chemotherapeutics [149- 183].

Preclinical models provided ample evidence that cannabinoids are cytotoxic against cancer cells [149- 183]. Among the best studied phytocannabinoids, cannabidiol (CBD) is most promising for the treatment of cancer as it lacks the psychotomimetic properties of delta-9-tetrahydrocannabinol (THC) [149- 183-215]. *In vitro* studies and animal experiments point to a concentration- (dose-) dependent anticancer effect. The effectiveness of pure compounds versus extracts is the subject of an ongoing debate [149- 183]. Actual results demonstrated that CBD-rich hemp extracts must be distinguished from THC-rich cannabis preparations [149- 183-215]. Whereas pure CBD was superior to CBD-rich extracts in most *in vitro* experiments, the opposite was observed for pure THC and THC-rich extracts, although exceptions were noted [149- 183-215]. The cytotoxic effects of CBD, THC and extracts seem to depend not only on the nature of cannabinoids and the presence of other phytochemicals but also largely on the nature of cell lines and test conditions [149- 183-215]. Neither CBD nor THC are universally efficacious in reducing cancer cell viability. The combination of pure cannabinoids may have advantages over single agents, although the optimal ratio seems to depend on the nature of cancer cells, the existence of a 'one size fits all' ratio is very unlikely [149- 183-215]. As cannabinoids interfere with the endocannabinoid system (ECS), a better understanding of the circadian rhythmicity of the ECS, particularly endocannabinoids and receptors, as well as of the rhythmicity of biological processes related to the growth of cancer cells. This could enhance the efficacy of a therapy with cannabinoids by optimization of the timing of the administration, as has already been reported for some of the canonical chemotherapeutics[149- 183-215]. Theoretically, a CBD dose administered at noon could increase the peak of anandamide and therefore, the effects triggered by this agent [149- 183-219]. Despite the abundance of preclinical articles published over the last 2 decades, well-designed controlled clinical trials on CBD in cancer are still missing [149- 183-215]. The number of observations in cancer patients, paired with the anticancer activity repeatedly reported in preclinical *in vitro* and in vivo studies warrants serious scientific exploration moving forward [149- 183-219]. CBD in glioblastoma and other human carcinoma cell cultures has been repeatedly reported whereby the 'apoptotic threshold' likely varies, not only between different cancer cell types but also between cannabinoids [149- 183]. Furthermore, in a few experiments and in a few animals, complete eradication of tumor cells has been observed with CBD, as well as with THC [149- 183-215]. However, it is worth noting that there are distinct differences between animal experiments and patients. Tumors in animals are artificial, and in humans they are anti-inflammatory processes [149- 183-215]. One study found 1204 gene transcripts that were significantly up- or down regulated by CBD, many of them being directly involved in Zn homeostasis, whereas only 94 gene transcripts were regulated by THC [149- 183-215]. CBD also interacts with a wide range of transient receptor potential ion channels known to play a role in carcinogenesis, such as TRPA1, TRPM8, TRPV1, TRPV2, TRPV3, TRPV4, as well as the voltage-dependent anion channel VDAC1, the peroxisome proliferator-activated receptor-γ (PPARγ) and the G-protein coupled 'orphan' receptor, with GPR55 being the most relevant [149- 183-215].

CBD acts as an agonist of PPARγ, but is an antagonist of GPR55. Activation of PPARγ suppresses nuclear factor-κB (NF-κB), which is constitutively active in cancer cells and is responsible for cancer cell proliferation and the formation of inflammatory cytokines such as tumor necrosis factor (TNF)-α [149- 183-215]. In addition, overall effects are further influenced by endocannabinoids (anandamide, 2-arachidonoylglycerol), which demonstrated anticancer activities of their own [149- 183-215]. Anandamide and 2-arachidonoylglycerol bind to both receptors (CB1 and CB2; anandamide more to CB1 than CB2, and 2-arachidonoylglycerol more to CB2 than CB1) [149- 183]. Furthermore, CBD is able to interfere with fatty acid amide hydrolase (FAAH) and to increase blood levels of anandamide. CBD also inhibits, concentration dependently, ID-1, which controls cancer invasiveness and metastasis [149- 183-215]. On a molecular basis, CBD is also able to induce programmed cell death by autophagy as well as by apoptosis. Compared with healthy tissues, the expression of cannabinoid receptors is altered in tumors, and the effects of cannabinoids therefore, vary among cancer cell lines [149- 183-215].

A major goal in cancer therapy is to kill malignant cells via the natural process of apoptosis, avoiding eventual ecyclation' of cancer cells by autophagy [149- 183-215]. In addition to tumor-selective action of agents, this requires intact immune competence of the host. In diseased subjects, the ECS is dysregulated [149- 183-215]. As an example, although the results were somewhat conflicting, anandamide levels seemed to be lower and 2-arachidonoylglycerol levels were up-regulated in glioblastomas [149- 183]. Glioma invasiveness has been linked to the tumor suppressor p38 MAPK; the anti-invasive effect of CBD interferes with this pathway [149- 183-215]. Increased expression and activity of p38 MAPK correlates with poor prognosis in GBM. Intriguingly, the levels of phosphorylated p38 MAPK are significantly reduced in clock-deficient glioma cells, indicating that the circadian clock plays an important role in the activation of this pathway [149- 183]. Other big players on inflammation and cell survival, such as PPARγ, also exhibit circadian expression [149- 183-215].

In vitro, a dose-dependent increase in the apoptotic effects of CBD in glioblastoma and other human carcinoma cell cultures has been repeatedly reported whereby the 'apoptotic threshold' likely varies, not only between different cancer cell types but also between cannabinoids [149- 183-215]. Furthermore, in a few experiments and in a few animals, complete eradication of tumor cells has been observed with CBD as well as with THC [149- 183]. However, it is worth noting that there are distinct differences between animal experiments and patients [149- 183-215]. Tumors in animals are artificial, whereas they are spontaneous in man [149- 183]. Cannabinoids have been administered during the day, which corresponds to the rest/sleep phase in mice and rats. Products are most often injected once daily in animals, resulting in 100% bioavailability, and the frequency of applications varied between two and seven times per week [149-183]. In contrast, oral bioavailability in man is low and administration is usually on a continuous, twice-daily basis [149-183-215]. Patients referred to in the studies by Likar et al., as an example, have been advised to take CBD daily after breakfast and after the evening meal [149- 183]. In any case, the problem of translatability from preclinical results to therapy in man remains. Therefore, there are considerable physiological differences between humans and animals that impact drug effects [149- 183-215].

There is mounting evidence from preclinical studies that phytocannabinoids are effective against cancer cells [149- 183-215]. An increasing number of *in vitro* studies have described not only the cytotoxic effects of cannabinoids against numerous cancer cell lines but also putative mechanisms that finally lead to an inhibition of metastasization, angiogenesis, tumor growth, enhancement of autophagy, and, ultimately, to apoptosis of cancer cells [149- 183]. Observations in man support the idea of a potential life extension of cancer patients. Nonetheless, there is currently no medical proof of a long-term cancer cure in man [149- 183-215]. Although sensitivities vary between cell lines and vary in the dependence of the nature of the cannabinoid, most articles report dose- (or concentration-) dependent effects [149- 183]. In general, CBD has demonstrated a favorable overall efficacy and safety profile, with a potency that has exceeded, in many *in vitro* tests, that of its psycho-tomimetic counterpart THC and of extracts [149- 183-215]. Combinations of cannabinoids have been reported to increase potency further, although the optimal also been observed for the expression of CB1 and CB2 receptors in liver tissue of rats, with the highest amount of RNA during the resting (light) phase [149- 183-215]. Circadian rhythmicity can be disrupted by a number of conditions, such as obesity, after intensive exercise, after sleep restriction, and in tumor cells where the circadian clock may even be suppressed [149-183-215].

Drugs that target the endocannabinoid system are of interest as pharmacological options to combat cancer and to improve the life quality of cancer patients. From this perspective, cannabinoid compounds have been successfully tested as a systemic therapeutic option in a number of preclinical models over the past decades [149-181-215]. As a result of these efforts, a large body of data suggests that the anticancer effects of cannabinoids are exerted at multiple levels of tumor progression via different signal transduction mechanisms[149-181-215]. Accordingly, there is considerable evidence for cannabinoid-mediated inhibition of tumor cell proliferation, tumor invasion and metastasis, angiogenesis and chemoresistance, as well as induction of apoptosis and autophagy. Further studies showed that cannabinoids could be potential combination partners for established chemotherapeutic agents or other therapeutic interventions in cancer treatment [149-181-215]. Research in recent years has yielded several compounds that exert promising effects on tumor cells and tissues in addition to the psychoactive Δ9-tetrahydrocannabinol, such as the non-psychoactive phytocannabinoid cannabidiol and inhibitors of endocannabinoid degradation [149-181-215].

Phytocannabinoids have been used for centuries in several therapeutic applications. Regarding cancer, the use of phytocannabinoids has already been approved in several countries for the relief of chemotherapy-associated effects, but their clinical potential is greater than initially thought, and their clinical interest has been rising in recent years [149-181-215]. Pre-clinical studies have demonstrated that phytocannabinoids exert important antitumor properties in the main breast cancer subtypes, particularly in TNBC, where different phytocannabinoids and synthetic cannabinoids have shown interesting therapeutic actions [149-183-215]. Therefore, more studies must be conducted, mainly on HER2+, luminal A and luminal B breast tumors, in order to better understand the mechanism of action of

these compounds, which would help to clarify the therapeutic potential in breast cancer subtypes, and even in other cancer types [149-183-215]. However, regarding luminal A breast cancer, some works have indicated that phytocannabinoids modulate key targets responsible for the survival and development of this breast cancer subtype, which are targeted by the therapies currently under clinical use [149-181]. Moreover, as mentioned, despite the fact that most of the studies have attributed antitumor actions to phytocannabinoids, some have demonstrated that they can also exert pro-tumor effects [149-181-215]. This biphasic behavior is correlated with the phytocannabinoid concentration used, since lower concentrations seem to induce cell proliferation and survival, while higher doses are associated with cell death and inhibition of cell growth [149-181-215]. Therefore, it is important to keep that in mind in order to develop better therapies able to exert the desired effects [149-183-215].

Despite this, most of the mechanisms of action induced by the phytocannabinoids appear to be common among the different breast cancer subtypes [149-183]. Regarding clinical trials, those developed thus far are focused on the safety and effectiveness of cannabinoids in several cancer types, and, currently, there are no clinical trials focused only on breast cancer, what would be an asset to better understand the potential of these molecules in this type of cancer[149-183-215]. Thus, it is possible to conclude that, despite the need for more studies focused on breast cancer, phytocannabinoids are promising therapeutic agents for the different breast cancer subtypes, being able to exert important actions on cell survival and metastasis [149-183]. In addition to the combination of cannabinoids, their dose timing and dosage schedule possibly influence the results [149-215]. Two *in vitro* studies suggest that an intermittent dosage of CBD could decrease the viability of cancer cell lines better than continuous dosing [149-183]. Until now, only one publication has also described the application of such a 'pulse dosing' regimen in man [149-183-215]. Remarkably, low daily doses of CBD have been administered according to a '3 days on, 3 days off' schedule and were considered to be successful [149-183-215].

The property of cannabinoids, in particular, to induce inhibition of tumor growth and spread at multiple levels of tumor progression argues for the use of these substances as an add-on option in tumor treatment. However, it should also be noted that research into the efficacy, dosage and drug safety of cannabinoids in tumor therapy still has a long way to go, especially with regard to human clinical trials to be conducted, through which alone the benefits and advantages for cancer patients but also possible risks can be defined [149-183-215].

17. Conclusion

Triple-negative breast cancer is a kind of breast cancer that does not have any of the receptors that are commonly found in breast cancer. Think of cancer cells as a house. The front door may have three kinds of locks, called *receptors*— One is for the female hormone estrogen. second one is for the female hormone progesterone. Third one is a protein called human epidermal growth factor (HER2). If patient cancer has any of these three locks, doctors have a few keys (like hormone therapy or other drugs) they can use to help to destroy the cancer cells. But if patient have triple-negative breast cancer, it means those three locks are not there. So doctors have fewer keys for treatment. Fortunately, chemotherapy is still an effective option. Often, patients first need to have the lump removed (a *lumpectomy*) or the entire breast removed (a *mastectomy*). Then they have chemotherapy treatments to target any cancer cells that can not be seen—cells remaining in the breast or that may have spread into other parts of the body. Sometimes doctors recommend chemotherapy before surgery to shrink the cancer. With lumpectomy, a surgeon removes the lump from patient breast. He or she also removes nearby lymph nodes (the little oval-shaped organs that are part of your immune system) to see if the cancer has spread. The surgery takes an hour or two. Most women spend the day at the hospital and usually do not need to stay overnight. For a mastectomy, the surgeon removes the breast and nearby lymph nodes to see if the cancer has spread. Some women choose to have breast reconstruction during the same surgery. Lumpectomies are usually followed by radiation therapy. This is where high-energy radiation is given to patient breast to kill any remaining cancer cells. It usually takes about 20 minutes per day. Most women go in four to five days a week for about six weeks. Cells from the cancer may have spread somewhere else in patients body. The goal of chemotherapy is to kill those cancer cells. Chemotherapy lowers the chance that patient cancer will grow or come back.

Triple negative breast cancer (TNBC) represents an aggressive subtype of breast cancer, considering its high proliferation index subtype of breast cancer, considering its high proliferation index and high rate of local and distant recurrence. Moreover, since TNBC tumors are negative for estrogen receptor (ER), progesterone receptor (PR) or human epidermal growth factor receptor 2 (HER2), they cannot be treated with targeted therapies, such as anti-hormonal or anti-HER2 drugs. Conventional therapies, such as neo-adjuvant chemotherapy, represent the only therapeutic choice. However, there is a high percentage of treated patients, which develop metastases, short recurrence after treatment, and a lower survival rate. PD-L1 positive TNBC. Thus, a new schedule for TNBC treatment is required. Particularly, the FDA has already approved the use of CB-based medicines tested in these studies, for clinical application in some countries. For instance, two Λ-9-tetrahydrocannabinol (THC) synthetic analogs, nabilone (Cesamet®), and

dronabinol (Marinol®), are officially used to overcome the undesirable effects (*e.g*., nausea, vomit) induced by chemotherapy. Nabiximols (Sativex®), a 1:1 mixture of THC and cannabidiol (CBD), is used in multiple sclerosis treatment (17), while CBD oil (Epidiolex®) is administered in pediatric patients suffering from epilepsy.

Moreover, different types of CBs, have been tested for cancer treatments. Some studies demonstrated that CBs, particularly endocannabinoids (ECs), possess a dual behavior on tumor proliferation, which is strictly related to the concentration used. The main cannabinoids are represented by phytocannabinoids, of which the most studied are the THC, with psychotropic activity, and the non-psychoactive CBD. CBD and THC are formed by the decarboxylation at high temperatures, of their acidic precursors, respectively, cannabidiolic acid (CBDA) and Δ9-tetrahydrocannabinolic acid (THCA). Other minor phytocannabinoids with relevant pharmacological features are cannabigerol (CBG), cannabinol (CBN), and cannabidivarin (CBDV).

Pre-clinical studies have demonstrated that phytocannabinoids exert important antitumor properties in the main breast cancer subtypes, particularly in TNBC. On the hand where different phytocannabinoids and synthetic cannabinoids have shown interesting therapeutic actions. However, it is worth noting that there are distinct differences between animal experiments and patients [149- 183-215]. Tumors in animals are artificial, whereas they are spontaneous in man. Tumors in animals are artificial, whereas they are anti-inflammatory processes. The number of observations in cancer patients, paired with the anticancer activity repeatedly reported in preclinical *in vitro* and in vivo studies warrants serious scientific exploration moving forward. Focusing on *in vitro* studies, the effect of CBD on cancer cell viability ranges from no effect, to a modest reduction, and to significant cytotoxicity depending on concentrations, cancer cell lines, cell growth conditions, the performed assays, and the time of CBD exposure. Despite the abundance of preclinical articles published over the last 2 decades, well-designed controlled clinical trials on CBD in cancer are still missing. However, besides the optimistic results obtained thus far, there is still a long way to go to fully understand the role of these molecules. Furthermore, it should also be noted that research into the efficacy, dosage and drug safety of cannabinoids in tumor therapy still has a long way to go, especially with regard to human clinical trials to be conducted, through which alone the benefits and advantages for cancer patients but also possible risks can be defined.

Compliance with ethical standards

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No conflict of interest to be disclosed.

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