Chapter 1

Introduction

1. Introduction

Breast cancer is considered the most diagnosed cancer in females, with more than 1 million women diagnosed and approximately 400000 women deceased every year globally [1]. Triple–negative breast cancer (TNBC) is a subtype of breast cancer characterized by the absence of estrogen receptors (ER), progesterone receptors (PR), and human epidermal growth factor receptor 2 (HER2). Representing only 10–20 % of all breast cancer cases, TNBC is both aggressive and relatively rare. Recently, it has garnered significant attention in biological and clinical research due to its aggressive nature, poor prognosis, and the lack of effective endocrine therapies [2].

1.1. Epidemiology

TNBC is more commonly seen in women with higher frequencies of BRCA gene mutations (BRCA1 and BRCA2). Geographically, it is diagnosed at higher rates among African American and Hispanic women, particularly those under the age of 40. Globally, TNBC accounts for approximately 12 % of all breast cancer cases, with a low survival rate of 8–16 % [3]. In India, the prevalence of TNBC is notably higher, ranging from 27–35 %, with an estimated average prevalence of around 31 % [4].

1.2. Heterogeneity of TNBC

Lehmann *et al*., 2011, identified six molecular subtypes of TNBC by gene expression analysis (Figure 1. 1A). Each subtype elucidates distinctive biology and *in vitro* responses optimal to various therapies. The TNBC subtypes comprised two basal–like subtypes, two mesenchymal subtypes, an immunomodulatory type, and a luminal subtype [5]. Gene expression analysis also identifies the cell lines representative of each TNBC subtype to evaluate their responses toward different treatments [6].

- **Basal–like 1**: Basal–like 1 (BL 1) subtype shows an elevated expression of ki67 mRNA, as characterized by their rapid cell division, enhanced proliferation, and loss of cell cycle control. They are responsive to various antimitotic agents.
- **Basal–like 2**: Basal–like 2 (BL 2) subtype shows the increased expression of growth factors signaling like EGFR, with myoepithelial markers like TP63, MET, etc. They are associated with the activation of glycolysis and gluconeogenesis routes.
- **Immunomodulatory**: Immunomodulatory (IM) subtype comprises signaling pathways responsive to the immune system, including the expression of antigens and cytokines.

- **Mesenchymal** and **Mesenchymal stem–like:** Mesenchymal (M) and Mesenchymal stem–like (MSL) subtypes are associated with the expression of epithelial–mesenchymal transition markers (PI3K, mTOR, SRC), including angiogenesis (VEGFR2), stemness and growth factors (FGFR, IGFR, PDGFR).
- **Luminal Androgen Receptor**: The Luminal Androgen Receptor (LAR) subtype is characterized by the expression of androgen receptors (AR). They maintain an elevated hormonal component regulated by steroid synthesis, porphyrin metabolism genes, and androgens.

Breast cancer that has a triple–negative phenotype includes a diverse set of histological subtypes (Figure 1. 1B). The majority of TNBC cases (~95 %) are classified as **invasive mammary carcinomas** without any specific type. The other subtypes include **medullary carcinoma,** which is rare (~0.4–1 %) and characterized by high lymphoplasmacytic infiltration, **adenoid cystic carcinoma**, **adenosquamous carcinoma,** and **spindle–cell metaplastic carcinomas** (~<1 %) [7].

It has been expressed that the tumor microenvironment (TME) is also recognized as an indicator of immunotherapy efficacy as well as patient prognosis (Figure 1. 1C). Further, the phenotype of TME is characterized by the prevalence of TILs, which were found to be in high levels in patients suffering from early–stage TNBC. It was observed that TILs in TNBC indicate enhanced overall survival, increased metastasis–free survival, and limited distant recurrence [7]. Based on these findings, Xiao *et al*., 2019, classified TNBC into three subtypes as per the plethora of microenvironment cells around the tumor site and TILs: (i) **immune–desert TNBC (Low)**, having less microenvironment cell infiltrations, (ii) **innate–immune activated TNBC (Intermediate)**, involving resting innate immune and non–immune stromal infiltrating cells, and (iii) **immune–inflamed TNBC (High)**, having immune cells infiltrations of both adaptive as well as the innate type [8].

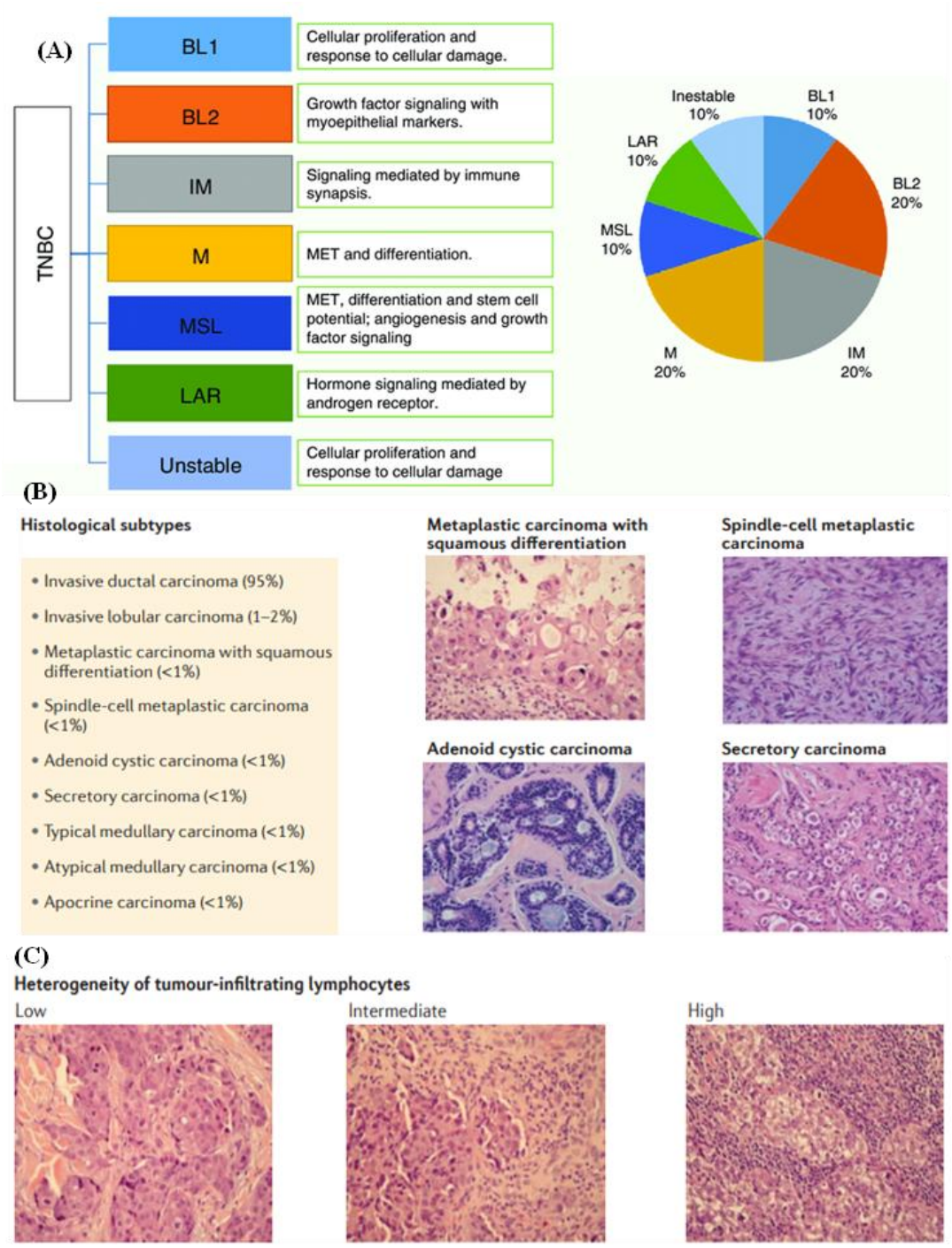

Figure 1. 1: The heterogenous landscape of TNBC: (A) Molecular subtypes defined by Lehmann *et al.,* 2011 (B) Histological subtypes, (C) Heterogeneity of tumor–infiltrating lymphocytes. Tumors with low, intermediate, and high lymphocyte infiltration. BL 1, basal–like 1; BL 2, basal–like 2; IM, immunomodulatory; M, mesenchymal; MSL, mesenchymal stem–like; and LAR, luminal androgen receptor. Adapted from Ref. [7].

1.3. Metastasis driven complexity

TNBC metastasis is a complex process outlined by genetic and epigenetic transformation, stroma and tumor interactions, angiogenesis, intravasation via the basal membrane into the blood or lymphatic circulation, and extravasation (Figure 1. 2) [9]. It is explained that upon genetic and epigenetic transformation, the TNBC cells acquire self–renewal and migration ability, which aid them in invading the locally surrounded normal tissues and the circulation. During the local invasion and intravasation, the TNBC cells experience various phenomena like epithelial–to–mesenchymal transition (EMT), the disintegration of cell–cell junctions, overexpression of mesenchymal genes, and reduced expression of epithelial markers [10]. This entire process of migration and intravasation into the systemic circulation is triggered by several transcription factors, namely SLUG, SNAIL, TWIST, etc. [11]. In another study, it was observed that the expression of TGFβ (Transforming growth factor–β)/Smad signaling activates EMT, which in turn regulates WAVE3 (actin–binding protein belonging to the WASP/WAVE family), and the overall process leads to the TNBC cells intravasation. Likewise, triggering of C–X–C chemokine receptor type 4 (CXCR4) by C–X–C motif chemokine ligand 12 (CXCL12) induces the signaling of Mixed lineage protein kinase 3 (MLK3) and Extracellular signal–regulated protein kinases 1 and 2 (Erk1/2) proteins, which develop intravasation and lead to lung and bone metastases [12]. Moreover, it was found that when the Tropomyosin receptor kinase B (TRKB) receptor binds with the brain–derived neurotrophic factor (BDNF), a metalloproteases network and calmodulin protein become regulated. As a result, the tumor–endothelial cell interaction gets altered, which leads to lung and brain metastasis in TNBC. It is further noted that other genes like Epiregulin (EREG), Cyclooxygenase 2 (COX2), and Matrix metalloproteinases (MMP1 and MMP2) are equally overexpressed in TNBC and play an important role in promoting lung metastasis. Most of the genes or proteins taking part in inducing TNBC metastasis have been observed to exhibit their respective roles during the early stages of TNBC, i.e., from migration to intravasation. This emphasizes the significant challenges faced during diagnosis and treatment in the early stage of TNBC. Therefore, identification of these TNBC metastasis–inducing genes and proteins provides an opportunity for the development of therapies against TNBC–metastasis [13].

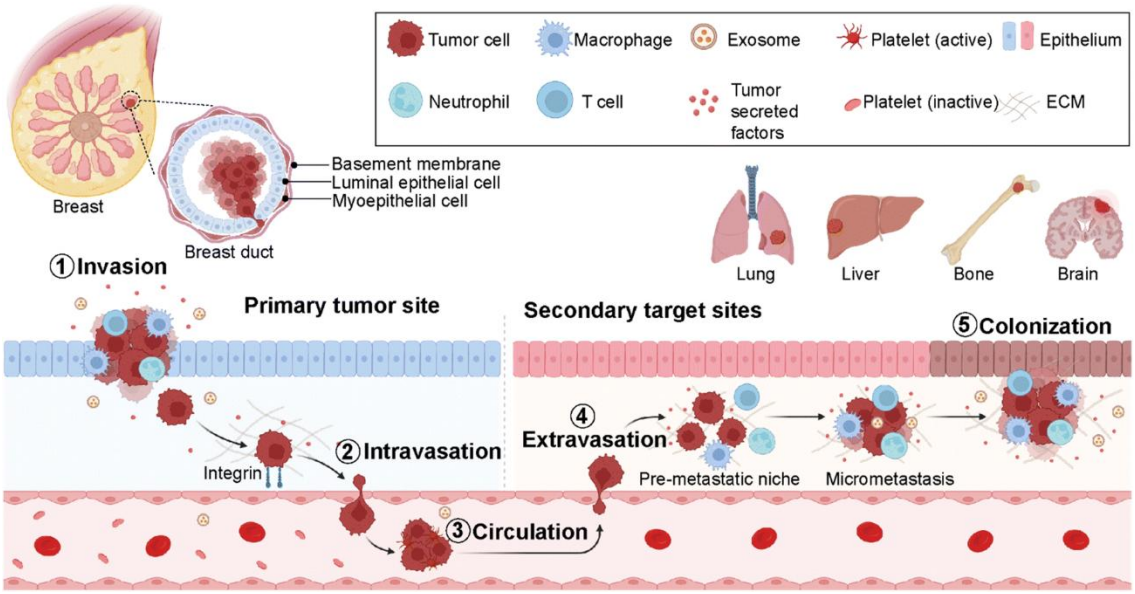

Figure 1. 2: TNBC metastatic cascade. Schematic representation of key components involved in breast cancer metastasis from the primary tumor site to secondary target sites like lung, liver, bone, and brain. Adapted from Ref. [14].

1.4. Treatments of TNBC

TNBC patients are treated with various therapeutic interventions, depending on their type and stage of cancer. Early–stage and low–risk TNBC treatment requires careful consideration of the balance between risks and benefits. TNBC is responsive to both immunotherapy and chemotherapy due to its characteristics, such as metastasis, mutational capacity, increased TILs, and programmed cell death. The diverse nature of TNBC provides multiple targets for treatment [15].

1.4.1. Surgery

TNBC is treated by surgery i.e., lumpectomy or mastectomy, and can be performed depending on the stage of the cancer [16]. Surgery is commonly employed in cases of locoregional or sentinel lymph node metastatic breast cancer to enhance treatment efficacy. Depending on the patient's clinical condition, it can be used alone or with chemotherapy. Surgery helps improve survival rates, reduce mortality risks, and address complications and metastases [17].

1.4.2. Radiation

TNBC patients seem to develop distant and regional recurrences at a higher rate than their non–TNBC counterparts [18]. Radiation therapy is frequently administered subsequent to mastectomy to eradicate residual cancer cells in the lymph nodes and breast. It is also

employed to address metastatic cancer. Nevertheless, radiation therapy may result in relapse in 7–12.6 % of patients within 5 years, and resistance can ensue. Consequently, a combination of radiotherapy and immunotherapy is commonly utilized. Trials are underway to investigate the potential of radioimmunotherapy in manipulating the immune system for TNBC, as it lacks expression of other molecular targets, to enhance treatment outcomes. Notably, a phase 1 study integrating pembrolizumab with intraoperative radiation therapy (IORT) is presently being conducted under open-label, single–arm, single–institution conditions in newly diagnosed triple–negative, node–negative breast cancer patients (NCT02977468) [19].

1.4.3. Chemotherapy

The standard approach for treating patients with TNBC involves a combination of chemotherapy and surgery. This approach effectively targets cancer cells throughout the body. Systemic chemotherapy, utilizing drugs such as anthracyclines (e.g., doxorubicin) and taxanes (e.g., paclitaxel) in the neoadjuvant (NACT) or adjuvant setting, is the established treatment for TNBC, as indicated in figure 1. 3. [20]. However, emerging studies indicate that chemotherapy should be tailored based on tumor size. Notably, Gupta *et al.,* 2020 observed favorable prognoses for TNBC tumors sized between 0.5 cm and 1.0 cm without lymph node involvement [21]. Conversely, another research group suggested that adding chemotherapy to TNBC tumors smaller than 1 cm in diameter may not yield significant benefits [22].

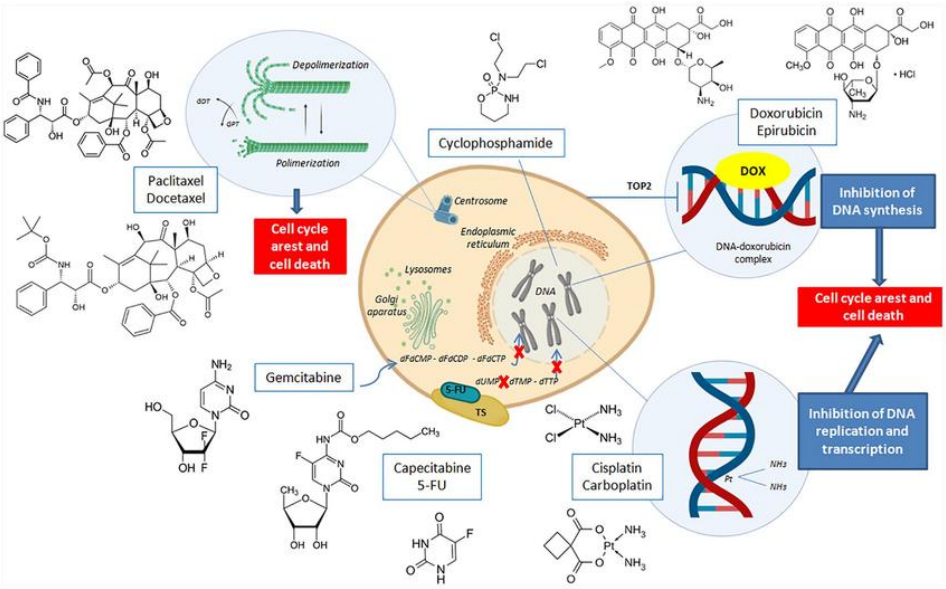

Figure 1. 3: Mechanisms of action of the main chemotherapeutic agents in TNBC. Adapted from [23]

Anthracyclines

The standard chemotherapy regimen for TNBC typically comprises anthracyclines and taxanes. Anthracyclines destabilize DNA through intercalation, leading to the degradation of the DNA repair cascade, which can confer benefits for TNBC [24]. Research indicates that anthracyclines directly eradicate cancer cells and activate the immune system by stimulating CD8+ T cells [25]. Studies have demonstrated that anthracyclines, such as doxorubicin and epirubicin, can enhance response rates and survival by several months. These agents have shown favorable outcomes and heightened sensitivity, whether employed as a standalone treatment or in the neoadjuvant setting. Patients with wild–type BRCA1/2 genes who have not undergone neoadjuvant or adjuvant treatment with these agents are commonly administered anthracyclines. Additionally, anthracycline–based therapy has exhibited a superior response rate [26]. It was observed that patients suffering from BL 1 or MSL subtype TNBC showed an increased rate of pCR, as compared to BL 2 and LAR subtypes, which are not sensitive to combination regimens [18].

Taxanes

The distinct mechanism of action of taxanes, coupled with their broad spectrum of applications in cancer therapy, positions them as a significant class of anticancer agents. Key molecular mechanisms of taxanes encompass the disruption of the mitotic spindle, induction of mitotic slippage, and inhibition of angiogenesis. Taxanes serve as anti–mitotic agents by impeding the depolymerization of microtubules, thereby arresting cell division at the prometaphase stage of the cell cycle [27]. This inhibition prevents the formation of spindles and spindle fibers, ultimately leading to the cessation of cell division. Additionally, taxanes' anti-cancer activity has been observed through activated macrophages [18]. Evidence suggests that taxanes, combined with anthracyclines, benefit TNBC compared to other subtypes, with pathological complete response (pCR) rates of at least 40 % [28]. Three taxanes, namely paclitaxel, docetaxel, and cabazitaxel, have received approval for clinical use [29]. Gene profiling analysis indicates that BL 1 and BL 2 subtypes of TNBC exhibit sensitivity to anti–mitotic drugs such as paclitaxel and docetaxel. Moreover, basal–like subtypes demonstrate a four–fold increase in remission rates compared to MSL and LAR subtypes of TNBC.

Platinum

The impact of platinum agents on DNA strand breaks and apoptosis is well documented. Their distinctive mechanism of action renders them particularly efficacious against cancerous tissues with impaired DNA repair processes, including those harboring detrimental BRCA variants. Patients afflicted with BRCA1/2 mutant TNBC and other deficiencies in homologous recombination have exhibited notable therapeutic advantages from platinum–based treatment regimens [26]. Cisplatin and carboplatin stand as widely utilized anticancer pharmaceuticals [30].

1.4.4. Immunotherapy

Immunotherapy has emerged as a treatment modality involving the administration of diverse agents to augment the localized function of the immune system within the tumor or to obstruct immune evasion pathways of tumor cells by normalizing the anti–tumor immune response, thereby facilitating the identification and eradication of tumor cells. These immunotherapeutic strategies encompass therapeutic cancer vaccines (TCVs) as active immunotherapies, targeted monoclonal antibodies (mAb) and their derivatives, such as antibody–drug conjugates (ADCs), adoptive cell therapies (ACTs), and cytokines as passive therapies. Immune checkpoint inhibitors (ICIs) are among the most widely recognized immunotherapy modalities. [30].

Anti–PD–1 /PD–L1 therapy

PD–1, found on immune cells like T, B, NK, and myeloid cells, triggers SHP2 when binding with PD–L1. This downregulates the PI3K/AKT axis, inhibiting T and B cell proliferation, inducing programmed T cell death, preventing autoimmune diseases, and enabling immune escape in tumor cells [31]. The Food and Drug Administration (FDA) has approved the following PD–1/ PD–L1 inhibitors: nivolumab, pembrolizumab, atezolizumab, cemiplimab, avelumab, and durvalumab [32]. Research findings indicate that PD–L1 is detected in approximately 20–30 % of TNBC cases. Furthermore, its presence is associated with lymphocyte infiltration and histological grades. [33].

Anti–CTLA–4 therapy

The CD28 and CTLA–4 receptors on T cells bind to CD80/CD86, providing positive and negative feedback for T cell activation, respectively. CTLA–4 impedes T cell growth, cell cycle progression, IL–2 production, and T cell differentiation, thereby serving as a specific

regulator of the CD4+ T cell response and maintaining T cell homeostasis. Notably, the expression of CTLA–4 is observed to be highest in TNBC [34].

Cytokines

Cytokines serve as pivotal regulators of both the innate and adaptive immune systems, governing leukocyte proliferation, differentiation, survival, and effector functions through localized communication in the paracrine and autocrine fashion within immune systems. They hold the potential to augment the anti–tumor immune response. The anti–IL–8 antibody HuMax–IL–8, also called BMS–986253, was engineered to effectively deplete tumor–secreted IL–8 and impede the mesenchymal properties of cancer stem cells. [35].

Monoclonal antibody and antibody–drug conjugate

Monoclonal antibodies (mAbs) and antibody–drug conjugates (ADCs) target tumor–specific antigens to kill tumor cells or inhibit tumor growth. Sacituzumab govitecan is an ADC employed for treating TNBC, targeting the human trophoblast cell–surface antigen 2 (Trop 2) and delivering SN–38, a topoisomerase I inhibitor. Other ADC targets for TNBC treatment include zinc transporter LIV–1 (SLC39A6) and folate receptor alpha (FRα) [36].

1.4.5. Targeted therapy

Overexpressed proteins in TNBC may be crucial for treating certain cases and could serve as future therapeutic targets. Aberrant signal pathways play a role in regulating growth, survival, and chemoresistance in TNBC, as depicted in figure 1. 4.

DNA damage and repair

Genetic alterations in the response to DNA damage are potential targets for treating TNBC. Cyclin–dependent Kinases (CDKs), Chk1, and BRCA1 are critical in regulating the cell cycle and DNA repair. Approximately 19.5 % of TNBC patients carry BRCA1/2 mutations. These mutations sensitize tumor cells to PARP inhibitors. Combining a PARP inhibitor with cisplatin or carboplatin has been shown to prolong the overall survival of mice with TNBC tumors [37]. CDKs, including CDK4 and CDK6, are altered in residual TNBC. A combination of an aromatase inhibitor and Ribociclib has been approved for hormone–receptor–positive/HER2–negative metastatic breast cancer. Inhibition of CDKs using

dinaciclib or purvalanol A had a lethal effect on TNBC cells. Additionally, CDK4 inhibition decreased chemotherapy–resistant cells and breast cancer stem cells in TNBC [38].

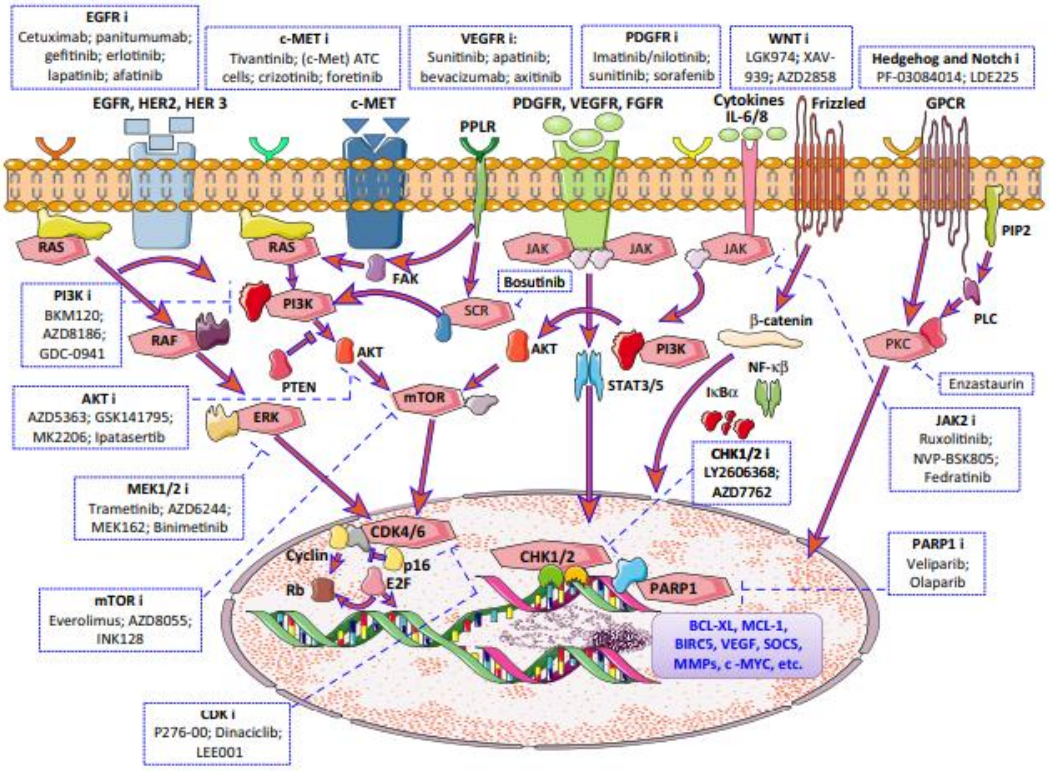

Figure 1.4: Schematic pathway–centric view of the molecular targets identified within TNBC and the potential target inhibitors that are in clinical trials: Numerous signaling cascades, including receptor tyrosine kinases (RTKs), non–RTKs, and downstream effectors, have been implicated in the pathogenesis of TNBC. These cascades comprise the EGFR, VEGFR, FGFR, WNT, Hedgehog, and Notch and the downstream signaling nodes of RAS, JAK/STATs, and PI3K. Therapeutic strategies involving cell cycle inhibition and DNA–damaging agents aimed at disrupting mechanisms within the nucleus could be combined with the inhibition of RTKs and downstream inhibitors. Adapted from Ref. [39].

Growth factors and angiogenesis

Epidermal growth factor receptors (EGFR), fibroblast growth factor receptors (FGFRs), and vascular endothelial growth factor receptors (VEGFRs) are growth factors overexpressed in TNBC. In clinical trials, EGFR inhibitors like cetuximab improved response rates in metastatic TNBC patients. Preliminary studies with PD173074, an FGFR inhibitor, showed inhibition of TNBC cell growth with amplified FGFR2 and CAL–51 xenografts [40]. Sunitinib and sorafenib, both anti–VEGFR tyrosine kinase inhibitors (TKIs), have exhibited notable efficacy in breast cancer based on clinical studies encompassing substantial populations of TNBC patients [41].

PI3K/AKT/mTOR pathway

In TNBC, PIK3CA mutations increase in residual TNBC and AR–positive TNBC. AKT and PTEN regulate the mammalian target of rapamycin (mTOR), and its activation is heightened in TNBC due to mutations in the phosphoinositide 3–kinase (PI3K) pathway and loss of PTEN [42]. In a current research investigation, everolimus is evaluated for its potential in treating advanced TNBC (NCT05563220). Additionally, other P13/AKT/mTOR inhibitors being assessed for TNBC treatment include PI3K inhibitors such as buparlisib and taselisib, AKT inhibitors including capivasertib and ipatasertib, as well as mTOR inhibitors like everolimus and temsirolimus [43].

Androgen receptor

TNBC lacks conventional hormonal receptors associated with breast cancer but contains other hormone receptors, such as glucocorticoid and androgen receptors (AR), with AR expression ranging from 12–36 % [44]. In addition to the androgen receptor, notable alternative targets include HSP90 and CYP17 lyase, which regulate androgen function and synthesis, respectively. Cochrane *et al.,* 2014, concluded that enzalutamide effectively treated AR–positive TNBC cells and ER+ cells *in vitro* and *in vivo* [45].

SRC, WNT signaling and DNMT1

In TNBC tumors, SRC detection at the membrane was significantly higher than non–TNBC tumors. TNBC cell lines, particularly in the M and MSL subtypes, showed increased sensitivity to the SRC inhibitor dasatinib. Additionally, WNT signaling was heightened in BL 2, M, and MSL subtypes of TNBC and correlated with lung and brain metastases [46]. LGK974 (a WNT–specific O–acyltransferase porcupine inhibitor) inhibited WNT signaling in a mouse model of breast cancer and has been proposed in a clinical trial in TNBC and other cancers (ClinicalTrials.gov number, NCT01351103). In TNBC, the gene DNMT1 is upregulated and plays a key role in methylation maintenance. Its expression is elevated in TNBC [47]. Combining the DNMT inhibitor decitabine with PARP inhibitors led to additional tumor inhibition in wild–type BRCA1 TNBC cells [48].

So, recent advancements in molecular profiling and the emergence of targeted therapy alongside chemotherapy enable us to provide more personalized therapy for TNBC patients. The table outlines recommended therapies for each TNBC subtype. By tailoring treatment to the specific TNBC subtype, these approaches aim to enhance therapeutic effectiveness and improve patient outcomes.

Table 1. 1 Different therapy for different TNBC subtypes [49]

TNBC subtypes	**Therapeutic approaches**	**Therapeutic classes**	**Examples**
Basal–like 1 (BL 1)	Inhibits cell division and interferes with DNA responses	Taxanes	Paclitaxel, and Docetaxel
		Platinum agents	Cisplatin, Carboplatin, and Oxaliplatin
		Anthracyclines	Doxorubicin, Daunorubicin, and Etoposide
		PARP inhibitors	Olaparib, Rucaparib, Talazoparib, and Niraparib
Basal–like 2 (BL 2)	Inhibits signaling of EGFR and MET	Platinum agents	Cisplatin, Carboplatin, Eptaplatin, and Oxaliplatin
		PARP inhibitors	Olaparib, Rucaparib, Talazoparib and Niraparib,
		Growth factor inhibitors	Erlotinib, Gefitinib, Afatinib, Cetuximab, Panitumumab, Bevacizumab, and Pertuzumab
		mTOR inhibitors	Rapamycin, Everolimus, and RapaLink–1
		Platinum agents	Cisplatin, Carboplatin, and Eptaplatin

			Nedaplatin, and Oxaliplatin
		PARP inhibitors	Olaparib, Rucaparib, Talazoparib and Niraparib
Immunomodulatory (IM)	Interferes or inhibits immune responses or signaling	Immune checkpoint inhibitors	Ipilimumab, Nivolumab, Pembrolizumab, Cemiplimab, Atezolizumab, Avelumab and Durvalumab
Mesenchymal (M)	Inhibit signaling pathways including Notch, Wnt, IGFR1, PI3K/AKT/mTOR, TGFβ, EGFR, Src, and EMT	Growth factor inhibitors	Erlotinib, Gefitinib, Afatinib, Avitinib, lapatinib, Cetuximab, Panitumumab, Vandetanib, Bevacizumab, and Pertuzumab
		mTOR inhibitors	Rapamycin, Everolimus, and RapaLink–1
		Src inhibitors	Dasatinib, and Bosutinib,
		PI3K inhibitors	Idelalisib, and Alpelisib
		AKT inhibitor	Ipatasertib, and Capivasertib
		Growth Factor inhibitors	Erlotinib, Gefitinib, Afatinib, Osimertinib, lapatinib, Cetuximab, Panitumumab,

Mesenchymal Stem–like (MSL)	Inhibit Wnt, PI3K/ mTOR, EGFR, MAPK, TGFβ, Src, and EMT		Vandetanib, Bevacizumab, and Pertuzumab
		mTOR inhibitors	Rapamycin, and Everolimus
		PI3K inhibitors	Idelalisib, and Alpelisib
		MAPK inhibitors	Trametinib, and Dabrafenib
		Scr inhibitors	Bosutinib, and Dasatinib
Luminal Androgen Receptor (LAR)	Inhibit AR signaling, PI3K/AKT/mTOR, and MAPK signaling, and FOXA1 signaling	Nonsteroidal antiandrogens	Enzalutamide, bicalutamide, and orteronel
		mTOR inhibitors	Rapamycin, and Everolimus
		PI3K inhibitors	Idelalisib, and taselisib

1.5. Novel drug delivery approaches for TNBC

Conventional pharmaceutical formulations like solution, suspension, or emulsions have limitations such as high dose requirements, low availability, first–pass metabolism, instability, and fluctuating drug levels. Novel carriers are needed to meet ideal drug delivery system requirements. Nanoparticle delivery systems have been proposed as promising drug carriers [50]. Nanocarriers are potent drug delivery, imaging, and diagnosis tools due to their small size and customizable surface properties. They include liposomes, dendrimers, nanocrystals, polymeric micelles, polymeric nanoparticles, carbon nanotubes, metallic nanoparticles, nanoemulsions, solid lipid nanoparticles, nanostructured lipid carriers, etc., all of which enhance drug bioavailability and reduce side effects [51].

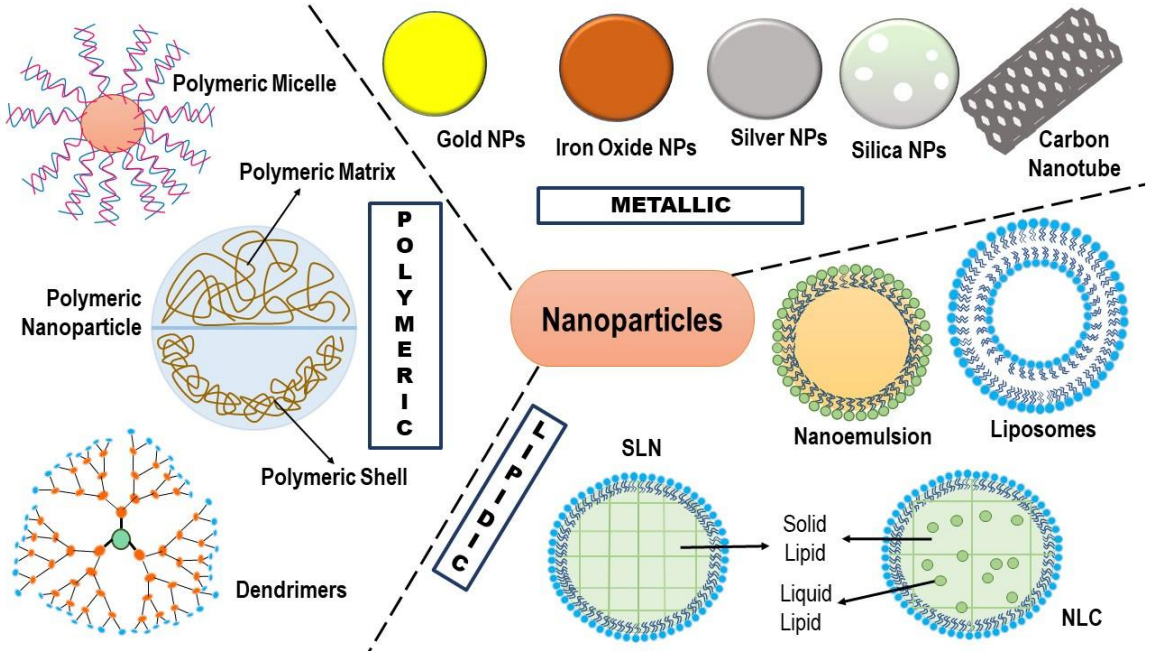

Figure 1. 5: Different types of nanoparticles administered to treat TNBC effectively. Adapted from [52]

Polymeric nanoparticles and polymeric micelles possess a notable advantage due to their biocompatibility. **Polymeric micelles** are supramolecular delivery systems composed of amphiphilic block copolymers. They have a semi–solid hydrophobic core made of biodegradable polymers like poly(L–lactide), poly(β–caprolactone), and PLGA and are ideal for delivering water–insoluble chemotherapies [53]. In addition to polymeric nanoparticles, **metal–based nanoparticles** offer significant versatility in drug delivery and diagnostic applications. **Mesoporous silica nanoparticles (MSNs)** have emerged as promising nanocarriers that can overcome limitations associated with conventional delivery systems. In a previous study, co–administration of DOX and siRNA via MSNs significantly reduced tumor growth *in vivo*, yielding synergistic effects compared to individual treatments with either the drug or siRNA alone [54]. **Liposomes** are a class of spherical vesicles characterized by a closed structure comprising an aqueous inner core and a membranous lipid bilayer. Typically measuring 50–100 nm in size, liposomes can encapsulate hydrophobic drugs within the lipid bilayer and hydrophilic drugs, genes, and siRNA (small interfering RNA) within the aqueous core. The benefits of liposomes include diminished systemic toxicity, prolonged circulation time, biocompatibility, and low susceptibility to recognition by the reticuloendothelial system, thereby reducing subsequent clearance [55]. The first FDA–approved nanomedicine, Doxil, consists of PEGylated liposomes loaded with

doxorubicin and is commonly used to treat Kaposi sarcoma, refractory breast cancer, and ovarian cancer [56]. In cancer chemotherapy, **dendrimers** have garnered significant attention as nanocarrier–based systems, particularly through ligand or receptor–mediated endocytosis, due to their capacity to confer targeting capabilities. Dendrimers exhibit a well–defined, homogeneous, monodisperse, highly branched, nanosized symmetrical structure, with a diameter ranging from 2–10 nm. These distinctive attributes render them well–suited as carriers for the precise delivery of therapeutic and diagnostic agents, offering notable advantages over traditional therapeutic approaches [57]. **Lipid–based nanoformulations (LNPs)** are thought to improve drug delivery to tumors, reduce adverse effects, and address multidrug resistance. These colloids, derived from various materials, offer therapeutic and diagnostic potential in cancer research, with advantages such as biodegradability, biocompatibility, and targeted delivery when formulated as NLCs, SLNs, and SMEDDS/SNEDDS [58]. **Carbon nanotubes (CNTs)**, fullerenes, and graphene have sparked interest due to their biocompatibility, chemical functionalization, and effective drug delivery. Recent reports suggest their potential for controlled drug delivery and use as contrast agents for diagnosing and imaging tumors, offering promise for cancer diagnosis and treatment [59]. **Exosomes** are membranous small vesicles with components that play roles in biological and pathological processes. Initially used to detect diseases like cancer, they are now being explored as drug delivery vehicles due to their non–immunogenic nature and long circulation time. Exosomes consist of various proteins and lipids found in the human body. Their outer surface can be modified for specific targeting, showing promise for clinical applications [60]. Chemotherapeutic agents such as adriamycin and docetaxel are utilized in the treatment of breast cancer. However, the emergence of drug resistance is a potential concern. This resistance can lead to the propagation of resistant cancer cells to other sensitive cancer cells through the exosome–mediated delivery of miRNA or associated proteins [61].

1.5.1. Cancer–targeted approaches

Targeted drug delivery systems have several advantages, including protecting healthy cells from cytotoxic compounds, reducing dose–limiting adverse effects, and combating drug–resistant cancer cells. Nanoparticles (NPs) deliver sensitive therapeutics to targeted lesions while minimizing accumulation in undesired organs and tissues. Meticulous nanoparticle design and optimization are essential for cellular and nuclear targeting [62].

Passive targeting

The blood vessel endothelium becomes more permeable under certain conditions, such as inflammation or tumor hypoxia. In response to hypoxia, tumors recruit new vessels or engulf existing ones, allowing selectively enhanced permeation of large macromolecules and nanosystems into the tumor stroma. The absence of normal lymphatic drainage in tumors leads to the retention of NPs. Encapsulating small molecule drugs in nanosized carriers extends their systemic circulation, provides some tumor selectivity, and reduces side effects. This passive tumor targeting relies on carrier characteristics and tumor biology. The EPR effect provides only modest tumor specificity, with a 20–30 % increase in delivery compared to normal organs (Figure 1. 5a). Biodistribution, pharmacokinetics, and toxicity profiles are influenced by the physicochemical properties of the developed nanocarrier or by the pathophysiological properties of the TME. Particle size and surface charge significantly impact nanoparticles' efficiency and cellular uptake pathways. The size of nanoparticles is a critical factor governing their permeation and retention within tumors, constrained by the fenestrations in tumor vessels (200–800 nm). The optimal size range typically falls within 20–200 nm. Surface chemistry and charge also significantly influence circulation time, as excessively hydrophobic or charged systems are promptly opsonized by the mononuclear phagocyte system (MPS). Therefore, it is advantageous for nanoparticle surfaces to mimic water, possessing hydrophilic and neutral or slightly anionic characteristics. To this end, water–soluble polymers, commonly PEGs, are grafted onto the nanocarrier surface [63].

Active targeting

After accumulating in the tumor region, drugs can be made more effective through active targeting (Figure 1. 5b). This involves decorating nanocarrier surfaces with ligands that bind to receptors overexpressed on tumor cells, enhancing drug penetration [64].

The **Androgen receptor (AR),** a steroid nuclear receptor, plays a crucial role as a transcription factor regulating gene expression in breast cancers. However, its role in TNBC is ambiguous due to significant variability in AR expression, ranging from 6.6–75 %. Some studies revealed that the TNBC is also associated with weight gain (obesity), which in turn is associated with the excess secretion of adipokine protein, named Leptin (16kDa), by the adipocytes in response to obesity–related stimuli [65]. It was observed that 70–80 % of TNBC cases show overexpression of **leptin receptors (LEPR)**. The LEPR is a prognostic–type biomarker of TNBC. It was found that Leptin induces the growth and proliferation of cancer and mediates drug resistance [66]. It was observed that the binding of Leptin to the

LEPR facilitates the recruitment of JAK2 kinase, which later leads to the phosphorylation of STAT3 (pSTAT3), activating various downstream signaling pathways (Notch, JAK2, PI3K/AKT/mTOR, and MAPK), genes (Wnt4, ADHFE1, RDH5, etc.), and RBP–JK transcription factor, which is responsible for cell growth, proliferation, migration, and angiogenesis. Leptin binding to the LEPR also increases the cell cycle's S–phase progression, apoptosis evasion, and chemoresistance. In TNBC, the transmembrane glycoprotein, **Intercellular adhesion molecule–1 (ICAM–1),** is up–regulated in basal–like TNBCs and under–expressed in luminal–like TNBCs. ICAM–1 is a glycoprotein belonging to the immunoglobulin superfamily that serves as an adhesion molecule. It was observed from various studies that TNBC was associated with metastasis to the lungs, bone, and brain, and it was revealed that ICAM–1 overexpression resulted in lung metastasis of the TNBC cells. It was further observed that endothelial ICAM–1 facilitates the adhesion of leukocyte to endothelium via ICAM–1–LFA1 (lymphocyte function–associated antigen 1) and ICAM–1–Mac1 (macrophage–1 antigen) intercellular interactions, further mediating leukocyte transendothelial migration (TEM). ICAM–1 signaling also sustains the expression of CDK6 and other related pathways related to cell cycle and cell survival [67]. **HA–CD44** interaction has been used for targeted delivery in TNBC treatment. HA plays a crucial role in tumor angiogenesis, cell migration, and tumor progression due to its high–affinity binding with CD44, HARE, and RHAMM. **Folate receptor alpha (FRα**) is one of the well–recognized prognostic biomarkers employed for diagnosing TNBC. It was also found that folate sustains metabolic reactions, creating a suitable TME essential for the growth of cancer cells. Various genomic studies have revealed that 30 % of the early–staged TNBC cases show the overexpression of FRα, whereas FRα is found to be overexpressed in 70–80 % of stage IV metastatic TNBC cases. Noteworthy attributes of folic acid encompass its non–immunogenic nature and its specific affinity for folic acid receptors [68]. Further, it was observed that in 98 % of human solid tumors, **Transferrin** (**TfR–1)** is found to be overexpressed. In addition, TNBC tumors show high overexpression of **interleukins (IL–3Rα, and IL–8).** From various clinical trials, it was observed that 55 % of TNBC patients showed overexpression of IL–3Rα. It was further observed that in TNBC, overexpression of IL–3Rα releases extracellular vesicles responsible for cell invasion, EMT, vascular mimicry (VM), and metastasis to secondary sites like the brain, bone, and lungs [69]. In TNBC, IL–8 production is associated with hypoxic conditions and aids in recruiting mesenchymal stem cells (MSCs) to the primary site of TNBC, creating a microenvironment

around the tumor. Such aberrant physiological conditions increase multidrug resistance and metastatic risk [70].

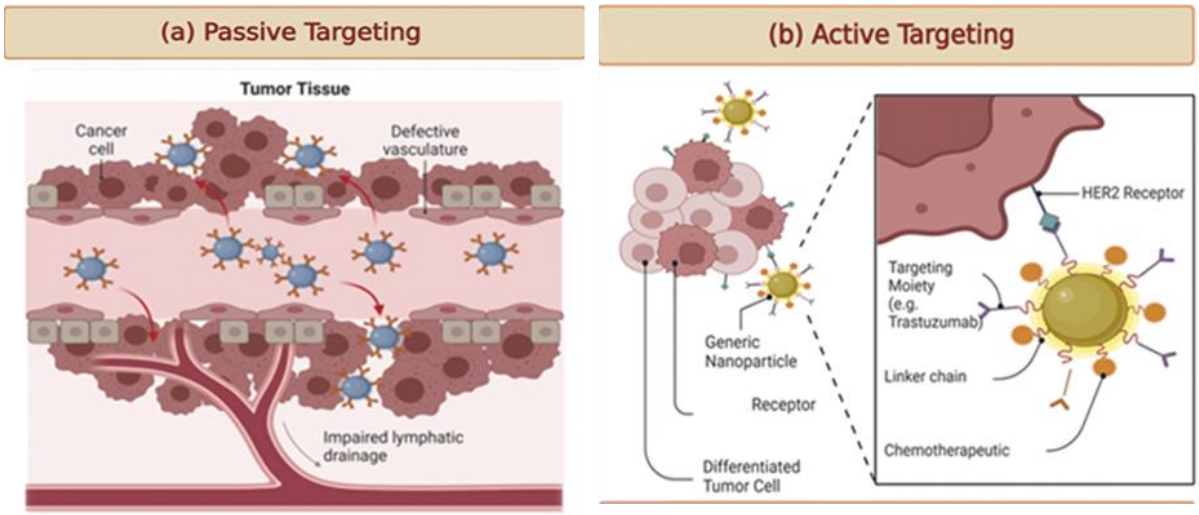

Figure 1. 6: A schematic representation highlighting three distinct approaches in drug delivery systems. (a) enhanced permeation and retention (EPR), (b) active targeting with ligands or antibodies. Adapted from Ref. [71].