INTRODUCTION

Chapter 1

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1.1 Cancer: The global burden

Cancer cases are on an alarming upsurge, especially in low-income and middle-income countries due to lack of resources for early detection and adequate treatment or due to the disparity in nutrition, lifestyle together with several environmental risk factors. The global cancer surveillance program reported 19.3 million new cancer cases and almost 10.0 million cancer deaths in 2020 out of which female incidences accounts for 9.2 million new cases and 4.4 million deaths. Globally the cancer burden is expected to be 28.4 million cases by 2040 (Sung et al., 2021). Recent studies project an increase in cancer burden to 29.8 million in India by the year 2025 (Kulothungan et al., 2022). Previous cancer studies from India (Mallath et al., 2014) indicates that the poor survival rate in patients (less than 5 years post diagnosis), contributes to the cancer mortality rate which is 68 percent of the total annual incidence. Therefore, to curb the cancer mortality, both, the decreased cancer incidences and increased cancer survival are quintessential. The foremost challenges, accounting to more than 90% of cancer deaths are resistance to the drug and its metastatic characteristics (Harbeck & Gnant, 2017). Despite extensive efforts being made to find the exact molecular mechanisms, the physiological conditions leading to the origin, metastasis and relapse of cancers is not understood.

Breast cancer is the topmost malignancy found in women (Harbeck et al., 2019) which is also the most common cause of cancer-related deaths in women worldwide (Hutchinson, 2010). In 2020, irrespective of gender, it accounted for roughly 12% of all malignancies highlighting a major global healthcare issue (data, 2022). Regardless of the economic status of a country, breast cancer is a burden that needs to be addressed on a war footing with an aim for better prevention, diagnosis and treatment.

1.1.1 Breast cancer statistics worldwide:

Global cancer data reveals that female breast cancer is most diagnosed cancer amongst both the sexes, with an estimate of 2.3 million new cases (11.7%), followed by lung (11.4%), colorectal (10.0 %), prostate (7.3%), and stomach (5.6%) cancers. Globocan 2020 reports that breast cancer accounts for 24.5% out of 9.2 million newly diagnosed cases with 15.5% out of 4.4 million cancer deaths. Breast cancer accounts for 1 in 4 cases and 1 in 6 deaths in women globally and ranks first for cases and deaths in most of the countries (Sung et al., 2021). Despite progress in medicine, about half-a-million women still succumb to breast cancer each year.

It is estimated that 287,850 new cases of invasive breast cancer will be diagnosed among women, as well as an estimated 51,400 additional cases of in situ breast cancer will be diagnosed leading to 43,780 deaths in US (socity, 2022). European countries (9.7% global population) represent 22.8% of the total cancer cases, and 19.6 % of cancer deaths. In contrast, African countries (17.2 % of the world population) represented 5.7% of the total new cases and 7.2% mortality. Although America represents 13.1 % of the world population, it accounts for 20.9% of new incidences and 14.2% mortality. Women living in transitioning countries (15.0 per 100,000) have 17% higher mortality rates compared with women in transitioned countries (12.8 per 100,000) (Sung et al., 2021). A recent report, suggested that among the Asian countries, which represents 59.5% of the global population, cancer incidences accounted for 49.3% of incidences, 58.3% of deaths (J. Huang et al., 2022). China had an ASIR of 39.1/100,000 of the population, and the number of new cases was 416,371 and number of deaths was 164,959, accounting for 32% of the total number of deaths due to female breast cancer ranking first amongst all countries. Israel topped age-standardized incidence rate (ASIR) (78.3/100,000), followed by Singapore (77.9/100,000), and Bhutan was at bottom with ASIR of 5/100,000 of the population worldwide. The age-standardized mortality rate (ASMR) in Singapore was 19.8/100,000 and was lowest in Bangladesh (2.6/100,000) (Luo et al., 2021). According to (Deepa, Venghateri, Khajanchi, Gadgil, & Roy, 2020), in developing countries like India, multicentre population-based data for the incidence and risk factors of various malignancies along with their geographical variations is negligible.

1.1.2. Breast cancer prevalence in India

The incidence rates of breast cancer (BC) are increasing, surpassing all the other cancers in developing countries (Torre et al., 2015). As per the Globocan data 2020, in India, BC accounted for 13.5% (178361) of all cancer cases and 10.6% (90408) of all deaths (Figure 1.1) with a cumulative risk of 2.81 (Sung et al., 2021).

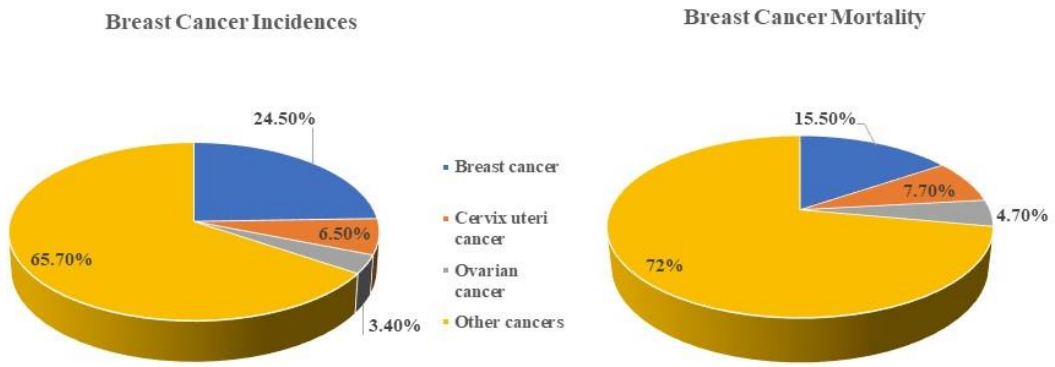

Figure 1.1: Distribution of major cancer incidences and major cancer mortality found amongst women. Breast cancer tops in incidences and mortality both, compared to cervical and ovarian cancer.

In India, metropolitan cities report increased numbers of breast cancer cases, while cervix cancer cases were on decline. Population based cancer registry (NCRP 2012-14) showed that breast cancer (19 PBCRs) and cervix uterine (7 PBCRs) as the most frequent neoplasm in women (Mathur et al., 2020). 5-year age distribution trends among different registries in India reported that the peak relative proportion in different registries was between 45 and 49 years whereas with north eastern registries the peak was seen in age group 35–39 (NCRP 2010–2012).

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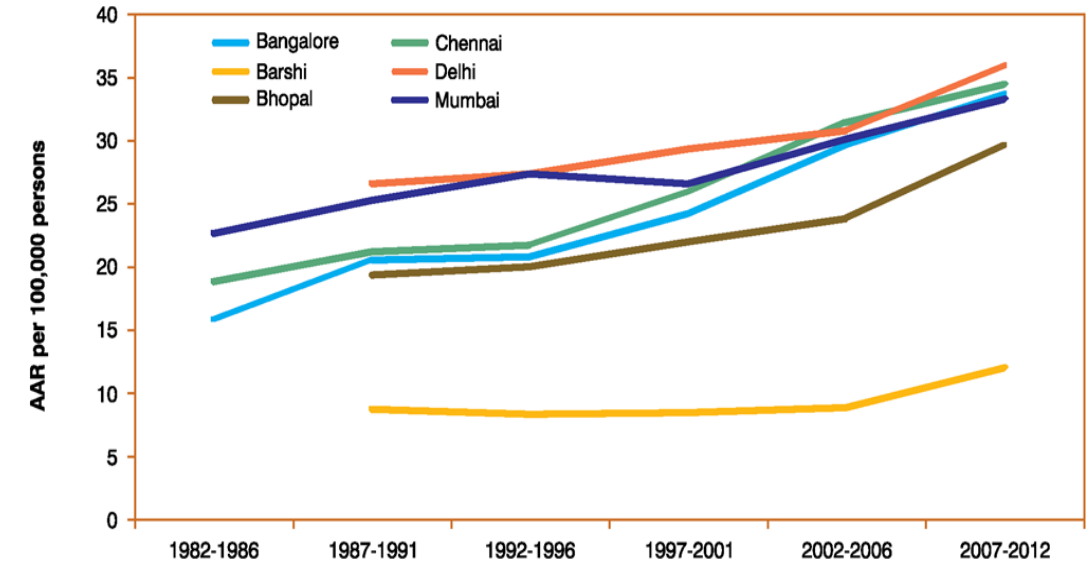

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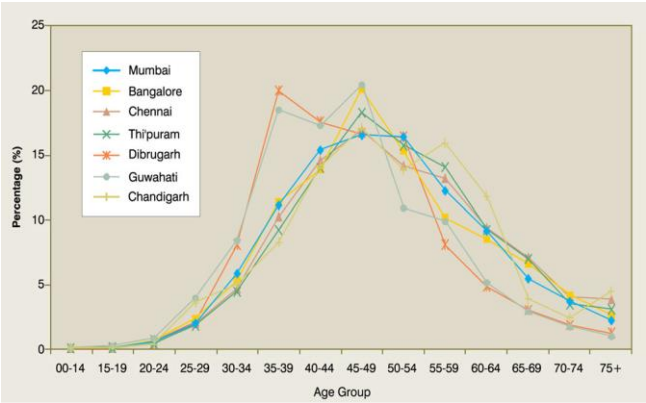

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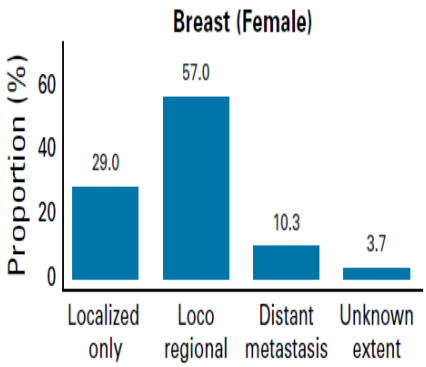

Figure 1.2: Breast cancer incidences in India. (a) Time trends of breast cancer over three decades from 1982 to 2012 among major PBCRs which showed significant and consistent increase in age adjusted rates (AAR) per 100,00 over the time. Adapted from : (b) 5-year age distribution trends among different registries in India. Adapted from (Malvia, Bagadi, Dubey, & Saxena, 2017) (c) Relative proportion (%) of patients according to clinical extent of disease, 2012-2016. Adapted from: (Mathur et al., 2020)

In India, the breast cancer incidences are high due to the dearth of awareness among women. This results into diagnosis of breast cancers at delayed stage mostly when it has advanced (Agarwal, Pradeep, Aggarwal, Yip, & Cheung, 2007). Insufficient cancer screening programs, improper or fragmented treatment along with lack of proper resources adds to the gravity of the disease (Maurya & Brahmachari, 2020). This also leads to poor survival rate of patients with breast cancer in India as compared to Western countries. Australia, New Zealand, Nordic countries, North America and Canada are on

the top for highest 5-year net survival (Allemani et al., 2018). As the stages advance, the 5-year overall survival chances decrease, a study reported it to be only 21% for stage IV patients whereas survival chances are 95% for stage I patients, decreasing to 92% for stage II and 70% for stage III (Arumugham, Raj, Nagarajan, & Vijilakshmi, 2014). For women diagnosed with breast cancer during 2010-2015, the 5-year net survival in India is as low as 66 percent as compared to more than 90 percent in USA and Australia (Mallath et al., 2014). Recent literature has focused on the limitations of the Indian Healthcare systems in regards to delayed early diagnosis, inadequate funding for the treatment of the disease as well as limited human resources (Mehrotra & Yadav, 2022). Hence, educational awareness, early diagnosis, ideal treatment and strengthening infrastructure and human resource is necessary to increase survival rate in Indian breast cancer patients.

1.2. Tumor heterogeneity of breast cancer

Breast cancer is broadly characterized into four major molecular subtypes based on immunohistochemical expression of triple markers which are steroid hormone receptors (estrogen receptor [ER], progesterone receptor [PR]) and growth factor receptor (HER2). They are classified as luminal A (ER+/PR+ and Ki67-low, HER2 negative), luminal B (ER+/PR+ and HER2+ or HER2–, and Ki67-high) and HER2 enriched, Claudin-low and Basal-like (triple negative breast cancer i.e. ER-PR negative and HER2 negative) (Visvader, 2009). The non-luminal (HER2 positive and ER-PR negative) or luminal (HER2 positive and ER or PR positive, or both) are potential cause for metastatic breast cancer (Harbeck & Gnant, 2017). Later based on gene copy number and expression profile ten different molecular subtypes have been characterized (Perou et al., 2000); (Sørlie et al., 2001). Advanced breast cancer (triple negative breast cancer) is usually an extremely heterogenous condition (Denkert, Liedtke, Tutt, & von Minckwitz, 2017), reinforcing oncogenic tumor progression through metastasis and resistance to therapy.

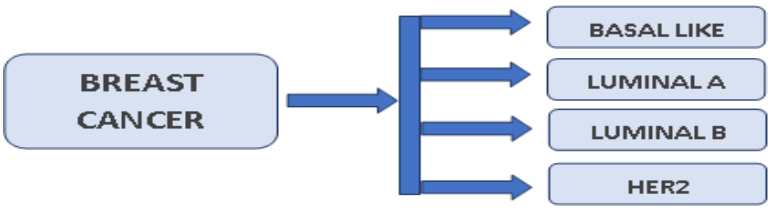

Figure 1.3: Schematic representation of molecular subtypes of breast cancer

- **Luminal A**: This subtype has estrogen-receptor and/or progesterone-receptor positive and HER2 negative properties. Luminal A cancers tend to grow slowly and have the good prognosis. Ki-67, cell proliferation marker is at low levels.
- **Luminal B**: In this subtype, estrogen-receptor and/or progesterone-receptor are positive, and either HER2 positive or HER2 negative. Due to high levels of Ki-67Luminal B cancers grow slightly faster than luminal A cancers and hence worsening their prognosis.
- **Triple-negative/basal-like** Hormone receptors: estrogen-receptor and progesterone-receptor are negative and HER2 is also negative. *BRCA1* gene mutations are more common in this kind of patients.
- **HER2-positive:** In this sub-type hormone-receptors are negative while HER2 is positive. Such type of cancers tends to grow at a faster rate compared to luminal cancers contributing to worse prognosis. Recovery rate of the patients can be improved by a drug known as Herceptin (also known as trastuzumab), a targeted therapy specifically aimed at HER2 protein.

Depending upon the site of origin and the ability of cancer cells to migrate to other sites, breast cancer has been categorized into following types:

1. Ductal carcinoma in-situ (DCIS): Ductal carcinoma in situ (DCIS) is the most common non-invasive stage of breast cancer where cancerous cells arise from cells lining the ducts. About 1 in 5 new breast cancer cases will be DCIS.
2. Lobular Carcinoma in situ (LCIS)**:** Lobular carcinoma in situ (LCIS) is also non-invasive stage of breast cancer where a cancer arises from cells lining the lobules.
3. Invasive ductal carcinoma (IDC): Invasive or infiltrating duct carcinoma (IDC) is the commonest form of breast cancer. It accounts for 85 to 90% of all cases. Invasive (or infiltrating) ductal carcinoma (IDC) starts in a milk duct of the breast, breaks through the wall of the duct, and grows into the fatty tissue of the breast. At this point, it may be able to spread (metastasize) to other parts of the body through the lymphatic system and bloodstream.
4. Invasive lobular carcinoma (ILC): This breast cancer type represents 5% of all diagnosis. Invasive lobular carcinoma (ILC) starts in the milk-producing glands (lobules). Lobular cancer cells break through the wall of the lobules and metastasize to

other parts of the body. Invasive lobular carcinoma may be harder to detect by a mammogram than invasive ductal carcinoma.

5. Inflammatory breast cancer (IBC): This uncommon type of invasive breast cancer accounts for about 1-3% of all breast cancers. Usually there is no single lump or tumour. Instead, IBC makes the skin of the breast look red and feel warm. It also may make the skin look thick and pitted, like orange peel. These changes are not caused by inflammation or infection, but by cancer cells blocking lymph vessels in the skin. The breast may get bigger, hard, tender or itchy.

Challenges exist for breast cancer diagnosis and the treatment because of the heterogeneity found in breast cancer. Heterogeneity is primarily due to the properties of cancer cells, like inter-clonal cooperativity and cell plasticity. Inter-clonal cooperativity occurs when two or more cancer clones exhibit an additional mutant phenotype in coexistence. Cell plasticity can be defined as an ability of cancer cells to adapt to tumor microenvironment (TME) signals, reprogramming their gene expression repository resulting in genotypic and phenotypic changes (Lüönd, Tiede, & Christofori, 2021). Heterogeneity can be either inter-tumor or intra-tumor heterogeneity (Skibinski & Kuperwasser, 2015). If the difference is from the tumors of different patients is it termed as inter-tumor heterogeneity (Turashvili & Brogi, 2017) whereas if the difference exists due to their stemness, plasticity, genetic, epigenetic, transcriptomic traits and tumor microenvironment among cancer cells within a single tumor, it is called intra-tumor heterogeneity. It is widely hypothesized that tumor heterogeneity triggers the phenomenon of therapeutic resistance and metastatic progression of breast cancer but our understanding of its mode of action is still fragmentary.

1.2.1 Tumor microenvironment as a premetastatic site

Normal cells gradually get transformed into neoplastic cells when there is a lack of coordination between molecular events like cell division, differentiation and apoptosis. Such dysregulation is marked by explicitly acquiring special characteristics like resisting antigrowth signals, sustained chronic proliferation, escaping apoptosis, incessant neovascularization, infinite replication capability and tissue invasion and metastasis, known as Hallmarks of cancer (Hanahan & Weinberg, 2000). Reprogramming the cellular energetics and evading immunological destruction (immune evasion) were the two compelling attributes that were further added to the list

of the previous hallmarks along with tumor empowering characteristics like genomic instability and tumor promoting inflammation. Tumor-promoting inflammation brings into limelight, the dual role of an immune system that can both boosts and supress the tumor advancement (Hanahan & Weinberg, 2011). Tumor repertoire constitutes of senescent cells, polymorphic microbiomes, together with cells capable of phenotypic plasticity and non-mutational epigenetic reprogramming (Hanahan, 2022).

Tumor can be defined as a heterogeneous assembly of resident host cells, infiltrating immune cells, and secreted factors whereas tumor microenvironment refers to the area surrounded by blood vessels, signalling molecules and the extracellular matrix (ECM) which aids in tumor progression (Truffi, Sorrentino, & Corsi, 2020). Tumor microenvironment forms when tumor cells induce a significant molecular, cellular and physical changes within the host tissues for the sustenance of tumor, its growth and development. Tumor microenvironment commonly consists of tumor cells, non-transformed cells, including the stromal cells such as fibroblasts, mesenchymal stem cells, endothelial cells, and immune cells like microglia, macrophages and lymphocytes along with collagen, fibronectin, hyaluronan, laminin forming extra cellular matrix. Cancer elimination or promotion including its initiation, growth, migration, metastasis, relapse and drug resistance are governed by crucial and complex cell-to-cell communications between tumor cells and the non-transformed cells (Kartikasari, Huertas, Mitchell, & Plebanski, 2021). Immune evasion caused by immunotolerant tumour cells is a major hurdle to immunotherapy. Immune suppression involves a lack of immune effector cells, impaired immune cell maturation, reduced antigen recognition or presentation, and immunosuppressive cell accumulation. It includes up-regulation of immune checkpoint modulators and synthesis of chemokines, inhibitory cytokines and ligands/receptors, which affect metabolism and worsen tumour microenvironment (H. J. Lin, Liu, Lofland, & Lin, 2022). Tumor microenvironment is established during tumorigenesis is a continuously growing dynamic site due to tissue remodelling and metabolic alterations, fostering neoplastic transformation and progression. Understanding tumor microenvironment would help in re-designing current anti-cancer treatments and also further allow the refinement of novel target specific therapeutic strategies for governing breast cancer.

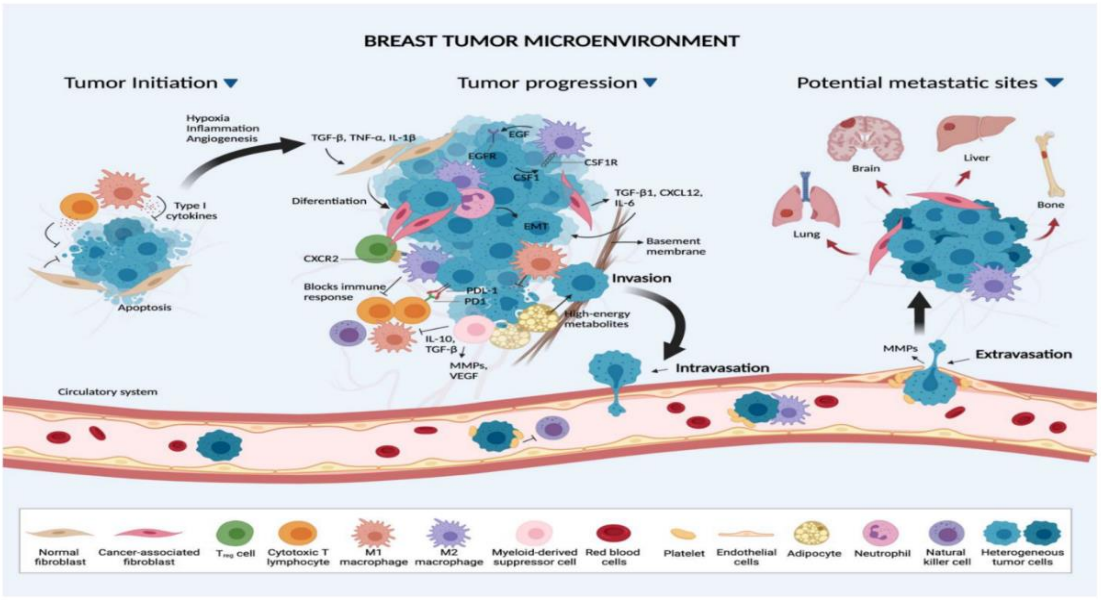

Figure 1.4: Tumor microenvironment in breast cancer. Adapted from (Terceiro et al., 2021)

1.2.2 Tumor microenvironment (TME) and breast cancer progression.

Tumor initiation overcomes growth-suppressive signals from inflammation, which are controlled by cytotoxic T-lymphocytes, M1 macrophages, and fibroblasts. Breast cancer cells escape these mechanisms by signaling stroma cells to gain pro-tumorigenic traits. Inflammatory cytokines (TGF-, IL-1, and TNF-) influence normal fibroblasts' development into cancer-associated fibroblasts (CAFs). These cells release extracellular matrix proteins and soluble factors (TGF-, CXCL12, IL-6) that promote EMT, tumour growth, and progression. Neutrophils secrete cytokines that promote EMT and tumour growth. Adipocytes release tumor-fuelling compounds. Tumor-associated macrophages (mostly M2 macrophages) secrete pro-tumorigenic cytokines and growth factors to encourage BC development and invasion. At the time of tumour growth, active cytokines in the environment (CXCL5-CXCR2, TGF-) recruit regulatory myeloid-derived suppressor cells (MDSCs) and T cells (Treg), which block natural killer cells, cytotoxic T lymphocytes, and M1 macrophages. These tumorigenic events provide invasive ability to tumour cells. MMPs and VEGF promote tumour cell intravasation where they interact with platelets and M2 macrophages to suppress immunological recognition. Platelets aide tumour cells to migrate at preferred metastatic site where endothelial cells enhance extravasation. Breast cancer subtype can influence the site of metastasis.

1.2.3 Tumor associated inflammation in breast cancer:

One of the most aggressive types of breast cancer is Inflammatory Breast Cancer (IBC). Inflammation can be stimulated either by extrinsic or intrinsic factors. The extrinsic factors can be bacteria, virus, allergens or pathogen-associated molecular patterns (PAMPs). In contrast, intrinsic factors can be damage associated molecular patterns (DAMPs) or cancer-initiating mutations contributing to neoplastic progression through the recruitment and activation of inflammatory response (Grivennikov & Karin, 2010); (Wertz & Dixit, 2010). Macrophages present in the breast tissue are an integral part of innate immune system but when they participate in facilitating breast tumorigenesis they are known as tumor associated macrophages (TAMs). When there is a disturbance in tissue homeostasis, tissue specific macrophages and mast cells produce mediators like cytokines, chemokines along with other bioactive mediators recruiting circulating leukocytes to the injured site. The macrophages together with other leukocytes, generate high levels of reactive oxygen and nitrogen species to fight infection (Maeda & Akaike, 1998). Macrophages are predominantly found in chronic inflammatory microenvironment. Those macrophages and T-lymphocytes may release tumor necrosis factor-alpha (TNF-α) and macrophage migration inhibitory factor to aggravate the DNA damage (Pollard, 2004). Thus, an inflammatory process is activated to heal the tissue injury (Medzhitov, 2008). Cytolytic T lymphocytes involved in acute tumorigenic inflammation protects against tumor growth, whereas chronic activation of humoral immunity brought about by infiltration by Th2 cells and with involvement of protumor-inclined inflammatory cells or by blocking anti-tumor immunity results in tumor promotion (DeNardo & Coussens, 2007); and (DeNardo & Coussens, 2007). Hence Inflammation is one of the crucial players of tumorigenesis from inception to its dissemination to secondary or multiple metastatic sites (Grivennikov, Greten, & Karin, 2010); (Hanahan & Weinberg, 2011); (Taniguchi & Karin, 2018).

Depending on the pathological conditions, the cells respond to the inflammatory mediators differently (Kemp, Griffiths, Campbell, & Lovell, 2013); (Medzhitov, 2008). Inflammatory cytokines also play a crucial role in neoplastic progression and intra-tumoral heterogeneity (Esquivel-Velázquez et al., 2015). Several studies reported that a network of inflammatory cytokines within the tumor microenvironment sustain proliferation of the cancer cells by preventing the activation of immunological effector function. Cytokines like IL-4, IL-13, and IL-10 are responsible for the adaptive immune

response suppression and promoting anti- inflammatory milieu (De Palma & Lewis, 2013) whereas pro-inflammatory cytokines like tumor necrosis factor (TNF)-α, interleukin (IL)-6, IL-8, IL-10, IL-18, macrophage migration inhibitory factor (MIF) and transforming growth factor (TGF)-β has found to be associated with invasive breast cancers enhancing angiogenesis and metastasis (B. E. Lippitz, 2013), (G. Landskron, M. De la Fuente, P. Thuwajit, C. Thuwajit, & M. A. Hermoso, 2014), (Allegrezza & Conejo-Garcia, 2017). The corelation of tumor growth, invasion and metastasis with an increased expression and secretion of proinflammatory cytokines and chemokines such as TNF-α, IL-6, IL-1α, IL-1β, TGF-β, MMP9 and CXCR4 was evident from the gene expression profiling of Ductal carcinoma in situ and invasive breast tumors. Higher tumor grade and lymph node metastasis can be co-related to increased concentration of intra-tumoral TNF-α (Esquivel-Velázquez et al., 2015). However, the understanding of TNF-α regulated immune response in the tumor progression is still scarce. Tumor associated macrophages, exhausted T cells and malignant cells releases proinflammatory mediators whose accumulation along with immune suppressor cells acts in an autocrine and paracrine manner encouraging T cell tolerization apparently supporting chronic inflammation. Tumor cells also modify its own cellular programming and utilizes essential growth factors to enable expansion as compared to the immune cells.

1.2.4 Cytokines induced by tumor microenvironment

Cytokines based on their structure and function, can be classified into diverse superfamilies like tumor necrosis factors (TNFs), interleukins (ILs), interferons (INFs), chemotactic cytokines (chemokines), tumor growth factors (TGFs), and colony-stimulating factors (CSFs) (Miller & Krangel, 1992). Immune cells, endocrine and nervous system cells secrete cytokines which facilitate inter-cellular and intra-cellular communication in the immune system and hence are key regulators of inflammatory response. Their biological activity is not restricted to a particular area only, as cytokines from TME can also exercise the systemic inflammation encouraging a further enhancement of oncogenic environment (Greten & Grivennikov, 2019). Besides exerting cell proliferation, the action of cytokine involves promoting cancer development, employing tumor-supportive stromal cells and immune-suppressive cells, exerting angiogenesis and metastasis, provoking anti-tumor immune response, and altering the efficacies towards medications(A. Mantovani, Allavena, Sica, & Balkwill,

2008); (Coussens & Werb, 2002). Conversely, metabolic re-programming, mutagenic transformation, hypoxia, apoptosis and anti-cancer treatment can also trigger cytokine synthesis (A. Mantovani et al., 2008). Thus in the tumor microenvironment, cytokines may inhibit tumor initiation or may contribute to chronic inflammation leading to tumor growth by tumor immune escape or tumor-induced immune suppression changing the dynamics of the TME (W. W. Lin & Karin, 2007).

Specific groups of cytokines can be associated with a distinct malignancy and stage of the oncogenic progression from the tumor microenvironment making them potentially suitable as biomarkers for cancer detection, anticipating the fate of the disease and for guiding therapeutic alternatives and responses. An enhanced and a dynamic network of the proinflammatory cytokines leading to dysfunctional immune response has been shown by patients with different tumor types (Galdiero, Varricchi, Loffredo, Mantovani, & Marone, 2018). Metastatic breast cancer studies suggested that malignant tumors exploit resident network of inflammatory cytokines within its microenvironment to evade and escape activation of immune effector function (Méndez-García et al., 2018). Increased level intra-tumoral cytokines such as tumor necrosis factor (TNF)-α, interleukin (IL)-6, IL-8, IL-17, IL-18, transforming growth factor (TGF)-β, and macrophage migration inhibitory factor (MIF) have been reported in several experimental and clinical breast tumors (Richard, Kindt, & Saussez, 2015). For predicting metastasis and prognosis of breast cancer, serum IL-6, IL-8, IL-10, SCC-Ag, and CYFRA 21-1 have emerged as candidate markers (H. Wang & Yang, 2017). Pro-inflammatory cytokines TNFα, IL-6, CSF-1, and IFNγ have been correlated with malignant cells in breast cancer (Semesiuk et al., 2013) whereas in patients with BC progression and metastasis, IL-1α could be a potential marker for the release of cancer cells. Increased IL-1β, IL-12, sFlt1 and PlGF has been linked with circulating tumor cells (CTCs) (VILSMAIER et al., 2016). Immunomodulatory role of the cytokines can be demonstrated from the specific cytokine signature pattern linked with poor prognosis with advanced breast cancer (Esquivel-Velázquez et al., 2015). TNF-α has appeared as a crucial cytokine and TNF-α-induced apoptosis can be either by the mitochondria-dependent pathway (intrinsic pathway) or by the mitochondria-independent pathway (extrinsic pathway) (Z. Jin & El-Deiry, 2005). Changes in mitochondrial structure and function can be co-related with chronic levels of TNF-α induced ROS production (K. Kim et al., 2010); (Yuan et al., 2017). However, there is lack of complete understanding

of the role of TNF-α governed immune crosstalk in regulating tumorigenicity of malignant cells. Delineating the intricate cytokine signaling pathways, controlling the chronic inflammatory response will bring better insights towards immune-stimulative and immunosuppressive pathways, ultimately helping in better therapeutic interventions.

1.2.5 TNF-α: significance to tumorigenesis

Solid tumor microenvironment is complex and comprises cells of many different origin including tumor cells, stromal cell, fibroblast and immune cells. Tumor microenvironment is complex however specific cytokines are high in the tumor microenvironment. It had been observed that TNF level is high in the tumor microenvironment and can be both pro-survival and can induce cell death. Although primary production of TNF-α is by macrophages and monocytes, it is also expressed by fibroblasts, and epithelial cells. TNF-α is induced via various stimuli, including cytokines, oxygen free-radical, LPS, and calcium flux (Spriggs et al., 1992). TNF-α is one of the important pleiotropic cytokines possessing multifunctional abilities like inflammatory and immunomodulatory functions. It is a crucial proinflammatory cytokine which can recruit cytokines, chemokines, adhesion molecules and activate leukocytes at infection site. (Varfolomeev & Ashkenazi, 2004). Another vital role of TNF-α is in regulation of inflammation, metabolism, tumor growth and survival, vascularization, invasion and apoptosis (Ji et al., 2014). Notably another important physiological aspect of TNF-α signaling is tolerization of macrophages and apoptosis of inflammatory cells (Kalliolias & Ivashkiv, 2016). Apart from its homeostatic functions, TNF-α has essential role against bacterial, viral and parasitic infections, hence the pathophysiological situation dictates the function of TNF-α whether it will send survival signals or death signals (Wajant, Pfizenmaier, & Scheurich, 2003); (Kalliolias & Ivashkiv, 2016); (Holbrook, Lara-Reyna, Jarosz-Griffiths, & McDermott, 2019). Thus, enforcing us to understand the fine regulation of TNF-α-induced signaling in different pathological conditions.

Tumor necrosis factor (TNF) superfamily (TNFSF) comprises of 19 ligands and 29 receptors in humans with additional three receptors in mice. TNF-α is produced as 26 kDa type II transmembrane protein and upon the proteolytic cleavage by TNF-α converting enzyme (TACE) produced as 19 kDa soluble forms. Soluble TNF-α in its homotrimeric form executes its cellular function upon binding to two signalling

receptors: TNFR1 and TNFR2 (H. Blaser, C. Dostert, T. W. Mak, & D. Brenner, 2016). Both receptors have been found in hematopoietic cells. TNFR1 (p55 or CD120a) receptor can be stimulated by both form of TNF-α and has a ubiquitous distribution on all cell types and whereas TNFR2(p75 or CD120b) receptor is expressed by immune and endothelial cells and mostly bind to the transmembrane form for its activation (Grell et al., 1995). TNF-binds to TNFR1 and R2 receptors can have different implications. TNF-R1 is expressed mostly in all the tissues and acts as the key mediator of TNF-α mediated signaling (Croft and Siegel, 2017; Holbrook et al., 2019; (Kalliolias & Ivashkiv, 2016; Wajant et al., 2003). After binding of TNF-α to TNFR1 activates two transcription factors NF-κB and AP-1 thus promoting production of stress and inflammatory response factors (Kalliolias & Ivashkiv, 2016); (Wajant et al., 2003). The pro-survival signalling pathway is activated upon binding of TNF-α to TNFR1 as it is a more effective activator of NF-κB transcription factor and MAPKs, than TNFR2. This mechanism has involvement of complex I and associated proteins [TNF receptor-associated protein with death domain (TRADD), TNF receptor associated factor 2 (TRAF2) and receptor-interacting protein kinase 1 (RIP1)] (Rath & Aggarwal, 1999); (L. Wang, Du, & Wang, 2008). On the other hand, the binding of complex II with Fas-associated death domain (FADD) activates caspase-8, initiating apoptosis cascade involving the mitochondria along with caspases as key regulators (Degterev, Boyce, & Yuan, 2003). The formation of Complex IIa and IIb leads to apoptosis, alternatively Complex IIc can lead to necroptosis and inflammation (Micheau & Tschopp, 2003).

1.2.6 TNF-α: an intricate link between inflammation and cell death

Acute inflammation differs from the chronic inflammation as the anti-inflammatory cytokines replaces the proinflammatory cytokines on the later stages of the healing process. Additionally, during the chronic inflammation tissue destruction and repair progresses at the same time along with the involvement of neutrophils, mononuclear cells, plasma cells, lymphocytes and macrophages (Coussens & Werb, 2002); (Shacter & Weitzman, 2002). TNF-α has a pivotal role in pro-inflammatory responses in innate immunity and immune surveillance, contradictorily it can also be produced by tumors functioning as an endogenous tumor promoter. Commencing from cellular transformation, tumor initiation, promotion, proliferation, invasion, angiogenesis, metastasis, and resistance to therapy, TNF- α is involved in each step of tumorigenesis. TNF- α can be acclaimed as master regulator of an intricate signalling network

orchestrating cell survival and apoptosis (L. Wang et al., 2008). The paradoxical role of TNF-α makes it the most exemplified cytokine (Balkwill, 2009).

A continuous low production of TNF- α is mainly responsible for tumor growth and tumor advancement of primary breast neoplasm. TNF- α has been found to be strongly associated with cancer cachexia and advanced cancer with poor survival rate (Han et al., 2012). Reports from clinical data showed a pre-dominant, chronic levels of intra-tumoral TNF-α, which is consistently associated with more aggressive tumor phenotype in patients with breast cancer (B. E. Lippitz, 2013). Hence in TME, TNF- α is the key proinflammatory cytokine impairing cytotoxic macrophages and anti-tumor effector functions of T cells in addition to regulation and recruitment of monocytes and neutrophils (Alberto Mantovani & Dejana, 1989). It also promotes activation of adhesion molecules on the vascular endothelial cells enhancing the tumor niche (Newton & Dixit, 2012). Metastatic sites and its development have been found to increase with administration of TNF- α (endogenous and exogenous) in mice models of metastasis (Orosz et al., 1993). As an alternative, blocking agents like etanercept, infliximab, and adalimumab have been clinically tested in combination with chemotherapy against TNF- α, however it did not show the desired results thus requiring more studies (Mercogliano, Bruni, Mauro, Elizalde, & Schillaci, 2021).

1.2.7 The paradoxical role of TNF-α in breast cancer

Tumour necrosis factor-α is a crucial pro-inflammatory cytokine found in breast tumor microenvironment (Komori et al., 1993). TNF-α acts differentially according to the stages of tumor progression. Reports have showed dual role of TNF-α in cancer (Cruceriu, Baldasici, Balacescu, & Berindan-Neagoe, 2020). Earlier studies showed that TNF-α, as its name suggests, promoted necrosis of the cancer cells (Kalliolias & Ivashkiv, 2016). Also, TNF-α promoted necroptosis in leukemia (Sawai, 2014) and colon cancer ((Moriwaki, Bertin, Gough, Orlowski, & Chan, 2015) by RIP3 mediated large signaling complex leading to cell death. Cell proliferative effects of TNF-α have been found on T47D breast cancer cells (Rubio, López-Muñoz, & Alamo, 2006); (Rivas et al., 2008). Contradictorily, TNF-α has been found to display anti-mitogenic (Jeoung, Tang, & Sonenberg, 1995) and pro-apoptotic (Y. Wang et al., 2012) properties in MCF-7 cells. TNF-α is known to inhibit proliferation and induce apoptosis of MCF-7 breast carcinoma cells (Jeoung et al., 1995). TNF-α exhibited cell cycle arrest in MCF-7 cells by upregulating the expression of cell cycle inhibitory protein Cip1 (p21) and tumor

suppressor protein p53 (Simstein, Burow, Parker, Weldon, & Beckman, 2003). Studies also exists suggesting role of TNF-α in cancer cell proliferation or survival via activation of transcription factors like NF-κB and AP-1 (Waters, Pober, & Bradley, 2013). TNF-α regulates metastasis related genes enhancing the invasive ability of MCF-7 cells (X. Chen, Bäumel, Männel, Howard, & Oppenheim, 2007). TNF-α aids in tumor metastasis by enhancing its invasion and migration properties (Wolczyk et al., 2016), also promoting epithelial to mesenchymal transition via upregulating matrix metalloproteinases (MMPs) (Kalliolias & Ivashkiv, 2016). TNF-α also causes chemotherapy and radiotherapy sensitization in breast cancer cells (X. Wu et al., 2017). These findings indicate that the TNF- α mediated activation of different signalling pathways and TNF- α -induced differential gene expression TNF-α may result into diverse outcomes affecting tumor growth and progression. Therefore, systematic investigation of TNF- α-induced genes and their role in associated signalling pathway may provide critical evidences regarding the role of TNF-α in cancer pathogenesis. Hence TNF-α acts as a dual edged sword in case of cancer including breast cancer, therefore, the balance of TNF-α in inducing survival and death-signalling is pivotal in determining the fate of TNF-α responding cells. Modulating this balance could help to prevent cancer development and facilitate using TNF-α for cancer therapy.

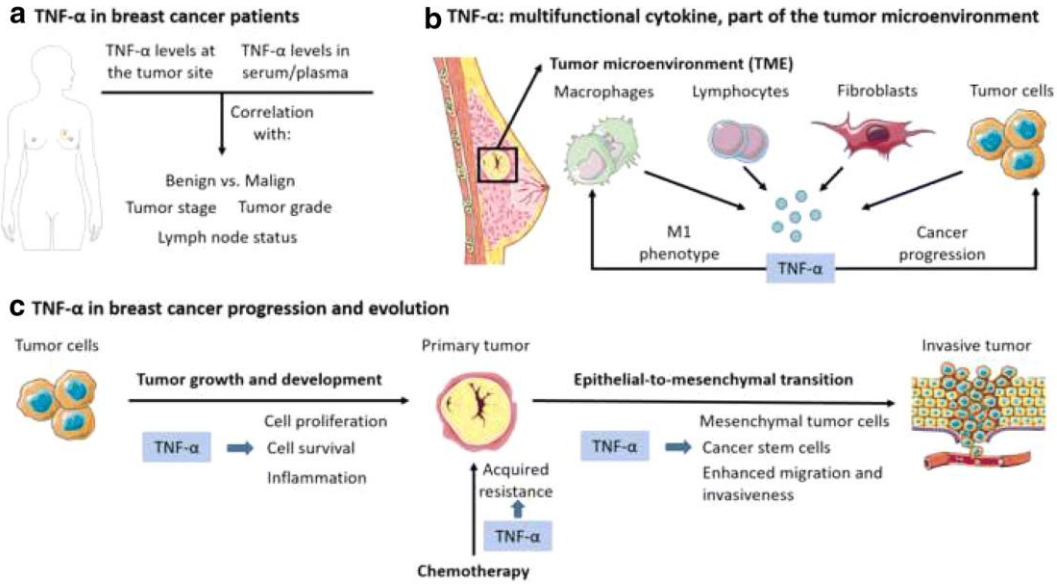

Figure 1.5: Overview of TNF-α relevance in breast cancer progression. A). TNF-α levels in breast cancer patients are correlated with clinical status and outcome; B). TNF-α is secreted in the tumor microenvironment (TME) by multiple types of cells, C). TNF-α is involved in all stages of breast cancer tumorigenesis Adapted from: (Cruceriu et al., 2020)

1.2.8 TNF-α influences ROS production in cancer:

TNF-α signalling comprises of two main pathways: One is the pro-survival pathway and the other is pro-apoptotic pathway. The pro-survival pathway functions through TNF-α-induced signaling complex I via activation of NF-κB and MAPK (Micheau & Tschopp, 2003) whereas in proapoptotic pathway which acts through TNF-α-induced signaling complex II, down-stream mediators are reactive oxygen species (ROS), a caspase cascade, and the mitochondria and. Activation of NF-κB blocks this TNF-α-induced cell death pathway.

TNF-α enhances tumor initiation and tumor progression by generating free molecules such as reactive oxygen species (ROS), indicating ROS as a signalling mediator for TNF-α. It has been reported to be involved in both cell survival and cell death in different cell lines (Shoji, Uedono, Ishikura, Takeyama, & Tanaka, 1995); (Lo & Cruz, 1995); (Sundaresan et al., 1996); (Corda, Laplace, Vicaut, & Duranteau, 2001). The genotoxicity produced leads to genomic instability and DNA damage (H. Blaser et al., 2016). Remarkably, in the TNF-α- dependent programmed cell death pathway, ROS is either generated from mitochondrial or non-mitochondrial sources. Reports indicate that after TNF-α treatment, NADPH oxidase is the source of ROS generation (Y. S. Kim, Morgan, Choksi, & Liu, 2007). However, the major resource of ROS generation is the mitochondrion that contributes to TNF-α-induced cell death (Meier et al., 1989); (Shoji et al., 1995); (Corda et al., 2001). A positive feedback loop regulates both TNF-α signaling and ROS production (H. Blaser et al., 2016). ROS are also known to cause cell death by activating c-Jun N-terminal kinase (JNK) and causing permeabilization of mitochondrial membrane (Sakon et al., 2003); (Tobiume et al., 2001). One of the reports suggests that initially there is early decrease in mitochondrial membrane potential ($\Delta\psi m$), but after ROS generation, a late decrease in mitochondrial membrane potential is promoted (Gottlieb, Vander Heiden, & Thompson, 2000). Therefore, understanding the molecular connections between TNF-α signaling and mitochondrial ROS generation is important to decipher the regulation of the tumor cell survival and promotion in tumor microenvironment. Unravelling the cross-talk between the inflammatory pathways and cancer cell metabolism could help in reinstating the tumoricidal immunity via novel therapeutic targets for malignant tumors.

1.2.9 Mitochondria: Regulator of cell death

The power house of the cell, the mitochondria; is known as a chief regulator of cells energy metabolism. Yet there are number of growing evidences showing its role in inflammatory and cell death pathways. Mitochondrial proteins in the inter-membrane space play important role in mitochondrial biogenesis, energy metabolism, as well as in cell death (Candé et al., 2004). Mitochondria serves as a platform for assemblage of signalling complexes and also involves certain mitochondrial proteins for critical regulation of inflammatory pathways viz NF-κB and IFNs pathway (O'Dea & Hoffmann, 2009). The activation of transcription factors leads to the downstream expression of various cytokines which also attracts immune cells at the site of tumor favouring further cytokine release and henceforth aggravating the inflammation in tumor microenvironment. The progression of inflammatory response leads to the advancement of angiogenesis and tumor growth.

Cell death can occur via apoptosis, necrosis, or autophagy. Apoptosis, a type of programmed cell death can be described as either extrinsic pathway or intrinsic pathway (mitochondrial pathway). In the extrinsic pathway, the danger signals are being sensed by specialized ligands (e.g. FasL, TNF, and TRAIL) which binds and activates their respective receptors which are a part to TNF family receptors consisting of extracellular domain and a cytoplasmic domain (or death domain) (Ashkenazi & Dixit, 1998). The death domain is a crucial element for transmitting the death signal to the intracellular signalling pathways from the cell surface. Activated receptors bind to their adaptor proteins viz., TNF receptor associated death receptor (TRADD) to TNF receptor and Fas associated death domain protein (FADD) to Fas receptor. The multimeric complexes form death inducing signalling complex (DISC) by recruitment of pro-caspase-8 to, which is processed to its active form subsequently leading to proteolytic cascade (Kischkel et al., 1995).

Any variations in physiological or stress conditions can activate intra-cellular damages triggering intrinsic pathways of apoptosis where mitochondria play pivotal role by the release of inter-membrane proteins in cytosol. The proteins released due to mitochondrial permeability transitions can initiate apoptosis process and exaggerate the process by relieving the inhibitors of apoptosis (IAPs) (Bonora & Pinton, 2014). Apoptotic stimulus results into the release of cytochrome c from mitochondria.

Cytochrome c forms a complex known as apoptosome, with Apaf-1 (Apoptotic Protease Activating Factor), procaspase-9 (inactive form) in presence of dATP. Caspase-9 is one of the initiator caspases which further activates downstream executioner caspases -3 and caspases -7 in the intrinsic pathway of cell death (L. Wang et al., 2008).

Primarily, the cancer cells reprogrammes its own cellular metabolism and alters the immune-metabolic interaction to promote and sustain cancer-associated inflammation and immunosuppression, and secondly, by surpassing other cells for key nutrients of tumor microenvironment. Importantly, TNF-α has arose as a chief cytokine that can regulate mitochondrial function and influence the cellular metabolism (Bell et al., 2013). Altered mitochondrial structure and function along with increased ROS generation are the repercussions of chronic levels of TNF-α (K. Kim et al., 2010); (Yuan et al., 2017). Despite of active research ongoing in this area, the understanding of the crosstalk between the mitochondrial metabolism regulation and TNF-α-associated immune response in controlling tumorigenicity of cancer cells is still evolving.

Ideally, there is a fine regulation between cell growth and cell death through modulation of growth inhibitory or stimulatory signals by genetic control system. Any disbalance caused, either by genetic alterations or mutations leads to discrepancy in cell proliferation and cell death, as a consequence resulting into cancer cell growth and proliferation. In spite of notable advances in primary screening and diagnosis, there is systematic need to explore the cause, pathogenesis and progression of breast cancer to bring down the incidence rate and mortality rate. Extensive efforts are being made to comprehend the molecular mechanisms and progression of breast cancer to curb the disease at early stage. Despite advances in curative measures like surgery, radiotherapy and chemotherapy; high mortality associated with cancer has not been restrained. Further efforts are needed to discover potential targets for successful therapy. Moreover, multidrug resistance (MDR) is also one of the factors hindering anticancer therapy. These hurdles are the driving force for the quest of novel anti-cancerous compounds. Identification of newer therapeutic agents is crucial to effectively inhibit cancer progression with minimal side effects, which at present is associated with the stringent treatment regime for this challenging disease.

1.3 Phytocomponents as a source for potential therapeutic approach:

Ayurvedic pharmacopoeia is a rich compendium of Medicinal plants. Traditional medicine systems have been using medicinal plants as a source of therapeutic tools showing effectiveness against many diseases including cancer. The medicinal properties of plant species, which was popular in a few countries have now started to gain global importance due to demonstration of exceptional therapeutic potential through the drug discovery processes using techniques in modern science. Nevertheless, except for a few compounds, there is lack of scientific evidence to substantiate the efficiency of the said natural plants. Some of the well worked out examples are the discovery of well-known drugs; Vincristine and Vinblastine from Catharanthus roseus, Taxol from Taxus brevifolia and Campothecin from Camptotheca acuminate (Khazir et al., 2013); (Kingston, 2011). Some naturally derived anti-cancer agents which are in widespread clinical use are paclitaxel, epothilone, topotecan, vinblastine and cisplastin.

The contemporary world is looking for such more plant-derived compounds with more efficacy against various types of cancer. Thus, medicinal plants have generated a separate niche for themselves in scientific research amidst the modern health care systems and have a great potential as rich source for new drugs. Phytocomponents identified for use in other cancers and their mechanism of action are shown in the Table 1.1.

Indian medicinal plants used for anti-cancer studies:

The mentioned list (Table 1.2) of natural sources of phytocomponents are from plants being used in India since ancient times. Allium Sativum commonly known as, garlic is used as flavouring agent in the food has anti-oxidant, anti-asthamatic, anti-cholestrol anti-thrombotic, diuretic properties and anticancer property. The Ethanolic extract of Neem leaves (*Azadirachta indica*) possess immune-modulatory, anti-inflammatory properties, and anti-carcinogenic properties against prostate cancer. *Berberis vulgaris* L. (Barberry) belonging to family Berberidaceae is used against various liver, stomach and urinary tract related ailments. Beetroot known for its anti-oxidant property has potential against oesophagus and breast cancer The rhizomes of *Curcuma longa* L. (Haldi), one of the main ingredients of Indian cuisine and tradition is known for its anti-mutagenic and anti-

carcinogenic properties. Phytocompound Curcumin, the most potent compound in turmeric, is found to possess multiple potentials against several ailments. Mango possess immunostimulant, immunomodulatory, anti-viral and analgesic properties. Tulsi (*Ocimum sanctum* L.) leaves is useful due to its anti-oxidant, anti-bacterial and radio protective properties. Damro (*Ocimum basilicum* L.) is known for its anti-carcinogenic properties in breast cancer. Both, pomegranate and ginger are known to possess anti-inflammatory and anti-oxidant properties. Ginger has added benefits of being diaphoretic, diuretic and digestive, anti-spasmodic, expectorant, peripheral circulatory stimulant, astringent, appetite stimulant.

Table 1.1: Phytocomponents identified for use in other cancers and their mechanism of action:

Sr no.	Cancer type	Model	Natural Agent	Source	Mechanism	Reference
1	Colorectal cancer HeLa and HepG2 Mechanism of action	Human clinical trial	Artesunate	*Artemisia annua*	Prevents angiogenesis via VEGF inhibition	(Krishna et al., 2015) (Yang et al., 2014)
2	Multiple myeloma Malignant melanoma	Multiple myeloma cancer stem cells Human clinical trial	Bruceantin	*Brucea antidysen-terica*	Inhibition of protein synthesis	(Issa, Berndt, Carpentier, Pezzuto, & Cuendet, 2016)
3	Malignant solid tumor Metastatic colorectal cancer	L1210 cells and SV40-infected monkey cells Human clinical trial	Camptothecin	Nyssaceae	Interferes with DNA topoisomerase I	(Shimada et al., 1993)
4	Gastric cancer	Gastric cancercell lines Human gastric cancer cell line	Colchicine	Meadow saffron	Microtubule Destabiliser	(Z. Y. Lin, Kuo, Wu, & Chuang, 2016) (T. Zhang et al., 2019)
5	Bladder cancer	Human bladder cancer cells Murine bladder cancer model Cancer cell lines	Combretastatin	South African Combretum caffrum tree	Anti-mitotic agent, inhibits binding to colchicine binding to tubulin	(Shen et al., 2010) (Pettit et al., 1995)
6	Leukemia, neuroblastoma	leukemia HL-60 and CCRF-CEM cells, neuroblastomaIMR-32, UKFNB-3 and UKFNB-4 cells and U87MG glioblastoma cells	Elipticine	*Ochrosia elliptica*	Disruption of the cell cycle via regulation of kinases	(Stiborová et al., 2011)
7	Oral squamous carcinoma	C6 glioma cells HSC-3 cancer cells	Geniposide	Gardenia Fructus	Activation of apoptotic cascades PKCd/ JNK/ Fas/ caspase8 and caspase 3	(Peng et al., 2004)

8	Acute myeloid leukemia/mechanism of action Non small cell lung Adenocarcinoma	Mouse model of acute myeloid leukemia Murine lung tumor models	Homoharrig-tonine	*Cephalotaxus harringtonia*	Inhibits cell growth, induces cell cycle arrest and apoptosis	(C. Li et al., 2020) (Weng et al., 2018)
9	Lung cancer	Lung cancer cell lines	Podophyllo-toxin	American Mandrake or May Apple	Drisruption of cell cycle via DNA interactions with topisomerase II	(A. Y. Chen & Chen, 2013)
12	Lung cancer Ovarian cancer	Human clinical trials Human clinical trials CHO cells	Taxanes	Yew trees	Block cell cycle progression and trigger apoptosis	(Rigas, 2004)
13	Prostate cancer Esophageal squamous cell carcinoma	Prostate cancer cells Prostate and breast cancer cells SEC62 overexpressing tumor cells Esophageal squamous cell carcinoma cells	Thapsigargin	*Thapsia garganica*	Releases apoptotic factors including caspase 3 from the mitochondria	(F. Huang, Wang, & Wang, 2018) (Sehgal et al., 2017) (Ma et al., 2016)
14	Lung cancer	Human clinical trial HeLa cells	Vinca (Catharanthus) Alkaloids	Madagasgar Periwinkle	Interacts with tubulin and disrupts microtubule function preventing proliferation	(Furuse et al., 1994) (Jordan, Thrower, & Wilson, 1991)
15	Pancreatic cancer Lung carcinoma	Human clinical trial Human lung carcinoma cell line A549 6 different human cancer cell lines	Viscum Album extract	Mistletoe plant	Activates the Caspase pathways associated with apoptosis	(Tröger et al., 2013) (Siegle, Fritz, McClellan, Gutzeit, & Mürdter, 2001)

Table 1.2: Some Indian medicinal plants used for anti-cancer studies (Chanda & Nagani, 2013):

No.	Cancer type	Common name	Scientific Name	Traditional and reported uses
1	Oral cancer cell, sarcoma 180 cancer cell	Garlic	*Allium Sativum* L. (Liliaceae)	Anti-oxidant properties, anti-asthamatic, anti-cholesterolemic, cholagogue, diaphoretic and diuretic, antiseptic, anti-thrombotic, anti-cancer
2	Prostrate cancer	Neem	*Azadirachta indica* J. (Neemeliac)	anti-inflammatory, anti-ulcer, anti-malarial, anti-fungal, anti bacterial, anti- viral, ,anti-oxidant, Immunomodulatory, anti-mutagenic and anti-carcinogenic properties.
3	Breast cancer	Barberry	*Berberis vulgaris* L. (Berberidaceae)	Anti-oxidant, active against diseases such as diarrhea,, liver&gall bladder dysfunctions, leishmaniasis, malaria, stomach problems and urinary tract infections.
4	Skin and lung cancer	Beet	*Beta vulgaris* L. (Chenopodiaceae)	Anti-oxidant, leukemia, cancer such as breast, oesophagus glands, head, intestine and leg
5	Colon cancer cells	Turmeric	*Curcuma longa* L. (Zingiberaceae)	Anti-mutagenic, anti-carcinogenic
6	Lung cancer	Mango	*Mangifera indica* L. (Anacardiaceae)	Anti-tumor, anti-oxidant, anti-viral, anti-bacterial, analgesic, anti-inflammatory, anti-diarrhoeal, anti-amoebic, spasmolytic, immunostimulant and immunomodulatory properties.
7	Skin cancer	Tulsi	*Ocimum sanctum* L. (Lamiaceae)	Anti-stress, anti-oxidant, hepatoprotective, anti-inflammatory, anti-bacterial and radio protective properties
8	Breast cancer	Damro	*Ocimum basilicum* L. (Lamiaceae)	Chemopreventive, anti-carcinogenic, radioprotective and numerous others pharmacological uses.
9	Prostate carcinoma cell	Pomegranate	*Punica granatum* L. (Lythraceae)	anti-oxidant and anti-inflammatory
10	Prostrate cancer	Ginger	*Zingiber officinale* Rosc. (Zingiberaceae)	Carminative, anti-oxidant, diaphoretic, diuretic and digestive, anti-spasmodic, expectorant, peripheral circulatory stimulant, astringent, appetite stimulant, anti-inflammatory

1.4 Potential phytocompounds against breast cancer and their mode of action:

As there is dearth of scientific support to the herbal plant therapy, there is unwillingness for its usage. However, work has been initiated in this regard world-wide. Effect of a few groups of phytochemicals from apple, *Gmelina asiatica* and Pterostilbene from blueberries have induced cell cycle arrest and apoptosis in breast cancer cells of different histopathological subtypes. Such reports on spontaneous animal models or cell-line models of cancer using phytochemicals encourage us to understand and explore the mechanism which results in such phenotypic changes to the cancerous cells.

There are several studies showing role of phytocomponents in Breast cancer and their mechanism of action has been explored (Table 1.3)

Role of Flavonoids, Iso flavones, catechins and Polyphenols have been explored in breast cancer (Table 1.4)

Role of isolated phytocomponents and their role in breast cancer has been mentioned in Table 1.5.

Table 1.3: Studies showing role of phytocomponents in Breast cancer and their mechanism of action:

Sr no.	Breast cancer Model	Natural Agent	Source	Mechanism	Reference
1	Human clinical trial	Bruceantin	*Brucea antidysenterica*	Inhibition of protein synthesis	(Wiseman, Yap, Bedikian, Bodey, & Blumenschein, 1982)
2	Human breast adenocarcinoma MCF-7 cells,	Elipticine	*Ochrosia elliptica*	Disruption of the cell cycle via regulation of kinases	(Stiborová et al., 2011)
3	Breast epithelial cells	Roscovitine	*Raphanus sativus*	Inhibits cyclin dependent Kinases ,directly competes at the ATP-binding site	(Nair, Vallabhaneni, Tekmal, & Vadlamudi, 2011)
4	Human breast carcinoma orthotopic metastasis model Solid tumor models and xenografts	Salvicine	*Salvia prionitis*	Inhibition of DNA topoisomerase II and ROS generation	(Lang et al., 2004), (L. H. Meng, Zhang, & Ding, 2001)
5	Human clinical trial	Taxanes	Yew trees	Block cell cycle progression and trigger apoptosis	(Burstein et al., 2007)
6	Human clinical trial	Viscum Album extract	Mistletoe plant	Activates the Caspase pathways associated with apoptosis	(Kovacs, Hajto, & Hostanska, 1991)

Table 1.4: Role of Flavonoids, Iso flavones, catechins and Polyphenols in breast cancer: (Siddiqui et al., 2015)

Sr. No.	Flavonoids	Signaling Pathway	Iso flavones & catechins	Signaling Pathways	Polyphenols	Signaling Pathway
1	Anthocyanindins (Cyanidin,delphinidin, malvidin, pelargonidin, peonidin, petunidin)	Ras-EKR-MAPKs and PI3K/AKT-mTOR-p OS6K	Biochanin A	ER and HER-2 pathway	Carotenoids	p38-MAPK pathway
2	Apigenin	HER-2/PI-3K/AKT Pathway	Daidzein	ER and HER-2 pathway75	CBD	ER and stress/ ERK and reactive oxygen species (ROS) pathways
3	Hesperitin	Bone Metastatic Pathway Glycolytic pathway	EGCG	ErbB2/ErbB3-PIK/AKT/ HIF-1α/NF-κB/VEGF	Curcumin	NF-κB and Wnt/β-catenin HER2/Akt/MAPK pathway
4	Kaempferol	MAPK pathway Drug efflux system	Gabridin	FAK/ SRC/AKT/ ERK1/2 signaling pathway.	Lignans	HER-2 and IGF-IR pathway
5	Luteolin	ER pathway IGF-IR signaling	Genistein	ER/HER-2/EGFR	Resveratrol	ER Pathway, EGFR/PI3K/ and ERK1/ERK2 pathway
6	Naringenin	ERK/PI3-K/AKT pathway	Glycetin	Not known		
7	Quercetin	Wnt/β-catenin, phosphatidyl inositol, 4-phosphate 5-kinase	Ipriflavone	Bone Metastatic Pathway.		

These phytocomponents inhibits the proliferation of cancer cells in breast cancer, kills the cells by apoptosis and hence reducing the risk of metastasis, curbing the disease spread and outgrowth.

Table 1.5: Isolated phytocomponents and their role in breast cancer: (Ranjan et al., 2019)

Sr. No.	Flavonoids	Major Sources	Iso flavones & catechins	Major Sources	Polyphenols	Major Sources
1	Quercetin	Onions, kale, broccoli, apples, cherries, fennel, sorrel, berries, tea	Genistein	Coffee, soybeans	Resvera-trol	Red wine, grapes, skin, peanuts, and several other foods/drinks
2	Kaempferol		Daidzein	Tofu, soybeans	Lignans	Cereals and grain products, vegetables, fruit, berries and beverages
3	Apigenin	Celery, Parsley, thyme, red pepper	Glycetin	soy	Curcumin	Turmeric
4	Luteolin		Gabridin	Licorice	Carotenoids	Tomatoes, carrots, Apricots, spinach,broccoli
5	Hesperitin	Citrus, prunes	Bio-chanin A	Red clover, peanuts	CBD	Cannabis
6	Naringenin		Ipri-flavone	Alfalfa		
7	Anthocyanindins (Cyanidin,delphinidin, malvidin, pelargonidin, peonidin, petunidin)	Cherries, Grapes	EGCG			

1.5 Effect of various plant-derived bioactive substances and their mode of action in the process of mammary carcinogenesis.

Different phytochemicals involved in regulating a wide range of molecular processes in order to modulate cancer.

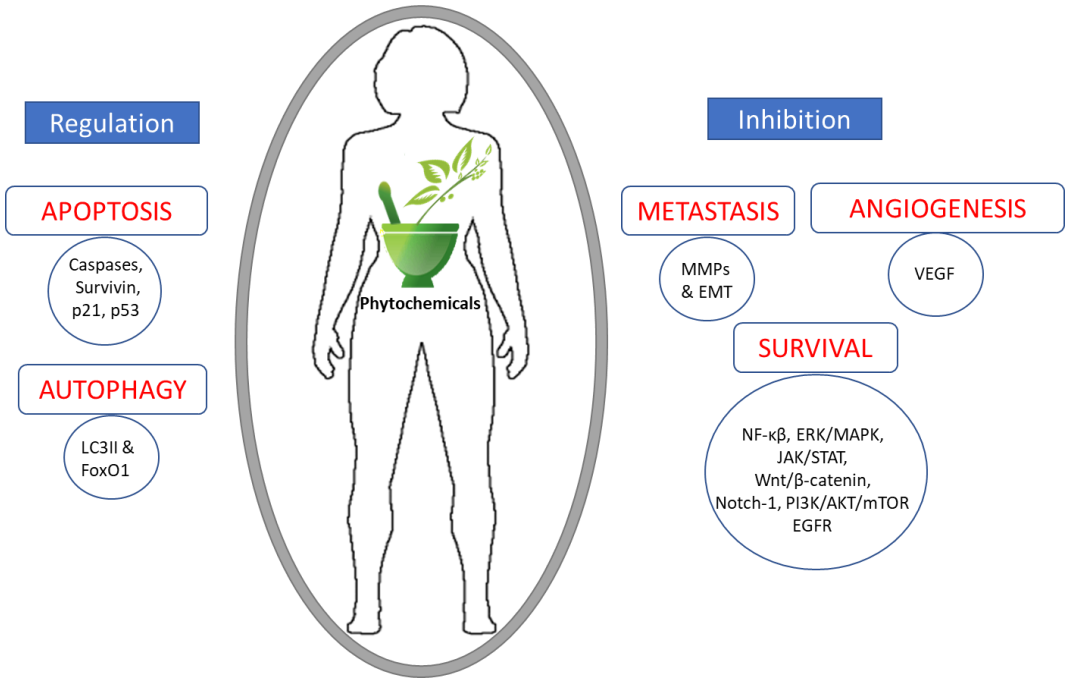

Figure 1.6: Holistic approach against cancer progression.

1.5.1 Impact on inflammation leading to carcinogenesis:

Inflammatory breast cancer is a rare and very aggressive disease in which cancer cells block lymph vessels in the skin of the breast. This type of breast cancer is called "inflammatory" because the breast often looks swollen and red, or inflamed (NIH). There is clear evidence that the immune system and inflammation play a critical role in the process of carcinogenesis and that inflammatory microenvironment is an essential component of all tumors (A. Mantovani et al., 2008). Various immune cells are frequently found accumulated in tumors. The important link between inflammation and carcinogenesis is the pro-inflammatory transcription factor, NF-κB (S. C. Gupta, Kim, Prasad, & Aggarwal, 2010). Moreover, the inflammatory mediators such as pro-inflammatory cytokines stimulate the survival and proliferation of premalignant cells and activate oncogenic transcription factors (Grivennikov et al., 2010), (Yu, Kortylewski, & Pardoll, 2007), (Karin & Greten, 2005).

Many plant-derived compounds have been found to play an important role in reduction of inflammation in breast cancer (Marín et al., 2007), (Min et al., 2007), (Kowalski, Samojedny, Paul, Pietsz, & Wilczok, 2005), (Xu, Shen, Chen, Gélinas, & Kong, 2005), (Lang et al., 2004), (Shishodia, Majumdar, Banerjee, & Aggarwal, 2003). (Table 1.6))

For example, perillyl alcohol showed impact on reduction of NF-κB DNA-binding activity and target gene induction in ER-negative mammary cells *in vitro* (S. Wang, Yang, & Lippman, 2003); (Yoon & Liu, 2007) showed that curcumin (at doses of 10–20 μM) and apple extracts (at dose of 5 mg/mL) significantly blocked the TNF-α-induced members of the Bcl-2 family of proteins including pro-apoptotic Bax and antiapoptotic Bcl-2. Resveratrol reduced expression of COX-2 and MMP-9, accompanied by reduced NF-κB activation in rat breast cancer tumors in an in vivo study (Banerjee, Bueso-Ramos, & Aggarwal, 2002). In other study, (Subbaramaiah et al., 2013), showed that the mixture of several dietary polyphenols from Zyflamend®, including resveratrol, EGCG, and curcumin suppressed levels of pro-inflammatory mediators (TNF-α, IL-1β, COX-2, phospho-Akt, phospho-p65, NF-κB-binding activity) in the mouse model of obesity-associated mammary gland inflammation.

Table 1.6: Role of phytocomponents effective against inflammation in breast cancer.

Sr No.	Phytochemical	Mode of action	Study Type	References
1	Perillyl alcohol	showed impact on reduction of NF-κB DNA-binding activity and target gene induction in ER-negative mammary cells	*In vitro*	(S. Wang et al., 2003)
2	Curcumin, Apple extracts	significantly blocked the TNF-α-induced NF-κB activation in MCF-7 cells by inhibiting the proteasomal activities.	*In vitro*	(Yoon & Liu, 2007)
3	Resveratrol	reduced expression of COX-2 and MMP-9, accompanied by reduced NF-κB activation in rat breast cancer tumors	*In vivo*	(Banerjee et al., 2002)
4	the mixture of several polyphenols from Zyflamend®, including resveratrol, EGCG, and curcumin	suppressed levels of pro-inflammatory mediators (TNF-α, IL-1β, COX-2, phospho-Akt, phospho-p65, NF-κB-binding activity) in the mouse model of obesity-associated mammary gland inflammation.	*In vivo*	(Subbaramaiah et al., 2013)

1.5.2 Induction of apoptosis leading to carcinogenesis

Apoptosis (programmed cell death), is a regulated process which serves to control cell turnover numbers Hallmarks of apoptosis are the fragmentation of DNA, activation of caspases (cysteine dependent, aspartate specific family of proteases), development of apoptotic bodies from fragmented cellular contents. These bodies can further phagocytize and be utilized by other cells present in the periphery. This feature allows the removal of damaged cells in a controlled manner with no inflammation (Sun & Peng, 2009). Inappropriate apoptosis is a characteristic feature that precedes many types of cancer. Cancer cells tend to not undergo apoptosis, allowing tumors to grow in a rapid and uncontrolled manner. The tumor suppressor gene TP53 plays one of the most important roles in this process. Its mutation leads to functional inactivation of the p53 protein, and thus, the cell loses the important DNA damage sensor capability that normally triggers the apoptotic cascade. Some other important players in apoptosis are the members of the Bcl-2 family of proteins including pro-apoptotic Bax and antiapoptotic Bcl-2 (Kapinova et al., 2018) (Table 1.7).

1.5.3 Inhibition of proliferation leading to protection against metastasis

Cell proliferation depends on how quickly a cancer cell copies its DNA and divides into 2 cells. If the cancer cells divide more rapidly, it means the cancer is growing faster or more aggressive. One of the major characteristics of carcinogenesis is a dysregulated and aggressive proliferation and rapid growth of the tumor cells. The case of normal healthy cells is their proliferation finely regulated through a balance between the growth and antigrowth signals. However, cancer cells develop the ability to grow uncontrollably, and they generate their own growth signals and become insensitive to antigrowth signals (Gupta SC et al. 2010), (Hanahan D et al. 2000). The important factors that regulate the cell through its natural progression are the cyclins, cyclin-dependent kinases, COX-2, and c-Myc. In case of cancer, they can be upregulated causing uncontrollable cell proliferation (Table 1.8).

Table 1.7: Role of phytocomponents effective against carcinogenesis in breast cancer.

Sr No.	Phytochemical	Mode of action	Study Type	References
1	Some isolated phytochemicals	effectively trigger the activation of effector caspases in the process of apoptosis, such as caspase-3 and caspase-7, or the others, and increased Bax/Bcl-2 pro-apoptotic ratio	*In vitro*	(Y.-J. Zhang et al., 2015)
2	Acetoxychavicol acetate	In breast carcinoma-derived MCF-7 and MDA-MB-231 cell line, their results showed decrease of tumor cell viability through a caspase-3-dependent increase in apoptosis.	*In vitro*	(Campbell et al., 2007)
3	Sanguinarine	induced apoptosis in MDA-MB-231 human breast carcinoma cells through several mechanisms, including activation of caspase-3 and caspase-9	*In vitro*	(Choi, Kim, Lee, & Choi, 2008)
4	Sulforaphane	induced cell type-specific apoptosis in various human breast cancer cell lines.	*In vitro*	(Pledgie-Tracy, Sobolewski, & Davidson, 2007)
5	Pterostilbene	induced apoptosis of MCF-7 and MDA-MB-231 breast cancer cells through Bax activation	*In vitro*	(McCormack & McFadden, 2013)
6	Quercetin	induce apoptosis and necroptosis in MCF-7 cells	*In vitro*	(Khorsandi et al., 2017)
7	Lutein	inhibits growth of mammary tumors in female BALB/c mice by regulating apoptosis.	*In vivo*	(Krinsky, Landrum, & Bone, 2003)

Table 1.8: Role of phytocomponents effective against proliferation in breast cancer.

Sr No.	Phytochemical	Mode of action	Study Type	References
1	carotenoids	Showed antiproliferative effects. They cause cell cycle arrest at various stages of the cell cycle, many of them just by affecting cyclins.	*In vitro*	(J. A. Kim, Jang, & Lee, 2021)
2	Sesamin	downregulate cyclin D1 expression in a wide variety of tumors, including human breast cancer in *in vitro* testing	*In vitro*	(Yokota et al., 2007)
3	Beta-sitosterol and crocin	inhibition of growth in human breast cancer cell lines	*In vitro*	(Awad, Williams, & Fink, 2001)
4	Genistein	induced G2/M cell cycle arrest in MCF-7 breast cancer cell line	*In vitro*	(Pagliacci et al., 1994)

1.5.4 Impact on metastasis and angiogenesis:

Cancer cell invasion and metastasis are processes which involve growth, adhesion, and migration of cancer cells, and also proteolytic degradation of tissue barriers– extracellular matrix and basement membrane (S. C. Gupta et al., 2010). Some matrix metalloproteinases (MMP-2, MMP-9) and intercellular adhesion molecule (ICAM-1) participating in the degradation of these barriers (Sternlicht & Werb, 2001). Recent reports from breast cancer studies have demonstrated antimetastatic and antiangiogenic effects of various phytochemicals (Kapinova et al., 2018) (Table 1.9).

Table 1.9: Role of phytocomponents effective against metastasis and angiogenesis in breast cancer.

Sr No.	Phytochemical	Mode of action	Study Type	References
1	two chalcones–2-hydroxychalcone and xanthohumol	inhibited the growth and invasiveness of triple negative breast cancer cell line MDA-MB-231. These chalcones were able to decrease the secreted level of MMP-9 in cancer cells.	*In vitro*	(S. Y. Kim, Lee, & Moon, 2013)
2	Apigenin	play an important role in inhibition of adhesion and motility of breast cancer cells. It showed the ability to mediate the HER2-HER3-PI3K-AKT pathway in this experiment.	*In vitro*	(Way, Kao, & Lin, 2005)
3	diindolylmethane	decreased the CXCR4 and CXCL12 levels, in MCF-7 and MDA-MB-231 breast cancer cell lines. The chemokine receptor CXCR4 and its ligand CXCL12 are desired for metastatic activity of mammary cells	*In vitro*	(Hsu et al., 2009)
4	Flavopiridol	inhibited the secretion of MMP-2 and MMP-9 in mammary cancer cells	*In vitro*	(L. Wang et al., 2006)
5	sanguinarine, ganoderic acids, genistein, [6]-gingerol, silibinin, phytic acid, and indole-3-carbinol	suppress invasive behavior of breast cancer cells	*In vitro*	(Xiang et al., 2016)
6	Flavin7 (flavonoids)	flavonoids inhibit the growth of mammary tumor cells by suppressing of the VEGF/VEGFR-2 signalling pathways. Flavin7 inhibited endothelial cell migration and capillary tube formation that indicates its potential antiangiogenic properties and also inhibited the activity of matrix metalloproteinases (MMP-9 and MMP-2) which play an important role in tumor cell invasion. In HUVEC-lines	*In vitro*	(Mojžiš et al., 2008)

1.5.5 Impact on breast cancer stem cells

Cancer stem cells (CSCs), sometimes referred to as "tumor-initiating" or "tumor propagating" cells, are a small but aggressive population of cells within the tumor mass which have the ability of self-renewal, differentiation into tumor cells, invasiveness, and metastatic activity (Velasco-Velázquez MA et al. 2012) (Kai K et al. 2010) (Wu CH et al. 2015). The subpopulation of putative human breast cancer stem cells (BCSCs) have a specific cell-surface antigen profile. Their identification from tumor samples and mammary cancer cell lines has been based mainly on CD44, CD24, and ALDH1 phenotypes. BCSCs are generally CD44 positive/CD24 negative (CD44+/CD24−) and ALDH1 positive (ALDH1+). Furthermore, BCSCs express higher levels of oxidative stress-responsive genes, which could be also responsible for their ability to resist anticancer therapy, than non-CSCs (Velasco-Velázquez MA et al. 2012) (Kai K et al. 2010) (Al-Hajj M et al. 2003). Only few *in vitro* or in vivo studies have evaluated the effects of plant-derived compounds (isolated or mixture) on BCSCs (Table 1.10)

There is a lack of data confirming anti-CSC action of phytochemicals to this date; further preclinical and clinical studies and validation of cell signaling pathways are needed in this research area. (Kapinova, A et al.2018)

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Table 1.10: Role of phytocomponents effective against breast cancer stem cells.

Sr No.	Phytochemical	Mode of action	Study Type	References
1	Pterostilbene and 6-shogaol	the expression of CD44 in BCSCs. promoted β-catenin phosphorylation through the inhibition of hedgehog/Akt/GSK3β signaling, and this way decreased the protein expression of downstream c-Myc and cyclin D1 and reduced BCSCs	*In vitro*	(C.-H. Wu, Hong, Ho, Yen, & chemistry, 2015)
2	combination of curcumin, genistein, indol-3-carbinol, c-phycocyanin, resveratrol, and quercetin	downregulated the expression of several oncostatic markers, including CD44 in MCF-7 and MDA-MB-231 breast cancer cell lines.	*In vitro*	(Ouhtit et al., 2013)
3	curcumin (alone or in combination with piperine)	Anti-BCSC	*In vitro*	(Kakarala et al., 2010)
4	Sulforaphane	eliminated BCSCs *in vitro* and *in vivo* as well. This compound decreased ALDH1-positive cells in human breast cancer cell line and reduced the number and size of primary mammospheres. It eliminated also BCSCs abrogating tumor growth in mouse model. Moreover, researchers showed that sulforaphane downregulated the Wnt/β-catenin self-renewal pathway.	*In vitro*	(Y. Li et al., 2010)
5	oregano	confirmed the inhibitory effect of oregano against BCSCs in rat mammary breast cancer model. The immunoexpression of CSCs markers–CD24, and EpCAM were significantly decreased in rat mammary cancer cells after oregano treatment.	*In vivo*	(Kubatka et al., 2017)
6	Cloves (active component not mentioned.)	In the rat model, cloves significantly decreased CD24 and CD44 markers, however increased ALDH1 expression in mammary carcinoma cells	*In vivo*	(Kubatka et al., 2017)

1.6 Plant derived Drugs in pipeline for breast cancer

Breast cancer has continued to cause high cancer death rates among women worldwide. The use of plants' natural products in breast cancer treatment has received more attention in recent years due to their potentially wider safety margin and the potential to complement conventional chemotherapeutic drugs. Plant based products have demonstrated anticancer potential through different biological pathways including modulation of the immune system. Immunomodulatory properties of medicinal plants have been shown to mitigate breast cancer cell growth. Different immune cell types participate in this process especially cytotoxic T cells and natural killer cells, and cytokines including chemokines and tumor necrosis factor-α. Medicinal plants such as Glycyrrhiza glabra, Uncaria tomentosa, Camellia sinensis, Panax ginseng, Prunus armenaica (apricot), Allium sativum, Arctium lappa and Curcuma longa were reported to hold strong potential in breast cancer treatment in various parts of the world. Interestingly, research findings have shown that these plants possess bioactive immunomodulators as their main constituents producing the anticancer effects. These immunomodulatory compounds include ajoene, arctigenin, β-carotene, curcumin, epigallocatechin-3-gallate, ginsan, glabridin and quinic acid. This review, discusses the ability of these eight immunomodulators in regulating the immune system potentially applicable in breast cancer treatment via anti-inflammatory (curcumin, arctigenin, glabridin and ajoene) and lymphocytes activation (β-carotene, epigallocatechin-3-gallate, quinic acid and ginsan) properties, as well as future research direction in their use for breast cancer treatment (Baraya, Wong, & Yaacob, 2017).(Table 1.11)

Examples of natural therapeutics that have been tested in clinical trials for cancer treatment .are mentioned in Table 1.12

Table 1.11: Pre-clinical studies of plant-derived bioactive substances and their mode of action in the process of mammary carcinogenesis.

	Apoptosis	BCSCS	Inflammation	Metastasis and angiogenesis	Non-coding RNAs	Proliferation
Acetoxychavicol acetate	+					
Apple extracts			+			
Apigenin				+		
Artemisinin					+	
Beta-Sitoterol						+
Chlorella	+			+		
Cloves						+
Curcumin		+	+		+	
Curcumin with Piperine		+				
Di-indolylmethane				+		
EGCG			+			
Flavopiridol				+		
Fruit peel polyphenols	+			+		+
Ganoderic acids				+		
Genistein				+		+
[6]-Gingerol				+		

Glyceollin					+	
2-Hydroxy Chalcone				+		
Indol-3-carbinol				+	+	
Lutein	+					
Oregano	+	+		+		+
Perillyl alcohol			+			
Phytic Acid				+		
Pterostilbene	+	+				
Quercetin	+					
Resveratrol			+		+	
Sanguinarine	+			+		
Sesamine						+
6-Shogaol		+				
Silibinin				+		
Sulphoraphane	+	+				
Xanthohumol				+		
Young Barley	+					+

Table 1.12: Natural therapeutics tested in clinical trials for cancer treatment.

No	Cancer type	Plant/phytocomponent	Therapeutic strategy			Clinical trial/stage	Outcome
			Chemotherapy	Radiotherapy	Other		
1	Prostate	Curcuma Longa/ Curcumin	-	yes	-	I	Unknown
	Breast		-	-		I	Positive
	Lung		-	-	tyrosine kinase inhibitors	I	
	Colon		yes	-	-	I	Ongoing
	Colorectal		-	-	Curcumin alone	I	Ongoing
	Head and neck		-	-	Curcumin supplemented with turmeric extract	I	Unknown
	Advanced cancers		-	-	Water soluble curcumin Combination with other cancer drugs	I	Unknown
2	Cancers	Camellia sinensis/ As Green Tea	-	-	Green tea vs placebo	N/A: interventional	unknown
	Colorectal		-	-	Green tea and milk thistle supplements	I	Ongoing
	Prostate		-	-	Green tea with plant phenol Quercetin	I	Ongoing
	Breast		-	-	Green tea extract supplement	II	Unsuccessful

	Head and neck		-	-	tyrosine kinase inhibitor	I	Unknown4
3	Breast	Sorghum bicolour/ Jobelyn	yes	-	-	I/II	Unknown
4	Pancreas	Ocoxin	yes	-	-	II	Recruiting
	Advanced cancers		yes	-	-	II	Recruiting
	Stomach		yes	-	-	II	Recruiting
	Breast		yes	-	-	II	Recruiting
	Ovarian		yes	-	-	II	Recruiting
5	Prostate	Sage	-	-	To regulate hot flashes	I	Unknown
6	Lung	Cruciferous plants/Sulphorane	-	-	Sulforaphane vs placebo	II	
	Prostate		-	-	Sulforaphane alone	II	Recruiting
	Bladder		-	-	Sulforaphane vs placebo	II	To begin
	Breast		-	-	Sulforaphane from broccoli extract in mango juice	II	Positive
7	Prostrate	Tomato/Tangerine	-	-	Tangerine tomato juice alone	N/A: interventional	Unknown

1.7 Cancer therapy approaches: With the advancement in cancer research, new strategies used to treat cancer, combining different disciplines to obtain the most efficient and personalised therapy for patients (figure no. 1.7)

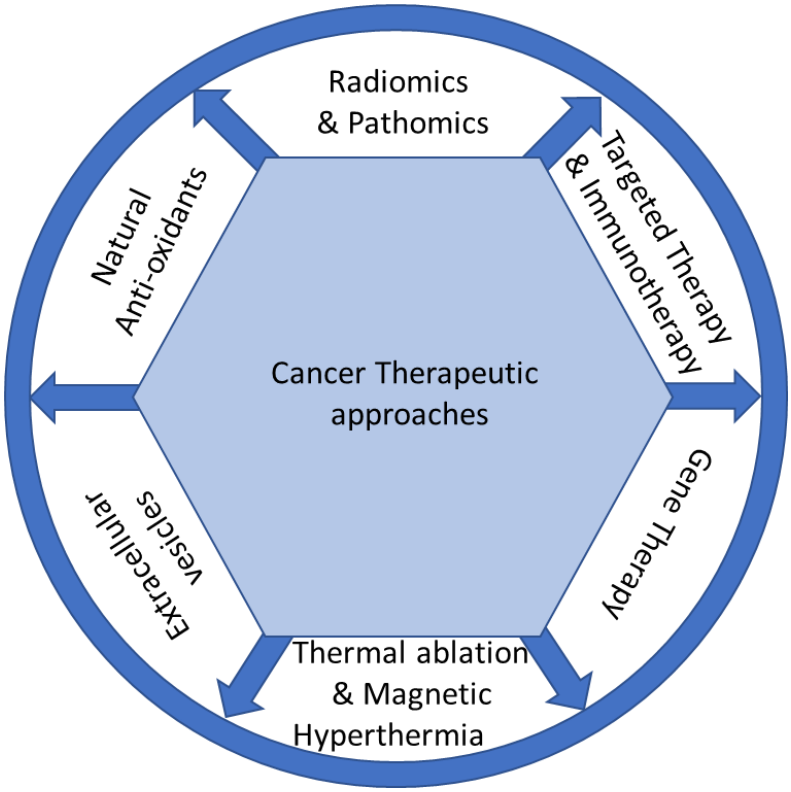

Figure 1.7: Innovative approaches for cancer treatment (Nagai & Kim, 2017); (Pucci, Martinelli, & Ciofani, 2019)

Despite advances in research against cancer like targeted therapy and immunotherapy, gene therapy, thermal ablation and magnetic hyperthermia, radiomics and pathomics, cancer is still unconquered. Among the various research leads, an effort to identify novel drugs is most pursued due to the side effects of the known anti-cancer compounds. Hence, there still exists a need to identify novel compounds with greater therapeutic potential. Phytocompounds acting individually or synergistically having ability to halt cancer at either early or late stages are the most promising.

Extensive literature survey, provide evidence that *Bauhinia variegata* L. is a plant with multitude potentials. It has good anti-oxidant and radical scavenging property, a property which has been seen in most molecules with good therapeutic value (P. K. Gupta & Sahu, 2012). Ethanolic extract of Bauhinia has shown an increase survival

time of Swiss albino mice with Dalton's ascetic lymphoma (DAL), proving its efficacy against tumors (B. Rajkapoor, B. Jayakar, & N. Murugesh, 2003). Additionally, there is a report depicting the decrease in cyclophosphamide induced micronucleus formation in Swiss Mouse Bone Marrow cells (P. R. Pandey et al., 2011). The bark of stem is also known to possess anti-carcinogenic property as described in an investigation where it was observed that there was reduction and delayed papilloma formation in the skin papilloma Swiss Albino Mice model, treated with Bauhinia extract (Agrawal & Pandey, 2009). Antitumour activity of ethanolic extract of *B. variegata* was also reported against N-nitrosodiethylamine induced liver tumour in rats (Rajkapoor, Jayakar, Murugesh, & Sakthisekaran, 2006).

In vitro studies have also been reported with the leaves of this plant which demonstrates that aqueous extract of *Bauhinia variegata* L. has significant cytotoxic potential on MCF-7 and T47 D cell lines compared to other extracts (K. Mishra, Ojha, & Chaudhury, 2012). Gunalan and Vijayalakshmi, 2016 reported n-hexane and ethyl-acetate-methanol fractions of *B.* variegata leaf to possess good cytotoxicity against colon cancer cells. It induced DNA damage and cell cycle arrest at sub G1 and G2/M phase respectively in COLO 320 cells. ID7 fraction isolated from *Bauhinia variegata* L. stem was found to inhibit cell viability, migration, invasion in the 4T1 and MDA-MB-231cells. It increased the late apoptosis, adhesion, expression of PARP, caspase-7, caspase-8, RIP and TNF-R1and reduced the secreted active gelatinases indicating attenuated tumour volume and weight, reduced inflammation in the liver and metastasis in lung (Monteiro et al., 2019). Similar results with *Bauhinia variegata* stem fraction on human peripheral blood mononuclear cells (PBMCs) and human cervical tumor cells (HeLa), inducing cell death process by activating Caspase-3, TNF-R1 and RIP have also been reported (K. M. Santos et al., 2018). *Bauhinia variegata* L. ethanolic bark extract showed the cell cycle arrest of HeLa cell lines in G0/G1 phase and apoptotic cell death by flow cytometric analysis (B. Kumar & Bhat, 2014). The methanolic bark extract exhibited cytotoxicity against C-6 glioma rat brain, HCT-15 colon cancer and MCF-7 breast cancer cell lines (N. Sharma et al., 2019).