Clinical Advances in TNBCs Treatment: Focus on PLGA Nanoparticles

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Significance Statement: This mini review summarizes the progress on chemotherapeutics and nanoparticles delivery for treatment of TNBC and specifically highlights the lead compounds that are in clinical trials.

List of Abbreviations

adenosine (ADN)
androgen receptor (AR)
antibody-drug conjugates (ADCs)
basal-like (BL1 and BL2)
B-cell lymphoma 2 (BCL 2)
breast cancers (BCs)
breast cancer susceptibility 1 (BRCA1)
chromosomal region maintenance 1 (CRM1)
datopotamab deruxtecan (Dato-DXd)
doxycycline (DOX)
drug delivery system (DDS)
enhanced permeability and retention (EPR)
electrohydrodynamic (EHD)
epidermal growth factor receptor (EGFR)
estrogen receptor (ER)
extportin 1 (XPO1)
Food and Drug Administration (FDA)
5-fluourouracil (5-FU)
heparanase (HPA)
human epidermal growth factor receptor 2 (HER2)
human umbilical venous endothelial cells (HUVEC)
hyaluronic acid (HA)
indocyanine green (ICG)
luminal androgen receptor (LAR)
mesenchymal (M)
mesenchymal stem-like (MSL)
nanoparticles (NPs)
near infra-red (NIR)
paclitaxel (PTX)
poly (ADP-ribose) polymerase) inhibitors (PARPi)
poly (L-lactide-coglycolide) (PLGA)
polyethylene glycol (PEG)
progesterone receptor (PR)
programmed death-ligand 1 (PD-L1)
sacituzumab govitecan (SG)
standard-of-care (SOC)
thymoquinone (TQ)
triple negative breast cancer (TNBC)
tumor-infiltrating lymphocytes (TIL)
tyrosine kinase inhibitors (TKIs)
rotating magnetic field (RMF)
unclassified (UNC)
vascular endothelial growth factor (VEGF) receptor (VEGFR)
Abstract

Triple Negative Breast Cancer (TNBC) is the most aggressive type of breast cancer and is associated with high probability of metastasis and poor prognosis. Chemotherapeutics and surgery remain the most common options for TNBC patients; however, chemotherapeutic resistance and relapse of tumors limit the progression free survival and patient life span. This review provides an overview of recent chemotherapeutics that are in clinical trial, and the combination of drugs that are being investigated to overcome the drug resistance and to improve patient survival in different molecular subtypes of TNBCs. Nanotherapeutics have emerged as a promising platform for TNBC treatment and aim to improve the selectivity and solubility of drugs, reduce systemic side effects, and overcome multi-drug resistance. The study explores the role of nanoparticles for TNBC treatment and summarizes the types of nanoparticles that are in clinical trials. Poly(1-lactide-co-glycolide) (PLGA) is the most studied polymeric carrier for drug delivery and for TNBC treatment in research and in clinics. This review is about providing recent advancements in PLGA nanotherapeutic formulations in their application to help treat TNBC. Some background on current chemotherapies and pathway inhibitors is provided so that the readers are aware of what is currently considered for TNBC. Some of the pathway inhibitors may also be of importance for nanotherapeutics development.

1. Introduction to TNBC

Triple-negative breast cancer (TNBC) is a distinct subtype of breast cancer characterized by the absence of estrogen receptor (ER), progesterone receptor (PR), and the human epidermal growth factor receptor 2 (HER2) protein (Bottaro, Larsen and Madhur, 2008; Anders and Carey, 2009; Derakhshan and Reis-filho, 2022). TNBC is often aggressive and has a higher probability of metastasis, particularly in the brain and lungs, compared to other breast cancer types. It is more common in younger women, particularly in African-American ethnicities and those with breast cancer susceptibility 1 (BRCA1) gene mutations, accounting for approximately 15-20% of all breast cancers (BCs). Survival rates for TNBCs tend to be lower, with a higher risk of recurrence within the first 3-5 years after treatment (Lehmann et al., 2016). TNBCs are heterogeneous at the molecular level, with various subtypes identified through gene expression profiling. The molecular subtypes of TNBC include: Basal-like (BL1 and BL2), Mesenchymal (M), Luminal Androgen Receptor (LAR) and unclassified (Lehmann et al., 2011, 2016; Ahn et al., 2016; Wang et al., 2019; Zhao et al., 2020; Bissanum et al., 2021).

**Basal-like (BL1 and BL2):** These subtypes are characterized by high expression of genes associated with cell division and DNA damage response. BL1 cancers exhibit high expression of genes involved in cell cycle and DNA replication processes, whereas BL2 subtypes show dysregulation in growth factor signaling and myoepithelial markers. They are typically responsive to chemotherapy (Lehmann et al., 2016).
Mesenchymal (M) and Mesenchymal Stem-Like (MSL): Both subtypes show elevated expression of genes involved in epithelial-mesenchymal-transition and growth factor pathways, but only the MSL subtype demonstrates a reduction in the expression of genes related to proliferation and may be involved in resistance to conventional therapies. Compared to the other subtypes, M subtypes have the lowest immunomodulatory correlation where tumor microenvironment (TME) is non-permissive to immune cell infiltration (Lehmann et al., 2016; Wang et al., 2019; Bissanum et al., 2021).

Luminal Androgen Receptor (LAR): Despite the triple-negative status, the LAR subtype expresses androgen receptor (AR) and displays a luminal gene expression pattern. This subtype may benefit from anti-androgen therapies (Lehmann et al., 2016; Wang et al., 2019; Bissanum et al., 2021).

Unclassified (UNC): Tumors that do not clearly fit into the above categories are termed unclassified and may require more individualized research to determine specific characteristics (Wang et al., 2019; Bissanum et al., 2021).

Each molecular subtype of TNBC can influence the prognosis and may eventually guide tailored therapeutic approaches. For instance, the BL-1 subtypes tend to have a better response to neoadjuvant chemotherapy, while the LAR subtype is indicative of unique hormone receptor status and opens opportunities for targeted hormonal treatments (Anders and Carey, 2009; Yin et al., 2020).

Although TNBC only accounts for 15-20% of newly diagnosed cases, this BC group results in the most BC-related deaths, mainly due to insufficient targeted treatment alternatives (Borri and Granaglia, 2021; Siegel et al., 2021; Jabbarzadeh Kaboli et al., 2022; Luo et al., 2022; Zhu et al., 2023). Surgical resection followed by systemic chemotherapy and radiation are currently the standard-of-care (SOC) treatment protocols used for TNBC (Bianchini et al., 2022; Luo et al., 2022) Approximately 50% of TNBC patients respond positively to chemotherapy, which makes this approach the major treatment option (Mittendorf et al., 2014; Beniey et al., 2019; Bianchini et al., 2022; Jabbarzadeh Kaboli et al., 2022).

There are several recent or ongoing clinical trials for potential targeted treatment regimens for TNBC. These include immune check-point blockade strategies (e.g. inhibiting PD-L1 (programmed death-ligand 1)), PARP (poly (ADP-ribose) polymerase) inhibitors (PARPi), PI3K/AKT/mTOR inhibitors, epidermal growth factor receptor (EGFR) inhibitors, Notch inhibitors, and antibody-drug conjugates (ADCs), with some of these combined with chemotherapy (Bianchini et al., 2022; Luo and Keyomarsi, 2022; Zhu et al., 2023) and can be used based upon the molecular subtypes of TNBCs.

As noted above, ~50% of TNBC patients are treated with chemotherapy. Of the remaining TNBC cases, about 10-15% of TNBC patients can be treated with PARP inhibitors (McCann and Hurvitz, 2018; Jabbarzadeh Kaboli et al., 2022) and approximately 20% are treated with PD-L1 inhibitors (Jiao et al., 2017; Jabbarzadeh Kaboli et al., 2022). Specifically, regarding the treatment approaches for the TNBC subtypes, PI3K/mTOR and Notch inhibitors are considered
for both the ML and MSL subtypes (Lehmann et al., 2011; Jiang et al., 2019; Zhu et al., 2023). PARP inhibitors can be used against both BL1 and BL2 subtypes, and in addition for the BL2 subtype, treatment strategies may include EGFR, PI3K/AKT and/or mTOR inhibitors (Lehmann et al., 2011; Jiang et al., 2019; Zhu et al., 2023). AR and PI3K/mTOR inhibitors can be utilized as treatment options for the LAR subtype (Lehmann et al., 2011; Jiang et al., 2019; Zhu et al., 2023). The IM subset of TNBC allows the possibility of incorporating immune checkpoint inhibitors (Jiang et al., 2019; Luo et al., 2022; Zhu et al., 2023).

2. Clinical trials – recent and current treatments for TNBC

Inhibitors against PARP are used to suppress its activity and block DNA damage repair in tumor cells, particularly in BRCA1/2-mutant cancers (Turner and Reis-Filho, 2013; Luo et al., 2022). The FDA (U.S. Food and Drug Administration) has recently approved PARP inhibitors olaparib (Robson et al., 2017) and talazoparib (Litton et al., 2018) for HER2 negative advanced or metastatic BC with BRCA mutations (Lehmann et al., 2011; Liu et al., 2016; Zhu et al., 2023). Pamiparib is beneficial for patients with advanced or metastatic HER2-negative BC with germline BRCA mutations (Xu et al., 2023). Due to PARP inhibitor resistance, PARPi have been combined with cell-cycle checkpoint inhibitors (e.g. against PI3K, mTOR, CDK12/13, ATM, CHK, WEE1, DNA methyltransferase and histone deacetylase (which suppresses HR; restored with PARPi resistance)) (Luo and Keyomarsi, 2022). PARPi combined with immune checkpoint inhibitors include niraparib and pembrolizumab (anti-PD1 antibody) (Vinayak et al., 2019); or olaparib + pembrolizumab (see Table 1). Talazoparib has been combined with an ADC sacituzumab govitecan (SG) (Trodelvy), which targets Trop-2, for metastatic TNBC (Jabbarzadeh Kaboli et al., 2022; Luo and Keyomarsi, 2022; Zhu et al., 2023). Olaparib is also combined with a Bcl-2 (B-cell lymphoma 2, apoptosis regulator protein) inhibitor (navitoclax) (Table 1). PARPi talazoparib is also combined with an exportin 1 (XPO1, chromosomal region maintenance 1 (CRM1)), and mediates nuclear export of proteins/RNAs) blocker (Selinexor) (Table 1).

Approximately 10% of TNBC have PI3KCA mutations and are predominantly found in ML/MSL and LAR subtypes (Zhu et al., 2023). There are several PI3K inhibitors that have been investigated in clinical trials, with only a few found to be promising, however when combined with chemotherapy these were found to be more effective (e.g. alpelisib + Nab-paclitaxel: NCT04216472 (Zhu et al., 2023); or copanlisib + eribulin) (Table 1). PI3K inhibitors combined with other pathway inhibitors also seem promising. Alpelisib is also combined with an iNOS inhibitor and Nab-paclitaxel in patients with HER2 negative metaplastic BC (see Table 1). Alternatively, PI3K/mTOR dual inhibitor therapy (e.g. apitolisib, dactolysisib, sarmolysisib, voltarricoxib) has been shown to be more effective than individual inhibitors (Wu et al., 2022; Zhu et al., 2023). Dactolisib effectively inhibits both wild-type and mutant forms of PI3KCA, which may be beneficial for mesenchymal and LAR TNBC subtypes (Zaytseva et al., 2012).
There are also a number of AKT inhibitors that have been investigated, including combination therapy (Zhu et al., 2023), but these single inhibitors have not been found to be as effective. There may be some benefit in targeting multiple tumor growth pathways, as well as chemotherapeutic combination therapies with AKT inhibitors.

AR positive expression is found in about 12% of patients that are both ER and PR (progesterone receptor) negative (Zhu et al., 2023). The AR inhibitor enzalutamide is recommended for AR positive patients (Traina et al., 2018; Zhu et al., 2023). Another AR inhibitor, bicalutamide, is combined with the CDK inhibitor ribociclib, which may hold some promise (Table 1).

Tyrosine kinase receptor, EGFR, is an effective target for 89% of TNBC patients, and tyrosine kinase inhibitors (TKIs) (e.g. gefitinib) or anti-EGFR monoclonal antibodies (e.g. cetuximab) combined with chemotherapeutic agents (e.g. carboplatin or cisplatin) have improved survival in patients with metastatic TNBC (particularly the BL2 TNBC subtype) in past clinical trials (Carey et al., 2012; Baselga et al., 2013; Zhu et al., 2023).

Increased expression of Notch-2 is associated with TNBC, particularly in BC stem cell involvement, and inhibitors have been investigated in a past clinical trial (Zhu et al., 2023). Targeting Notch-2 in combination with other therapies may be of some benefits for M and MSL TNBC subtypes, but may require further investigation.

Mechanism-of-action of immune checkpoint inhibitors (ICIs) is by blocking immunosuppressive receptors (e.g. PD-1 and PD-L1) which augments immune surveillance and antitumoral responses (Ribas and Wolchok, 2018; Luo and Keyomarsi, 2022; Jacob et al., 2023). Immune checkpoint blockade monotherapy demonstrated promising responses for patients with non-treated PD-L1 positive advanced TNBC, but was not advantageous when compared to chemotherapy alone (Howard et al., 2022). Regardless, pembrolizumab monotherapy is considered for some patients with advanced TNBC (Howard et al., 2022). Pembrolizumab or avelozimab and chemotherapy combination (chemoimmunotherapy) have been more successful (e.g. pembrolizumab + eribulin; pembrolizumab + Nab-paclitaxel, paclitaxel, or gemcitabine/carboplatin; avelozimab + Nab-paclitaxel), which are approved for PD-L1 positive advanced TNBC, as well as early-stage high-risk TNBC (Howard et al., 2022). Combining pembrolizumab and chemotherapy, guided by tumor-infiltrating lymphocytes (TILs) levels in resected early-stage TNBC is underway; or as a neoadjuvant therapy (Table 1). Atezolizumab and multiple chemotherapies (carboplatin, Nab-paclitaxel); combined with paclitaxel and bevacizumab (anti-VEGF antibody); or combined with carboplatin and gemcitabine, as well as the anti-VEGF antibody bevacizumab, are underway (Table 1). Anti-PD-1 antibody, sintilimab, combined with chemotherapies, taxane and carboplatin, is also underway (Table 1). PD-1 inhibitor, camrelizumab, combined with chemotherapy is ongoing (Table 1). PD-1 antibody, tislelizumab, is also combined with Nab-paclitaxel and TKI sitravatinib (Table 1). Atezolizumab combined with RP1 oncolytic immunotherapy (neoadjuvant) is also being considered (Table 1). Sintilimab combined with TKI anlotinib is ongoing (Table 1). PD-1 inhibitor camrelizumab is
combined with VEGFR2 inhibitor apatinib (Table 1). Pembrolizumab is also combined with SG-hziy (Table 1). PD-L1 inhibitor, avelumab, is also combined with SG (NCT03971409) or CDK 4/6 inhibitor, palbociclib (Table 1). Atezolizumab combined with capecitabine (metabolizes to 5-fluourouracil (5-FU)) is also underway, as is pembrolizumab with capecitabine following chemoinmunotherapy and surgery (Table 1). PD-1 inhibitor durvalumab is also combined with PARPi olaparib (Table 1). Immune checkpoint inhibitor targeting CTLA-4 is being assessed in a PD-1/CTLA-4 bispecific antibody, SI-B003 (Table 1). Antibody targeting CTLA-4, tremelimumab, is also combined with chemotherapy for metastatic TNBC (Table 1). Combining immune check point inhibitors with other pathway inhibitors and/or chemotherapeutic agents has improved overall patient survival, and holds promise for future therapeutic approaches.

ADCs consist of a monoclonal antibody targeting a surface protein (antigen internalization), a linker, and a payload that delivers a chemotherapeutic agent into the cancer cell cytoplasm (Vankemmelbeke and Durrant, 2016; Jabbarzadeh Kaboli et al., 2022). In addition to SG above, Datopotamab deruxtecan (Dato-DXd) (DS-1062a) (monoclonal antibody that targets Trop-2, a topoisomerase I inhibitor (SN-38), and a cleavable tetrapeptide junction), is considered for metastatic TNBC (Jabbarzadeh Kaboli et al., 2022; Luo et al., 2022; Zhu et al., 2023). Dato-DXd is also investigated in TNBC patients diagnosed with progressing brain metastases, and combined with durvalumab (Table 1).

Other alternative therapies under investigation include neoadjuvant tumor-infiltrating lymphocytes (TIL) and response-adapted chemotherapy; use of a Globo H vaccine (Adagloxad Simolenin); use of deferoxamine (aluminum and iron binding agent) with chemotherapy for metastatic TNBC; cisplatin with a histone deacetylase inhibitor, chidamide, or chidamine combined with vincristine metronomic chemotherapy; use of the histone deacetylase inhibitor, tucidinostat, and capecitabine (converts to 5-FU); or combining a TKI, apatinib, with albumin-bound paclitaxel (Table 1).

Although chemotherapy is somewhat effective for some TNBC patients, more directed therapies (immune checkpoint inhibitors; PARPi; targeting PI3K, mTOR, AKT, AR, Notch, EGFR, Trop-2, and Globo H), and particularly combination therapies (with VEGF/VEGFR2 inhibitors, histone deacetylase inhibitors, and TILs, as well as ADCs) are being investigated as a result of current genomic/proteomic data, molecular sub-typing, and our understanding of chemotherapeutic resistance, as well as the microenvironment heterogeneity. As indicated in Table 1, the most promising therapies currently under investigations involve: (1) the use of PARPi with either immune checkpoint inhibitors, an apoptosis modulator, an exportin 1 blocker, or an ADC; (2) PI3K inhibitors and chemotherapy with or without an iNOS inhibitor; (3) AR inhibitors with a CDK inhibitor; (4) immune checkpoint inhibitors combined with either chemotherapy, SG, TKIs, VEGF/VEGFR2 inhibitors, or PARPi; (5) ADC with or without immune checkpoint inhibitors; or (6) alternate strategies which include a Globo H vaccine, histone deacetylase inhibitors with or without chemotherapy, a TKI combined with chemotherapy, and lastly TILs and response-adapted chemotherapy.

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Challenges in conventional therapy for TNBCs stems from inefficient chemotherapies resulting in chemo-resistance due to single-target therapies, presence of a complex TME that can cause metastatic complications, and these factors can hinder drug delivery (Bianchini et al., 2022; Zhu et al., 2023). A more desirable approach could involve the use of nanotherapeutics with targeted ligands.

3. Nanotherapeutics for TNBCs Treatment

Types of nanomaterials for drug delivery applications can be broadly classified as lipid-based nanomaterials, polymeric and inorganic. Liposome, niosome, transfersome, lipid nanoparticles (NPs) and nanoemulsions are the main examples for lipid-based NPs which can be identified by spherical platforms with at least one lipid bilayer surrounding an internal aqueous compartment. Lipid-based nanostructures are the most popular type of drug delivery system, based on their characteristics, including formulation simplicity, self-assembly, biocompatibility, high bioavailability, and the capacity to transport substantial payloads. However, limited stability in physiological conditions and rapid uptake by the reticuloendothelial system necessitate surface modifications, shrinking their applications in clinical settings (Mitchell et al., 2021; Kasina et al., 2022).

Polymeric NPs, synthesized from natural or synthetic polymers, present varied structures like nanocapsules, nanosheet, nanofibers and nanospheres. Their advantages include biocompatibility and versatile drug delivery through encapsulation, entrapment, conjugation, or surface binding. Prominent types include polymersomes, polymeric micelles, and dendrimers with controlled three-dimensional architectures. Challenges involve an increased risk of particle aggregation, potential toxicity concerns, and limited FDA approval for polymeric nanomedicines (Mitchell et al., 2021; Kasina et al., 2022).

Inorganic NPs, including but not limited to metal, metal oxide, silica, and carbon-based nanomaterials, exhibit precise formulations with diverse sizes, structures, and geometries, featuring unique physical, electrical, magnetic, and optical properties. Inorganic NPs, such as iron oxide, are FDA-approved nanomedicines. However, use of inorganic NPs are limited mainly due to their solubility issues, low drug loading and toxicity concerns (Mitchell et al., 2021; Kasina et al., 2022).

Although the development of nanotherapeutics for TNBC is rapidly evolving, their translation into practice requires further validation through clinical trials to ensure efficacy and safety in this aggressive cancer subtype (Parodi et al., 2022). Nanoparticle albumin-bound (nab) paclitaxel (Ex. Abraxane) (Robidoux et al., 2010) and liposomal doxorubicin (Ex. Doxil) (Frenkel et al., 2006) are notable examples of clinically proven nanotherapeutic for TNBC. In addition to nab-paclitaxel, there are ongoing clinical trials assessing the efficacy of various chemotherapeutics/nucleic acid/ immunotherapeutic loaded nanocarriers that target the unique TME of TNBC. These nanoparticles are summarized in Table 2.
Poly-lactic-co-glycolic acid (PLGA), an FDA-approved copolymer, stands as a highly utilized polymer in the creation and development of drug delivery systems for biomedical purposes. This is attributed to its commendable characteristics such as biodegradability, biosafety, biocompatibility, as well as its adaptability in formulation and functionalization. Additionally, nanocarriers constructed from PLGA play a crucial role in optimizing the bioavailability of the encapsulated drug. They achieve this by safeguarding the drug from premature degradation within the biological environment, offering sustained and tunable degradation kinetics, targeted delivery, and enhanced intracellular penetration of bioactive compounds, thereby minimizing potential side effects (Ghitman et al., 2020; Cunha et al., 2021). In the context of TNBC, polyethylene glycol (PEG) coated polylactic acid (PLA), polyglycolic acid (PGA) and their copolymers, polylactic-co-glycolic acid (PLGA) are the most common delivery carriers in clinical trials. This section specifically highlights the advancements in PLGA formulations for TNBC treatment (Tang et al., 2017; Parodi et al., 2022).

Some problems and solutions associated with cargo delivery are that (1) many chemotherapy drugs are hydrophobic and have poor solubility, leading to challenges in their delivery, whereas encapsulation of nanoparticles, especially liposomes or micelles, can improve solubility and stability, enhancing drug delivery to target sites; (2) small inhibitors usually face rapid clearance and insufficient accumulation at the target site, whereas incorporating small inhibitors into nanoparticles can prolong circulation time and enhance their accumulation in tumor tissues through the enhanced permeability and retention (EPR) effect; (3) naked siRNA/miRNA face rapid degradation and poor cellular uptake, whereas nanoparticles offer protection to siRNA/miRNA, improve stability, and aid in targeted delivery, as well as facilitate endosomal escape, which overcomes a major hurdle in the intracellular delivery of nucleic acids (Tang et al., 2017; Valcourt and Day, 2020; Chiang et al., 2021; Camorani et al., 2022; Parodi et al., 2022; Zare et al., 2022; Dinakar et al., 2023; Yusuf et al., 2023).

4. Advances in PLGA formulations for TNBC Treatment

Advances in PLGA nanoformulations for TNBC treatment are summarized in Table 3.

a. **PLGA based controlled release carriers**: Sequential and controlled release of drugs can overcome several undesired effects of chemotherapeutics in TNBC patients. PEGylated PLGA nanoparticles loaded with 1,2-dioleoyl-sn-glycero-3-phosphate and DOX complexes, followed by sequential loading of Erlotinib showed enhanced chemotherapeutic effects in TNBC cell line (MDA-MB-468 cells), compared to single drug therapy and simultaneous release of both drugs. Instead of pure DOX, DOX-DOPA complexes enhanced the loading of DOX by 10-fold. The drug loaded nanoacarriers also showed superior localization in orthotropic mouse tumor model, compared to other organs. The sequential delivery of chemotherapeutics was achieved by suppressing the release kinetic of DOX encapsulated in lipid carrier, allowing faster release of Erlotinib, hence yielding controlled and improved pharmacokinetics and anticancer efficacies (Zhou et al., 2017). In another study, amphiphilic, cationic and thermoresponsive core shell
nanoparticles of PLGA were developed by ring opening esterification of PLGA with copolymer precursors of hydroxyl terminated poly(2-(2-methoxyethoxy) ethylmethacrylate-co-oligo (ethylene glycol) methacrylate-co-2-(dimethylamino) ethyl methacrylate). The nanoparticles obtained were coated with polydopamine to obtain spatiotemporal controlled release of chemotherapeutics and siRNA (survivin) in response to near infra-red (NIR) light and triggered release of cargo and TNBC regression in vitro (in MDA-M-231 cells) and in vivo in orthotropic breast cancer model. Poly(dopamine) coated nanoparticles are excellent photothermal agents that absorb light near NIR region and convert the light energy into heat allowing for on-demand drug release and enhanced anticancer efficacies due to chemo and photothermal therapy (PTT). In this study they showed a decrease in the chemotherapeutic drug dosage to about 1/20 of conventional dose (Ding et al., 2017). The Gao et al. in vitro investigation using PLGA loaded thymoquinone (TQ) NPs (PLGA-TQ-NPs) coated with chitosan (CS) (PLGA-CS-TQ-NPs) found decreased cell viabilities in two MDA-MB-231 and SUM-149 TNBC cell lines (Gao et al., 2023). They established that the additional controlled release of CS on the PLGA may decrease systemic cytotoxicity of TQ, rendering this assembly more effective for the target TNBC cells (Gao et al., 2023).

b. Targeted Nanoparticles: The overexpression of protein markers in heterogeneous tumor environment provides an alternative targeting strategy that is explored to develop nanoparticles for precise chemotherapeutics delivery in TME. Heparanase (HPA), a β-endoglucuronidase which degrades heparin sulfate in the extracellular matrix is overly expressed in TNBC (Vlodavsky et al., 1999; Chen et al., 2008; Duan et al., 2019). Paclitaxel (PTX)-encapsulated PEGylated PLGA nanoparticles were developed and then additionally surface-functionalized with a HPA aptamer (Apt (S1.5)-PTX-NP) (Duan et al., 2019). Apt (S1.5)-PTX-NP were evaluated in MDA-MB-231, where they bonded to the over-expressed HPA on the surface of TNBC cells, subsequently taken up by the cells, resulting in elevated cellular toxicity, compared to PTX-NP without the HPA aptamer (Duan et al., 2019). These NPs were also found to significantly affect tumor cell invasion (migration through pore membrane inserts) and angiogenesis (HUVEC (human umbilical venous endothelial cells) tube formation) in vitro, as well as reduced tumor volumes in nude mice orthotopically implanted with MDA-MB-231 cells in vivo, compared to other treatment groups (Duan et al., 2019). In addition, these NPs demonstrated sustained release of PTX in the cytoplasm of MDA-MB-231 cells in vitro (Duan et al., 2019). This approach may benefit TNBC tumors that over-express HPA. An advantage is that targeting heparanase enhances drug delivery selectivity to cancer cells. A limitation however, is that heparanase is also expressed in some normal tissues, resulting in possible unintended interactions that may lead to side effects (Duan et al., 2019).

Chaudhari et al. developed PTX-loaded ADN-conjugated PLGA-PEG NPs (PTX ADN-PEG-PLGA NPs) that could be used for treating TNBC, whereby adenosine (ADN) acts as a substrate for AR (overexpressed in some TNBC subtypes) (Chaudhari et al., 2023). ADN was able to assist in cytoplasmic internalization of the NPs via AR-mediated endocytosis that resulted in a substantial increase in MDA-MB-231 cytotoxicity and increased apoptosis in vitro (Chaudhari et
al., 2023). In vivo the PTX ADN-PEG-PLGA NP treatment resulted in a significant decrease in tumor burden (excised and measured) compared to controls (Chaudhari et al., 2023). Conclusively, the optimized NPs were found to be biocompatible, and were able to have an improved anti-TNBC activity (Chaudhari et al., 2023).

The overexpression of pro-survival proteins such as Bcl-2 and Notch-1 in TNBCs are associated with poor prognosis and may provide useful target to develop active therapeutics. Among different methods that have been studied for Bcl-2 inhibition, Bcl-2 homology 3 mimetics (ABT-737) that can bind with Bcl-2, causing apoptosis and dysregulation of p53 signaling by reinstating Bax mediated release of Cyt-C has received much attention. However, Notch signaling contributes to ABT-737 resistance and co-delivery of ABT-737 with Notch inhibitors can help improve TNBC treatments for patients with overexpression of Notch receptors. ABT loaded PLGA NPs, surface functionalized with Notch antibodies regulated Bcl-2 expression, enhanced cell death (in MDA-MB-321 cells, as was measured by MTT assay) and reduced tumor burden in mouse TNBC flank model. The results showed that Notch inhibitors can potentiate the effects of ABT-737 for TNBC treatment (Valcourt et al., 2020).

CXCR4 is a surface receptor overexpressed in TNBCs and is involved in tumor growth and metastasis. Targeting CXCR4 with Plerixafor decorated nanoparticles can provide selective delivery and release of chemotherapeutics in TNBCs. Surface coating of nanoparticles with proteins is a challenging task that may result in denaturation of targeted proteins during chemical/physical functionalization. Plerixafor coated PLGA NPs developed by electro hydrodynamic (EHD) cojetting allows formulation of carriers with multiple functionalities and loads on the surface of nanoparticles and without the need to utilize harsh chemical methods and solvents. Acrylate grafted PLGA nanoparticles were incorporated into EHD to surface immobilize Plerixafor by Michael addition reaction and ~100 nm nanocylinders showed effective targeting and delivery of drugs in CXCR4 expressing TNBCs (MDA-MB231 cells) (Misra et al., 2015).

c. **PLGA based combination therapies:** Combination therapies target and inhibit multiple essential pathways of tumor growth, invasiveness or metastasis and has great potential for enhanced therapeutic efficacies, with reduced drug toxicity, and to overcome drug resistance. PLGA based multifunctional polymeric NPs (HA-Olb-PPMNs), composed of polyethyleneimine-PLGA co-loaded with Olaparib (Olb) and MNPs were additionally surface coated with hyaluronic acid (HA). CD44-receptors targeting NPs were then subjected to a rotating magnetic field (RMF) that directly affected the MNPs, and subsequently resulting in magneto-cell-lysis and magneto-cell-apoptosis via cell membrane destruction and lysosome activation. In vivo biodistribution showed nanoparticles exhibited primarily higher liver accumulation and gradually enriched at the tumor site over time, owing to EPR effect (Zhang et al., 2020). This approach had cell-targeting via HA to CD44 on MBA-MB-231 TNBC cells, and a synergistic therapeutic effect that incorporated a mechanical force in the presence of RMF and Olb which exerted a dual anti-tumor effect (Zhang et al., 2020). A NIR fluorescent dye was
loaded into the NPs enabled imaging for drug distribution, and additionally MNPs were able to be detected by MRI via T\textsubscript{2}-contrast that allowed localization and detection of the tumors that had accumulated the HA-Olb-PPMNs (Zhang et al., 2020). HA-Olb-PPMNs exposed to RMF were found to have significantly smaller tumor volumes compared to all other treatment groups (saline, free Olb, Olb-PPMNs, HA-PPMNs and HA-Olb-PPMNs) in MDA-MB-231 tumor-bearing mice, and severe morphological changes and necrosis in tumor whereas minimal toxicities for normal tissues (Zhang et al., 2020). This multi-pronged approach, although in early preclinical investigations, could hold some promise for several therapeutic approaches against TNBCs.

Similarly, strategies to combine PTT with chemotherapy have showed promising anti-tumor efficacies in aggressive TNBC tumor models. ‘Triple-punch’ nanoplatform comprising of amphiphilic copolymers integrating chemotherapeutic, photothermal agents and gene therapy was designed for TNBC treatment. The thermos-responsive cationic copolymers prepared by ring opening esterification of PLGA with copolymer precursors of hydroxyl terminated poly (2-(2-methoxyethoxy)ethylmethacrylate-co-oligo(ethyleneglycol)ethacrylate-co-2-(dimethylamino)ethyl methacrylate) were modulated at different copolymer ratios to obtain thermoresponsive properties and burst drug release in response to high temperatures. Hydrophobic and hydrophilic cargoes (PTX and indocyanine green (ICG) respectively) were encapsulated in the nanoparticle core, while survivin siRNA was complexed by electrostatic interactions. MDA-MB-231 tumor growth was marginally restrained in monotherapy due to the modest drug dosages employed (ICG, 0.32 μmol/kg; PTX, 0.54 μmol/kg; siRNA, 1.5 mg/kg). In dual-therapy, initial significant tumor inhibition was observed, yet rapid recurrence ensued. Remarkably, the triple-therapy group demonstrated optimal efficacy, achieving complete growth inhibition and sustained remission without tumor recurrence. In comparison to inorganic photothermal agents, ICG is FDA approved NIR maging agent with excellent photothermal conversion properties, and low toxicity. The expression of survivin is closely related to lymphatic metastasis of BC and is the primary cause of poor prognosis for BC patients. Knockdown of survivin gene showed remarkable inhibition of tumor metastasis, enhanced sensitivity of BC-cells towards chemotherapy and minimized drug side effects (Su et al., 2015).

d. PLGA nanoparticles targeting tumor supporting environment of TNBCs: The heterogeneous environment of TNBCs contains various tumor supporting cells and extracellular factors including immune cells, adipose tissue and cancer associated fibroblasts that mediate tumor metastasis and progression. Circulating Tumor Cells (CTCs) are continuously recruited to pre-metastatic niche, in the presence of chronic proinflammatory TME and proinflammatory neutrophils play a major role in this context. With advances in cancer research, role TME in cancer progression is being highlighted and there is need to develop novel therapeutics for targeting TME. Neutrophils are terminally differentiated cell with half-life of 7 hours during culture experiments, hence targeting of neutrophils for therapeutic applications is not ideal. Neutrophil mimicking nanoplatform, comprising of PLGA-NPs encapsulated in anti-inflammatory neutrophil derived-membranes can enable ‘super neutrophil properties’ to target
and kill CTCs, as was shown by specific targeting of 4T1 cells under shear flow conditions. Carfilzomib (a second generation proteasome inhibitor) loaded PLGA NPs cloaked in neutrophil derived membranes showed minimum immunogenicity, prevented the formation of new metastatic niche and inhibited the already formed metastasis, in 4T1 mouse metastatic model (Kang et al., 2017).

Breast cancer stem cells (CSC) are capable of initiating new tumors and are key to tumor progression, metastasis and recurrence. Enrichment of CSCs in TNBCs is associated with tumor aggressiveness and is linked to Hippo signaling pathway. Yes associated protein (YAP) is downstream effector of Hippo signaling pathway and plays significant role in maintenance of stem cell like phenotype. YAP expression is correlated with cancer metastasis and is driver of CSCs. Verteporfin, an FDA approved drug inhibits cancer cell proliferation by inducing cytoplasmic sequestration of YAP. Combretastatin A4 (CA4) is vascular disrupting agent that is in clinical trials as anti-angiogenesis agent for various cancer treatments. Lipid polymer hybrid nanoparticles, composed of PEG PLGA, and lethicin prepared by nanoprecipitation method, were loaded with PTX, CA4 and veteporfin. The triple drug loaded nanoparticles inhibited CSC enrichment in tumors, while causing cytotoxic effects to cancer cells in vitro and in vivo by suppressing YAP genes. The nanoparticles also inhibited angiogenesis in zebra fish model, diminished CSC enrichment and showed enhanced anti-cancer efficacies in surgically engrafted patient derived xenografts in mice (El-Sahli et al., 2021).

CD155 upregulation in TNBC patients is associated with poor prognosis and acts as a ligand for costimulatory receptor DNAM-1 present on CD8+ T cells. CD155/DNAM interactions can trigger tumor killing by CD8+ T-cells. CD155 also act as co-inhibitory receptor for T cell immunoreceptor with Ig and ITIM domains (TIGIT) and CD96 in CD8+ T-cells. PDL1/PD1 and CD155/TGIT pathways are both negative regulators of DNAM-1 signaling in mouse and human CD8+ T-cells. The study of dynamics and cross-talk between PDL1/PD-1, CD155/CD96, TIGIT and CD155/DNAM-1 axes and their role in TME modulation can help develop new strategies for TNBC treatment. Both PD-L1 and CD155 are highly expressed on TNBCs. To understand their role in tumor progression, PLGA nanoparticles functionalized with PDL1 antibody and encapsulated with siCD155 were developed for dual blockade of two receptors, in an effort to explore the dynamic effects of multiple pathways on TME modulation. It was found that CD155 expression on TNBCs increases early stage tumor surveillance of T-cells in the presence of PDL-1 blockade. PDL-1 blockade also inhibited collaboration between T-cells and immune cells to facilitate tumor evasion (Chen et al., 2021).

Cancer Associated fibroblasts (CAFs) are primary driver of immunosuppressive TME through TGF-β signaling. Relaxin, an antifibrogen significantly improved TME by downregulating TGF-β. PDL-1 targeted lipid PLGA nanoparticles were developed to encapsulate relaxin plasmid DNA and further complexed with lipid poly-γ-glutamic to form nanoparticle to enhance in vivo stability. The relaxin expression in 4T1 tumors reduced stromal deposition and diminished CAFs expression and the system showed 2.2-fold increase in cytotoxic T cell infiltration and decreased immunosuppressive cells infiltration. Depletion of CAFs along with blocking of PDL-1 receptors...
significantly improved anti-tumoral immunity, as was seen by the recruitment of CD8$^+$ and CD4$^+$ T-cells in 4T1 mouse tumor models (Zhang et al., 2023).

Conclusions and Future Outlook

TNBC is an aggressive breast cancer sub-type that is associated with high metastasis rates in lungs and brain, is more common in American-African young females and is associated with poor patient survival. Although chemotherapy is somewhat effective for TNBC patients, more directed therapies, such as immune checkpoint inhibitors, and particularly combination therapies, such as with pathway inhibitors are being investigated, by taking advantage of our current insights into chemoresistance, and the microenvironment heterogeneity, and improvements in molecular sub-typing and updated genomic/proteomic data. The most promising therapies currently under investigations involve: (1) use of a PARPi with either immune checkpoint inhibitors, pathway inhibitors or an ADC; (2) PI3K inhibitors and chemotherapy with or without a pathway inhibitor; (3) AR inhibitors combined with a CDK inhibitor; (4) immune checkpoint inhibitors combined with either chemotherapy or pathway inhibitors or a PARP1i; (5) ADC with or without immune checkpoint inhibitors; or (6) alternate strategies, such as a Globo H vaccine, histone deacetylase inhibitors with or without chemotherapy, a TKI combined with chemotherapy, or TILS and response-adapted chemotherapy. The key is targeting multiple pathways based on genetic and proteomic data.

Considering the limitations of traditional chemotherapeutics in TNBC treatment, nanoparticles mediated chemotherapeutics delivery has been extensively investigated in research and a handful of nanoparticles for TNBC treatment are in clinical trials. Table 2 summarizes the types of nanoparticles that are in clinical trials for TNBC treatment. PLGA nanoparticles are an example biocompatible and biodegradable FDA approved nanocarriers that have been used in clinics for the delivery of various chemotherapeutics. PLGA formulations are intuitively modified for chemotherapeutics delivery in TNBC preclinical models, predominantly in MDA-MB-231 cells and in some instances using xenografts in nude mice. Different strategies have been used to optimize PLGA nanoformulations for TNBC treatment including development of sequential and controlled release carriers, stimuli responsive and on-demand drug release (heat light and magnetic field) nanocarriers, targeted nanoparticles by heparinase (HA) via aptamers, CD44-receptors via HA, ATG5 (an autophagy-related protein), Notch targeting via antibodies, CXCR4 targeting via Plerixafor, PDL-1 targeting via antibodies or AR via adenosine (ADN); and used therapeutic drugs such as paclitaxel (PTX), doxorubicin (DOX), docetaxel (DTX), thymoquinone (TQ), Verteporfin, Comberstatin-A4, Carfilzomib, ABT-737 (Bcl-2 inhibitor), Erlotinib or the PARP inhibitor Olaparib, to elicit anti-tumor activity. The chemotherapeutic encapsulated nanoparticles are often combined with other therapeutic modalities including nucleic acids (Survivin siRNA, siCD155, and relaxin pDNA) and imaging/stimuli responsive modalities (NIR dyes and photo thermal agents, magnetic nanoparticles, stimuli responsive polymers) to provide multi-pronged strategy for effective TNBC treatment. Nanotherapeutics provide an excellent platform to combine multiple new therapeutics in clinical trials as a single
drug modality and minimizing the drug toxicity and off-site effects. Hopefully some of these strategies can be translated to the clinic in the future as possible therapies for different TNBC subtypes. In addition, some important pathway inhibitors, such as immune check-point inhibitors combined with other pathway inhibitors can be considered in the development of future NPs as treatment alternatives for TNBC, which will provide multiple targets as well as the benefit of improved biodistribution.

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References:


Lehmann BD, Jovanović B, Chen X, Estrada M V., Johnson KN, Shyr Y, Moses HL, Sanders


**Footnotes**

Canadian Cancer Society Emerging Scholar Award [Grant # 707147]

No author has an actual or perceived conflict of interest with the contents of this article.

**Legends for Tables and Figures**

**Table 1:** Summary of drugs that are in clinical trials for TNBC treatment.

**Table 2:** Nanotherapeutics for TNBC treatment in clinical trials.

**Table 3:** Summary of advances in PLGA nanoformulations for TNBC treatment.
Figure 1: The depicted images illustrate diverse carriers constructed from poly(lactic-co-glycolic acid) (PLGA) polymers, developed in recent years, with a particular emphasis on the predominant types tailored for cancer-targeting applications, reproduced from Alsaab et al., 2022 under Creative Commons Attribution (CC BY) license.
Table 1: Summary of drugs that are in recent or current clinical trials for TNBC treatment. (ClinicalTrial.gov)

<table>
<thead>
<tr>
<th>Treatment regime</th>
<th>Description</th>
<th>Clinical trial no.</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>PARP inhibitors</td>
<td>niraparib (20 mg PO daily) + pembrolizumab (200 mg every 3 wks until progression or toxicity) (anti-PD1 antibody)</td>
<td>NCT04468061 NCT04230109 NCT04448886</td>
<td>Promising anti-tumor activity; safe and well tolerated</td>
</tr>
<tr>
<td></td>
<td>olaparib (300 mg oral 2x daily)+ pembrolizumab (200 mg every 3 weeks)</td>
<td>NCT05203445 NCT05174832</td>
<td>&gt;50% response rate</td>
</tr>
<tr>
<td></td>
<td>Olaparib (200 mg over 2 wks; repeat 21 d) + Bcl-2 inhibitor (navitoclax) (dose-escalated; 28 d cycles)</td>
<td>NCT05358639</td>
<td>Ongoing</td>
</tr>
<tr>
<td></td>
<td>talazoparib (1 mg daily; d 15-21; 28 d cycle) + antibody-drug conjugate (ADC) sacituzumab.govitecan (SG), Trodelvy (10 mg/kg d 1,8)</td>
<td>NCT04039230</td>
<td>Minimized toxicity and improved efficacy</td>
</tr>
<tr>
<td></td>
<td>talazoparib (1 mg daily; d 15-21; 28 d cycle) + exportin 1 blocker, selinexor (60 mg oral 2x weekly)</td>
<td>NCT05035745</td>
<td>Ongoing</td>
</tr>
<tr>
<td>PI3K and mTOR inhibitors</td>
<td>alpelisib (300 mg daily) + Nab-paclitaxel (100 m/m² iv)</td>
<td>NCT04216472</td>
<td>Some OS benefit observed; safe and tolearable</td>
</tr>
<tr>
<td></td>
<td>alpelisib (300 mg daily) + iNOS inhibitor (L-NMMA)(20 mg/kg 2x weekly; every 3 wks) +Nab-paclitaxel (100 m/m² iv)</td>
<td>NCT05660083</td>
<td>Ongoing</td>
</tr>
<tr>
<td></td>
<td>copanlisib (45 mg iv d 1,8; 21 d cycle) + eribulin (1.4 mg/m² d 1,8 iv)</td>
<td>NCT04345913</td>
<td>Ongoing</td>
</tr>
<tr>
<td>AR inhibitors</td>
<td>bicalutamide (150 mg oral; continuous) + CDK inhibitor ribociclib (600 mg daily for 21 days)</td>
<td>NCT03090165</td>
<td>Ongoing</td>
</tr>
</tbody>
</table>
### Immune checkpoint inhibitors (e.g. anti-PD1 antibodies: pembrolizumab, sintilimab, camrelizumab, tislelizumab, durvalumab)

<table>
<thead>
<tr>
<th>Drug Combination</th>
<th>Clinical Trial ID(s)</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>pembrolizumab (200 mg iv every 3 wks) and paclitaxel (125 mg/m² iv; 4 cycles)</td>
<td>NCT06078384</td>
<td>Increased OS with expected chemotoxicity</td>
</tr>
<tr>
<td>pembrolizumab (200 mg iv every 3 wks) + chemotherapy: neoadjuvant</td>
<td>NCT05681728</td>
<td>Increased OS with expected chemotoxicity</td>
</tr>
<tr>
<td>pembrolizumab (200 mg iv every 3 wks) + antibody against Trop-2 (Sacituzumab govitecan-hziy) (10 mg/kg)</td>
<td>NCT05382286, NCT05633654</td>
<td>Minimal toxicity; improved efficacy</td>
</tr>
<tr>
<td>pembrolizumab (200 mg iv every 3 wks) + capecitabine (1000 mg/m² oral 2x weekly; 21 d cycle) following chemo-immunotherapy and surgery</td>
<td>NCT05973864</td>
<td>Ongoing</td>
</tr>
<tr>
<td>sintilimab (200 mg/q3w iv) + taxane (75 mg/m²/q3w) + carboplatin (500 mg/q3w)</td>
<td>NCT05843292</td>
<td>Highly effective after failure of 1st-line chemotherapy</td>
</tr>
<tr>
<td>sintilimab (200 mg/q3w iv) + tyrosine kinase inhibitor, anlotinib (12 mg PO d 1-4 q3w)</td>
<td>NCT04877821</td>
<td>Favorable efficacy; acceptable safety profile</td>
</tr>
<tr>
<td>camrelizumab (200 mg d1; every 3 wks) + chemotherapy (Nab-paclitaxel) (125 mg/m² d1,8,15; every 3 wks)</td>
<td>NCT05999149, NCT05402722, NCT05088057, NCT04676997, NCT04907344, NCT04613674, NCT05670925, NCT05134194, NCT05475678</td>
<td>Promising efficacy; manageable safety; ongoing</td>
</tr>
<tr>
<td>camrelizumab (200 md d1; 21 d cycle) + VEGFR2 inhibitor (apatinib)(250 mg daily; 21d cycle)</td>
<td>NCT05556200</td>
<td>Promising efficacy; manageable safety profile</td>
</tr>
<tr>
<td>tislelizumab (200 mg iv every 3 wks) + Nab-paclitaxel (100 mg/m² iv 2x weekly; 3 wk cycle)+ tyrosine kinase inhibitor sitravatinib (120 mg oral; daily)</td>
<td>NCT04734262</td>
<td>Ongoing</td>
</tr>
<tr>
<td>durvalumab (1500 mg iv every 28d)+ PARPi Olaparib (300 mg 2x daily; oral; commenced 28d prior to 1st dose of durvalumab)</td>
<td>NCT03801369</td>
<td>No new toxicity concerns; good PFS and OS</td>
</tr>
</tbody>
</table>

**Cont. Table 1**: Summary of drugs that are in recent or current clinical trials for TNBC treatment. ([ClinicalTrial.gov](https://clinicaltrials.gov))
Cont. Table 1: Summary of drugs that are in recent or current clinical trials for TNBC treatment. (ClinicalTrial.gov)

<table>
<thead>
<tr>
<th>Immune checkpoint inhibitors (e.g. anti-PD-L1 antibodies: atezolizumab; avelumab)</th>
<th>NCT05266937</th>
<th>Improved OS</th>
</tr>
</thead>
<tbody>
<tr>
<td>atezolizumab (1200 mg iv every 3 wks) + multiple chemotherapies (carboplatin (AUC 6 mg/mL/min every 3 wks), Nab-paclitaxel (100 mg/m² iv every week))</td>
<td>NCT04408118</td>
<td>Ongoing</td>
</tr>
<tr>
<td>atezolizumab (1200 mg iv every 3 wks) + paclitaxel (175 mg/m²) + bevacizumab (15 mg/kg)</td>
<td>NCT04739670</td>
<td>Ongoing</td>
</tr>
<tr>
<td>atezolizumab (1200 mg iv every 3 wks) + carboplatin (AUC 6 mg/mL/min every 3 wks) + gemcitabine (1250 mg/m² on d1,8) + bevacizumab (15 mg/kg)</td>
<td>NCT06067061</td>
<td>Reduced toxicity; ongoing</td>
</tr>
<tr>
<td>atezolizumab (1200 mg iv every 3 wks) + RP1 oncolytic immunotherapy (240 mg q2w for 4 mon): neoadjuvant</td>
<td>NCT03756298</td>
<td>Ongoing</td>
</tr>
<tr>
<td>avelumab (10 mg/kg iv q2w) + Sacituzumab govitecan (10 mg/kg d1,8)</td>
<td>NCT03971409</td>
<td>Ongoing</td>
</tr>
<tr>
<td>avelumab (10 mg/kg iv q2w) + CDK 4/6 inhibitor, palbociclib (125 mg oral for 3 wks)</td>
<td>NCT04360941</td>
<td>Improved PFS; ongoing</td>
</tr>
<tr>
<td>(e.g. anti-CTLA-4 antibody: tremelimumab)</td>
<td>tremelimumab (75 mg iv; wk 0,3,6,9,16) + chemotherapy</td>
<td>NCT03606967</td>
</tr>
<tr>
<td>(e.g. PD-1/CTLA-4 bispecific antibody: SI-B003)</td>
<td>SI-B003 (10 mg/kg q2w)</td>
<td>NCT04606472</td>
</tr>
</tbody>
</table>
### Antibody-drug conjugates

<table>
<thead>
<tr>
<th>Drug Description</th>
<th>Clinical trials details</th>
<th>Results/Outcomes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Antibody-drug conjugates (e.g. Sacituzumab govitecan (see above), Dato-DXd)</td>
<td>Datopotamab deruxtecan (Dato-DXd) (DS-1062a) (antibody that targets Trop-2 + topoisomerase I inhibitor (SN-38) (6 mg/kg iv every 3 wks)</td>
<td>NCT03401385 NCT03742102</td>
</tr>
<tr>
<td></td>
<td>Dato-DXd (TNBC patients diagnosed with progressing brain metastases)(6 mg/kg iv every 3 wks)</td>
<td>NCT05866432</td>
</tr>
<tr>
<td></td>
<td>Dato-DXd (6 mg/kg iv every 3 wks) + PD-L1 antibody, durvalumab (1120 mg every 3 wks)</td>
<td>NCT05629585</td>
</tr>
</tbody>
</table>

### Alternative strategies

<table>
<thead>
<tr>
<th>Strategy Description</th>
<th>Clinical trials details</th>
<th>Results/Outcomes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neoadjuvant tumor-infiltrating lymphocytes (TIL) and response-adapted chemotherapy</td>
<td>Globo H vaccine (Adagloxad Simolenin) (30 µg/100 µg sc; 9 injections over 37 wks)</td>
<td>NCT03562637</td>
</tr>
<tr>
<td></td>
<td>Deferoxamine (50-60 mg/kg/day iv) + chemotherapy</td>
<td>NCT05300958</td>
</tr>
<tr>
<td></td>
<td>Cisplatin (75 mg/m2; 21d cycle) + histone deacetylase inhibitor, chidamide (20 mg 2x weekly; 21d)</td>
<td>NCT04192903</td>
</tr>
<tr>
<td></td>
<td>Chidamine (20 mg 2x weekly; 21d) + vincristine (2 mg iv) metronomic chemotherapy</td>
<td>NCT05747313</td>
</tr>
<tr>
<td></td>
<td>Histone deacetylase inhibitor, tucidinostat (40 mg/day; 2x weekly) + capecitabine (900 mg/m2 2x weekly; d1-15)</td>
<td>NCT05390476</td>
</tr>
<tr>
<td></td>
<td>TKI, apatinib (250 mg PO d1-21) + albumin-bound paclitaxel (260 mg/m2 iv; d1; 21d cycle)</td>
<td>NCT05019690</td>
</tr>
</tbody>
</table>

**Cont. Table 1**: Summary of drugs that are in recent or current clinical trials for TNBC treatment. (ClinicalTrial.gov)
Table 2: Nanotherapeutics for TNBC treatment in clinical trials (ClinicalTrials.gov).

<table>
<thead>
<tr>
<th>Nanoparticle Name</th>
<th>Brief Description</th>
<th>Trial outcome</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Inorganic Nanoparticles</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ELU001</td>
<td>Folic-Acid Functionalized C’Dot- exatecan-Conjugate for FRα overexpressing cancer including TNBC</td>
<td>Ongoing</td>
<td>NCT05001282</td>
</tr>
<tr>
<td><strong>Polymeric Nanoparticles</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Genexol-PM</td>
<td>PTX loaded monomethoxy-poly (ethylene glycol)-block-poly(D,L-lactide) micelle formulation with enhanced solubility and reduced systemic toxicity.</td>
<td>NCT00876486: Compared with standard paclitaxel, Genexol-PM demonstrated non-inferior and even superior clinical efficacy</td>
<td>NCT00876486, NCT01784120, NCT00912639, NCT02263495</td>
</tr>
<tr>
<td>Xyotax (CT-2103)</td>
<td>Conjugate of paclitaxel and poly-L-glutamic acid (Paclitaxel poliglumex) to improve tolerability and effectiveness</td>
<td>Not published</td>
<td>NCT00079876, NCT00148707</td>
</tr>
<tr>
<td>Nanoxel-PM</td>
<td>Analog of Genexol-PM loaded with docetaxel</td>
<td>Recruiting</td>
<td>NCT04066335</td>
</tr>
<tr>
<td>NK012</td>
<td>A polymeric micelle encapsulating the drug SN-38 (active metabolite of irinotecan), designed to increase the drug’s half-life and improve the antitumor activity.</td>
<td>Not published</td>
<td>NCT00951054</td>
</tr>
<tr>
<td>BIND-014</td>
<td>PSMA-targeted Accurin (polymeric nanoparticle) that contains docetaxel for improved efficacy and reduced toxicity.</td>
<td>BIND-014 demonstrated evidence of anti-tumor activity in tumors for which conventional docetaxel is known to have minimal activity</td>
<td>NCT01300533</td>
</tr>
<tr>
<td>---</td>
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<td>---</td>
<td>---</td>
</tr>
</tbody>
</table>

**Liposomal Nanoparticles**

<table>
<thead>
<tr>
<th>Doxil/Caelyx</th>
<th>Liposomal doxorubicin with an altered biodistribution and reduced cardiotoxicity profile.</th>
<th>Ongoing</th>
<th>NCT02456857</th>
</tr>
</thead>
<tbody>
<tr>
<td>ThermoDox</td>
<td>A temperature-sensitive liposomal doxorubicin, designed to release the drug in response to hyperthermia.</td>
<td>Low-temperature liposomal doxorubicin at 50 mg/m² given with mild local hyperthermia was safe and effective (48%) for heavily pretreated BC patients.</td>
<td>NCT00826085</td>
</tr>
<tr>
<td>Myocet</td>
<td>A non-pegylated liposomal doxorubicin with a reduced risk of cardiotoxicity</td>
<td>Not published</td>
<td>NCT00721747</td>
</tr>
<tr>
<td>LEP-ETU</td>
<td>Liposomal encapsulated paclitaxel aiming to provide better tolerability and improve on the pharmacokinetics of the drug.</td>
<td>Not published</td>
<td>NCT01190982</td>
</tr>
</tbody>
</table>
Table 3: Summary of advances in PLGA nanoformulations for TNBC treatment.

<table>
<thead>
<tr>
<th>Nanoparticles Specifications</th>
<th>Modifications to PLGA</th>
<th>Cargo</th>
<th>Models tested</th>
<th>Major findings</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controlled release carriers</td>
<td>PEGylated PLGA and DOPA-DOX encapsulation in lipids to achieve spatiotemporal release of two drugs</td>
<td>DOX and Erlotinib</td>
<td>MDA-MB-468 cells and orthotopic mouse tumor model for biodistribution of carriers</td>
<td>Increased loading (10 fold) capacity of DOPA-DOX compared to the pure DOX Only 20% of DOX released within the first 24 hrs 1.2 fold of NP accumulation inside the tumor compared to the liver</td>
<td>(Zhou et al., 2017)</td>
</tr>
<tr>
<td>NIR responsive carriers</td>
<td>esterification of PLGA with copolymer precursors of hydroxyl terminated poly (2-(2-methoxyethoxy) ethyl methacrylate-co-oligo (ethylene glycol) methacrylate-co-2- (dimethylamino) ethyl methacrylate) and coated with polydopamine</td>
<td>Survivin siRNA and DOX</td>
<td>MDA-M-231 cells and in vivo in orthotopic breast cancer model</td>
<td>Reduction of dose (20 times) compared to the free drug with NIR and superior tumor growth inhibition</td>
<td>(Ding et al., 2017)</td>
</tr>
<tr>
<td>Slow drug release</td>
<td>Chitosan coated PLGA</td>
<td>thymoquinone</td>
<td>MDA-MB-231 and SUM-149</td>
<td>Significantly higher drug loading (82%) with coated NP and slow release of the drug (29%) within 15 days</td>
<td>(Gao et al., 2023)</td>
</tr>
<tr>
<td>Heparanase targeting</td>
<td>(Apt (S1.5)-modified PEGylated PLGA</td>
<td>PTX</td>
<td>MDA-M-231 cells HUVEC cells for angiogenesis model orthotopically implanted tumor model</td>
<td>Apt(S1.5)-PTX-NP showed enhanced anti-invasive (5 fold) and superior anti-angiogenesis activity (3 fold) compared to PTX</td>
<td>(Duan et al., 2019)</td>
</tr>
<tr>
<td>AR targeting</td>
<td>ADN-conjugated PLGA-PEG NPs</td>
<td>PTX</td>
<td>MDA-MB-231 cells and tumor</td>
<td>PTX ADN-PEG-PLGA NPs showed</td>
<td>(Chaudhari et al., 2023)</td>
</tr>
<tr>
<td>Targeting Strategy</td>
<td>Targeting Strategy Details</td>
<td>Model</td>
<td>Results</td>
<td>Reference(s)</td>
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<td>Notch Targeting</td>
<td>Notch antibodies coated PLGA-NPs</td>
<td>ABT-737</td>
<td>5.22-fold decrease in %hemolysis, ~18.90-fold lower %tumor burden and 3.7 fold reduction in IC50 than control</td>
<td>(Valcourt et al., 2020)</td>
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<td>CXCR4 targeting</td>
<td>Plerixafor coated PLGA nanocylinders</td>
<td>MDA-MB231 cells</td>
<td>N1-ABT NPs showed a 3-fold accumulation in tumor site than IgG-ABT NPs</td>
<td>(Misra et al., 2015)</td>
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<tr>
<td>CD44-receptor targeting</td>
<td>Hyaluronic acid modified polyethyleneimine-PLGA NPs (PPMNP)</td>
<td>Olaparib and magnetic NPs</td>
<td>HA-PPMNP showed higher tumor accumulation and HA-Ola-PPMNP showed ~3 fold reduction of tumor volume compared to free Ola with rotating magnetic field</td>
<td>(Zhang et al., 2020)</td>
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<tr>
<td>Triple-punch (chemotherapy, photothermal therapy, and gene therapy) nanoplatform responsive to NIR</td>
<td>Esterification of PLGA with copolymer precursors of hydroxyl terminated poly (2-(2-methoxyethoxy) ethyl methacrylate-co-oligo (ethylene glycol)methacrylate-co-2-(dimethylamo) ethyl methacrylate) – (IPS)</td>
<td>MBA-MB-231, MDA-MB-231 tumor-bearing mice</td>
<td>NP-IPS exhibited complete ablation of the tumor xenografts) with low drug dose (ICG, 0.32 μmol/kg; PTX, 0.54 μmol/kg; siRNA, 1.5 mg/kg)</td>
<td>(Su et al., 2015)</td>
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<tr>
<td>Circulating tumor cells (CTC) targeting</td>
<td>Neutrophils cloaked PLGA NPs (NM-NP)</td>
<td>4T1 cells and metastatic tumor models</td>
<td>NM-NP-CFZ selectively depleted (~30 times lower) CTCs in the blood,</td>
<td>(Kang et al., 2017)</td>
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<tr>
<td>Lipid polymer hybrids for controlled drug release</td>
<td>Lethicin containing PEG-PLGA NPs</td>
<td>PTX, Combretastatin-A4, Verteporfin</td>
<td>MDA-MB-231, zebra fish model, surgically engrafted PDX tumors in mice</td>
<td>Triple drug-NP effectively inhibited the viability of PDX organotypic slide cultures ex vivo and stopped the growth of PDX tumors in vivo</td>
<td>(El-Sahli et al., 2021)</td>
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<td>PDL-1 targeting</td>
<td>PDL-1 antibody functionalized PLGA NPs</td>
<td>siCD155</td>
<td>Immune cells, 4T1 cells, 4T1 orthotopic tumor mode</td>
<td>P/PEALsiCD155 showed excellent TNBC targeting and induced CD8+ TILs-dominant intratumor antitumor immunity and efficiently inhibited TNBC progression and metastasis</td>
<td>(Chen et al., 2021)</td>
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<tr>
<td>PDL-1 targeting</td>
<td>PDL-1 targeted lipid PLGA</td>
<td>Relaxin plasmid DNA</td>
<td>4T1 cells, 4T1 orthotopic tumor mode</td>
<td>2.2-fold increase in cytotoxic T cell infiltration within the tumor and a decrease in immunosuppressive cells infiltration</td>
<td>(Zhang et al., 2023)</td>
</tr>
</tbody>
</table>
Different PLGA Nanoparticle-Mediated Targeted Drug Delivery Mechanisms, Responsiveness, and Release Pathways

**Delivery Platforms:**

- **A** PLGA Polymeric nanoparticles
- **B** PLGA Polymeric micelles
- **C** PLGA Polymerosomes
- **D** Polymeric Micelles
- **E** Lipid NPs with mRNAs
- **F** Polymersome

**Delivery Mechanism:**

**Active Targeting of cancer markers**
- Generic Nanoparticle
- Cancer Marker
- Differentiated Tumor Cell
- Targeting Moiety
- Linker chain
- Chemotherapeutic

**PLGA Stimuli-Responsive Drug Release**
- **External Stimuli**
  - Heat
  - Ultrasound
  - Magnetic field
  - Light
- **Internal Stimuli**
  - pH
  - Redox
  - Enzyme activity

**Stimulus**