Review article

# Breast cancer vaccines: New insights into immunomodulatory and nano-therapeutic approaches

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ABSTRACT

Breast cancer (BC) is known to be a highly heterogeneous disease that is clinically subdivided into four primary molecular subtypes, each having distinct morphology and clinical implications. These subtypes are principally defined by hormone receptors and other proteins involved (or not involved) in BC development. BC therapeutic vaccines [including peptide-based vaccines, protein-based vaccines, nucleic acid-based vaccines (DNA/RNA vaccines), bacterial/viral-based vaccines, and different immune cell-based vaccines] have emerged as an appealing class of cancer immunotherapeutics when used alone or combined with other immunotherapies. Employing the immune system to eliminate BC cells is a novel therapeutic modality. The benefit of active immunotherapies is that they develop protection against neoplastic tissue and readjust the immune system to an anti-tumor monitoring state. Such immunovaccines have not yet shown effectiveness for BC treatment in clinical trials. In recent years, nanomedicines have opened new windows to increase the effectiveness of vaccinations to treat BC. In this context, some nanoplatforms have been designed to efficiently deliver molecular, cellular, or subcellular vaccines to BC cells, increasing the efficacy and persistence of anti-tumor immunity while minimizing undesirable side effects. Immunostimulatory nano-adjuvants, liposomal-based vaccines, polymeric vaccines, virus-like particles, lipid/calcium/phosphate nanoparticles, chitosan-derived nanostructures, porous silicon microparticles, and selenium nanoparticles are among the newly designed nanostructures that have been used to facilitate antigen internalization and presentation by antigen-presenting cells, increase antigen stability, enhance vaccine antigenicity and remedial effectivity, promote antigen escape from the endosome, improve cytotoxic T lymphocyte responses, and produce humoral immune responses in BC cells. Here, we summarized the existing subtypes of BC and shed light on immunomodulatory and nano-therapeutic strategies for BC vaccination. Finally, we reviewed ongoing clinical trials on BC vaccination and highlighted near-term opportunities for moving forward.

## 1. Introduction

As a highly heterogeneous disease, breast cancer (BC) is the second most prevailing neoplasia behind pulmonary cancer and is a principal reason for death in 40 to 44-year-old women [1–3]. Studies have indicated that the disease contributes to the demise of 60–70% of these cases [1,4]. Findings also show that from 2014 to 2018, BC in females has slowly risen (0.5% per year) [5]. According to the World Human Organization (WHO), 2.3 million females were recognized with BC, and 685,000 fatalities were documented globally in 2020 [6]. Early detection of BC can reduce treatment expenditures and mortalities. Thus, developing efficacious methods for diagnosing BC is vital [7–9]. To

achieve this objective, researchers have detected some subtypes of BC varying in gene expression and clinical approaches, such as duct subtypes A and B (both of which are hormone receptor-positive), the HER-2 (ErbB-2) subtype, and the class comprehended as basal-like carcinoma (metaplastic carcinoma with chondroid differentiation) [10,11]. Immunoglobulins against the estrogen receptor (ER), progesterone receptor (PR), and human epidermal growth factor receptor 2 (EGFR2 or HER-2) are helpful for immunohistochemical (IHC) staining [12–14]. PR and ER receptors have been the first tumor markers to predict BC accurately. Patients positive for ER and PR hormone receptors are clinically susceptible to hormone therapy, and patients with HER-$2^+$ are vulnerable to targeted therapy [15]. Since the late 1990s, invasive
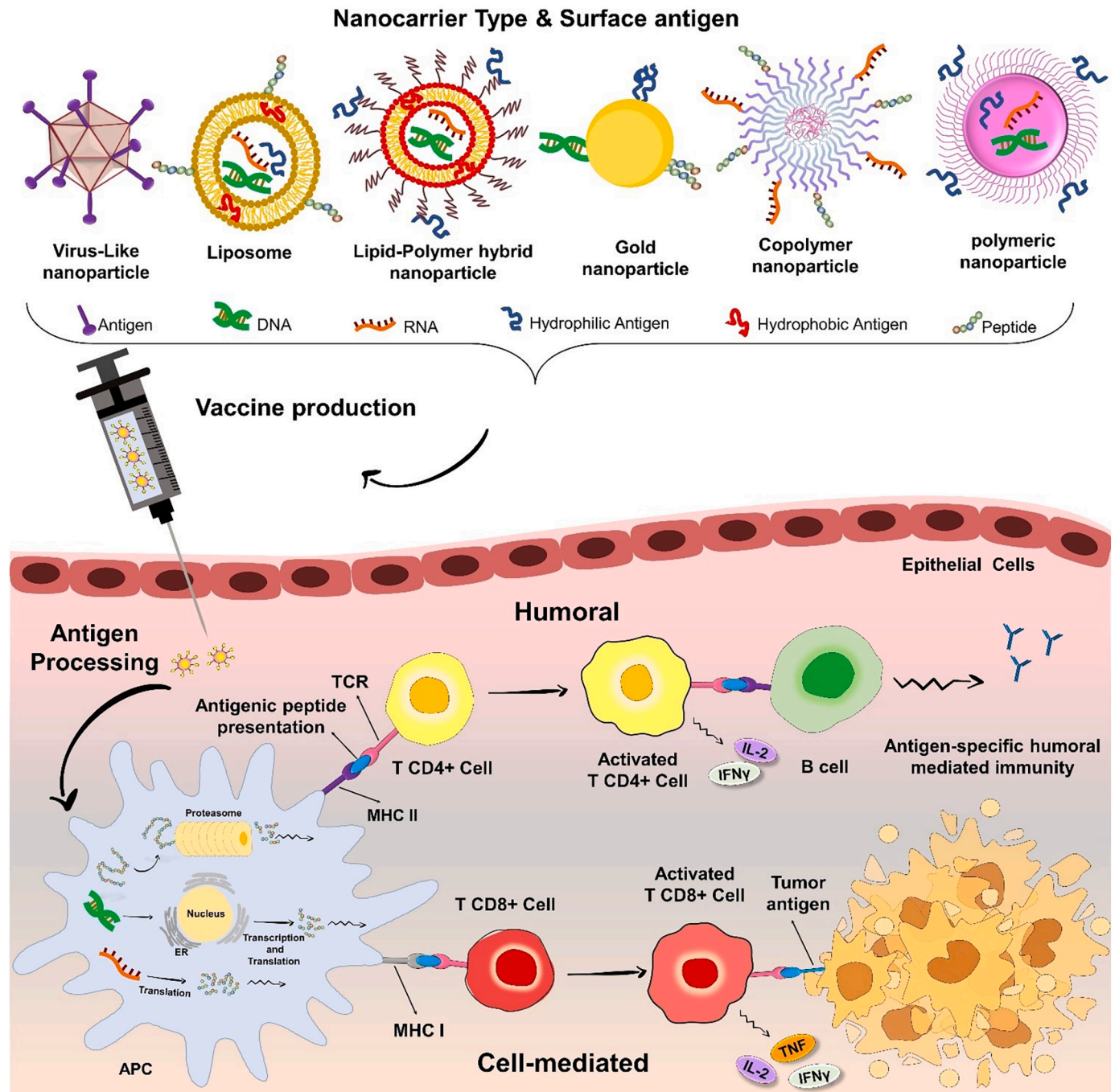


**Fig. 1.** A schematic representation of different NPs, including polymeric NPs, gold NPs (AuNPs), virus-like NPs (VLPs), inorganic NPs, lipid-, and protein/peptide-based NPs, have been widely employed as adjuvants, immunogens, and Ag delivery vehicles for activating the immune system. Antigens (Ags) of interest can be either encapsulated within or bound to the surface of NPs. Ag-containing NP cores are preserved against enzymatic degradation, whereas surface immobilization imitates Ag presentation by pathogens. APCs recognize Ags delivered with NPs and process them inside, inducing CD8$^+$ and CD4$^+$ T cell reactions. These Ags are then processed into the MHC class II-binding polypeptides by the endosome or proteasome's MHC class I-binding polypeptides. The MHC class I polypeptide epitopes are transmitted to the ergastoplasm by the TAP and then moved to the cell's surface through interaction with the MHC class I. Concurrently, tumor Ags are provided to CD8$^+$ and CD4$^+$ T cells by the professional APC in MHC classes I and II, respectively. The stimulated immune system can distinguish tumor Ags and kill malignant cells with CD8$^+$ T cells. Furthermore, by provoking B cells, Abs are secreted, and humoral immunity is activated. Ag, antigen; NP, nanoparticle; APC, antigen-presenting cell; CD, cluster of differentiation; MHC, major histocompatibility complex; TAP, transporter of antigen processing; Abs, Antibodies.

mammary carcinomas have been classified into molecular subgroups through related gene expression levels [10,16–19].

Ductal carcinoma *in situ* (DCIS) is defined as the growth of neoplastic cells that have not yet infiltrated the basal lamina of the mammary ducts and is a progenitor of invasive duct carcinoma (IDC) [20–24]. As the normal epithelium of the breast shifts to DCIS in a linear process, it results in invasive cancers such as IDC and then metastasis [25,26]. Tumor biomarkers are useful in the detection, therapy, and clinical care of patients because their presence in cancer tissues differs from that of healthy tissues [27,28]. Additionally, tumor markers potentially anticipate latent invading disease in at-risk patients [29]. Presently, immuno-oncology is at the vanguard of discovering novel approaches for treating cancer. Thus far, monoclonal antibodies (mAbs) have been the most triumphant investment outcome. Tumor-based cancer vaccines were one of the initial endeavors to engage the immune system in combating cancer [30]. The primary effort to apply cancer vaccines began over a century earlier. Vaccines have several benefits over chemotherapeutic agents and mABs. For instance, malignancy recurrence can be deterred through prompting prolonged immunologic memories with an efficacious vaccination protecting against diverse cancer antigens (Ags). Additionally, vaccines do not need to be employed constantly and are relatively more secure than chemotherapy [31].

Nanotechnology has given researchers unprecedented control over the design of devices down to the molecular level [32–34]. In this context, nanovaccines are obtained by combining pathogen-specific Ags with synthetic or natural nanostructures and have been studied to induce controllable immune responses. This approach requires using essential components of the pathogens called sub-units such as peptides, proteins, membranes, polysaccharides, and capsules to make vaccines more adjustable and safe [35]. Different nanoparticles (NPs), including polymeric, inorganic, lipid-, and protein/peptide-based, have been widely employed as adjuvants, immunogens, and Ag delivery vehicles for activating the immune system [36]. These nanostructures have shown high Ag loading capacity and less proteasome decomposition of antigenic subunits (Fig. 1) [37]. Proteasomes are polycatalytic proteinase compounds accounting for regulated proteolysis in the cytosol and are crucial for the restricted Ag-processing of Ag-presenting cells (APCs) *via* major histocompatibility complex (MHC) class 1. Free radicals, such as reactive oxygen species (ROS), enhance proteasome activity by wiping out oxidized proteins to sustain cellular protein homeostasis [38,39]. Smaller and more specific subunits often have low but sufficient immunogenicity when using adjuvants that co-stimulate or immuno-modulate signals. Their use is limited by individual-specific responses, immunotolerance to the target Ag, and undesired reactions towards self-Ags [40]. Therefore, NPs can be used as adjuvants, possibly reducing the need for conventional adjuvants and their side effects. In addition, cellular internalization of small subunits is low, and they are rapidly cleared from the body. NPs with tunable physicochemical characteristics can conquer this constraint by prolonging circulation time, bio-accumulation in lymphoid organs, and efficiently targeting immune cells. They can also increase cross-presentation by APCs and evoke the immune system at much lower doses [37]. Thus, the therapeutic use of the nanometric delivery system for vaccine carriers results in enhanced Ag delivery to the immune cells (or tumor cells); this, in turn, has an essential role in immune responses [41,42].

Accordingly, the purpose of this overview was to provide thorough and up-to-date information on tumor biomarker properties and designed vaccines to eradicate neoplastic cells. Herein, the first part precisely reviews diverse phenotypes and molecular markers of BC. Next, We will provide new perspectives on immunomodulatory and nanotherapeutic approaches toward developing BC vaccines.

## 2. Immunogenic phenotypes of BC

Even though the progression of normal mammary tissue to DCIS and eventually to invasive cancer has yet to be illuminated, multiplex proposed models of DCIS initiation and progression have provided scarce but precious data. Advanced imaging technologies and image analyses have determined presumptive histopathologic attributes and prognosis biomarkers [43]. The tumor markers with clinically-recommended utility comprise cancer antigen (CA) 15-3, CA 27.29, carcinoembryonic Ags (CEA, CD66e, or CEACAM-5), ER, PR, HER-2, urokinase-type plasminogen activator (uPA), and plasminogen activator inhibitor-1 (PAI-1) [44]. Although additional cancer biomarkers, such as TP53, cathepsin D, cyclin E, and nestin, can be used to screen BC, they lack sufficient proof to justify their routine application (Table 1) [45].

DCIS is a clonal growth of neoplastic cells involving the duct epithelium [93,94]. There is no proof that the basal lamina invades the stromal tissue adjacent to the duct. On another side, there is a broad spectrum of non-invasive malignancies at the peril of evolving into invasive cancer [94,95]. Epidemiological investigations have recorded that overall survival (OS) rates for DCIS are roughly 95% at ten years [96]. Thus, researchers pursue identifying types of patients for whom therapy can be lessened [97]. If left untreated, 40% of patients with low-grade DCIS evolve to invasive carcinoma [98]. The prevalence of molecular phenotypes of DCIS is remarkably diverse compared to invasive BC [99]. Utilizing the IHC method, researchers have realized that patients exhibit four distinct molecular phenotypes according to ER, PR, and HER-2 expression [100,101]: luminal A (ER$^+$/PR$^+$, HER-2$^-$), luminal B (ER$^+$/PR$^+$, HER-2$^+$), type HER-2$^+$ (ER$^-$/PR$^-$, HER-2$^+$), and negative triad (ER$^-$/PR$^-$, HER-2$^-$) [102,103]. In addition, many signaling pathways of these three biomarkers overlap, leading to the complex regulation of other genes and cellular mechanisms. Illuminating these pathways, clinicians can predict the clinical behaviors of BC (Fig. 2) [104].

### 2.1. HER-2$^+$

The HER-2 overexpression in BC is linked with inferior medical outcomes [105]. Patients with HER-2$^+$ BC are usually ER-negative; thus, treating these patients does not require antiestrogen hormone therapies [106,107]. HER-2 overexpression is also related to aggressive behavior in BC, invasion, relapse, and poor chemotherapeutic outcomes without alternatives to immunotherapy [108]. As evidence increases, it is clear that the interplay between cancerous cells and immune cells in HER-2$^+$ neoplasia is a fundamental stage in establishing the immune process in the host [109]. When HER-2 interacts with any existent tyrosine kinase binding receptor in BC cells, anti-HER-2 antibodies (Abs) and T cell reactions are induced. Invasive BC cases in the HER-2$^+$ subclass comprise 20–30% of all cases [110]. Patients with BC overexpressing HER-2 have endogenous HER-2$^+$-specified Abs and T-cell activities against HER-2, proposing that activating anti-HER-2 immune reactions might aid the treatment of HER-2$^+$ cancers [111,112]. In addition, HER-2 overexpression in malignant cells is considered a significant marker of tumor progression and provides potential targets for cancer immunotherapy [113].

### 2.2. Triple-negative BC (TNBC)

The negative triad phenotype of BC that lacks ER and PR expression (ER$^-$/PR$^-$) and erbB2 escalation [114] accounts for approximately 15% of total BCs [115]. Moreover, it has a high cell growth rate and inferior medical outcomes [17,116]. Triple-negative BCs (TNBCs), such as the basal-like subtype, emerge in patients of a definite age and racial groups, particularly juvenile black females [117], and respond to preoperative chemotherapy [118,119]. Furthermore, despite observing a complete pathological reaction in some patients with basal-like BC, these patients generally have an abysmal prognosis, probably associated with the greater possibility of recurrence in individuals who do not have a complete pathological response [119]. Overall, the immunotherapeutic targets in TNBC are referred to as tumor-associated antigens (TAAs) and

**Table 1**

A summary of BC biomarkers.

| Tumor Marker | Structure | Gene Locus | Function | Diagnostic Role |
|---|---|---|---|---|
| CEA | Glycoprotein (45–50% carbohydrates and 641 amino acids) [46] | Twenty-nine genes on chromosome 19ql3.2 [47] | 1. Cellular adhesion [47]<br>2. Participating in cancer invasion and metastases [48]<br>3. Recurrence after treatment [49] | 1. To diagnose relapse in post-operative patients<br>2. Follow-up on individuals receiving chemotherapy or radiation [50] |
| CA 15-3 (MUC1) | Glycoprotein [51] | 1q22 [52] | 1. Reducing cell-to-cell interaction<br>2. Impeding tumor cell lysis [53]<br>3. Developing mucous membranes on epithelial surfaces<br>4. Participating in intracellular signaling [1] | 1. Tumor aggressiveness and tumor growth [54]<br>2. Complementary in detecting recurrence [55]<br>3. CA 15-3 blood concentration is an autonomous predictor in metastatic mammary carcinoma [56] |
| CA 27.29 (MUC1) | Glycoprotein [57] | 1q22 [52,58] | 1. Developing mucous membranes on epithelial surfaces<br>2. Participating in intracellular signaling [1] | 1. Monitoring cancer development<br>2. Metastasis [59]<br>3. Tumor size predictor [60] |
| ER | Protein (a constituent of the nuclear steroid receptors)<br>The receptors may constitute ERa homodimers or ERp heterodimers [61] | ERa is on human chromosome 6q25.1-q25.2 [52]<br>Conversely, ERp is on chromosome 14q23.2-q23.3 [52], [61] | 1. Cellular growth<br>2. Proliferation<br>3. Differentiation [62] | 1. A key BC therapeutic response indicator [61]<br>2. As a predictor of hormonal resistance [63]<br>3. ERa anticipates delayed skeletal metastasis [64]<br>4. Managing carcinoma *in situ* (CIS) treatment [65] |
| PR | Protein [66] | 11q22.1 [52] | 1. Transcription<br>2. Steroid and lipid metabolism<br>3. Cell proliferation<br>4. Programmed cell death [67] | PR is among the most effective biomarkers for predicting hormone sensitivity in mammary carcinoma [68] |
| HER-2 | Protein (HER-2 comprises an extracellular ligand-binding domain E, a single transmembrane domain, and an intracellular protein-tyrosine kinase) [69] | 17q12 [52] | 1. Cell proliferation, differentiation, and survival [69]<br>2. Cell-cell communication [70] | 1. Helpful predictor of tumor mass [69]<br>2. The HER-2/neu receptor may detect recurrences and foretell BC metastases [71]<br>3. Overexpressed *HER-2/neu* is related to a more vigorous biologic behavior [72]<br>4. Resistance predictor to endocrine therapy<br>5. Selective resistance indicator to tamoxifen<br>6. Predicting resistance to specific cytotoxic factors, namely, sendoxan, MTX, and fluorouracil regimes<br>7. Predictor of anthracycline and anti-HER-2 treatments such as trastuzumab [70]<br>8. Mammary carcinomas without overexpressed *HER-2* generally metastasize to bone, while HER-2-negative ones typically disseminate to visceral organs, namely, lung, liver, and encephalon [73] |
| uPA and PAI-1 | Protein structures (enzyme)<br>uPA is a 53-kDa trypsin-like protease, and PAI-1 is a suppressant [74] | uPA: 10q22.2 [52]<br>PAI-1: 7q22.1 [52] | Various antagonists, namely PAI-1, PAI-2, and maspin, can suppress uPA catalytic function.<br>PAI-1 was assumed to be the principal uPA suppressor.<br>Besides adhering to uPA, PAI-1 might even bind to extracellular matrix proteins (EMPs), permitting it to influence cellular adhesion and migration [74] | uPA:<br>1. Spreading cancer *via* destroying the extracellular matrix, thus facilitating invasion and metastasis<br>2. Triggering angiogenesis, mitogenesis, and cell migration<br>3. Regulating cell adhesion<br>4. Precluding programmed cell death<br>5. Augmenting the longevity of neoplastic cells during the metastasis, thereby escalating the probability of establishing a secondary deposit [75,76].<br>Patients with elevated uPA and PAI-1 profit further from adjuvant therapy than those with lower concentrations.<br>Scientists increasingly regard the quantities of uPA, PAI-1, and uPAR as suitable for standard prognostic evaluation in patients with early BC [77]. |
| Tumor protein P53 | Protein (including 393 amino acids and seven domains) [78,79] | 17p13.1 [52] | 1. Participating in cell cycle regulation<br>2. Functioning as a tumor suppressor, precluding malignancies [78] | 1. In BCs, *TP53* gene mutations result in more severe disease and worse overall survival [80].<br>2. Survival *TP53* gene mutations could be correlated with aggressive cancers or distant metastases [80].<br>3. TP53 status might be administered as a prognostic factor of chemotherapy efficiency [81]. |
| CTSD | Protein [82] | 11p15.5 [52] | | |

**Table 1** (*continued*)

| Tumor Marker | Structure | Gene Locus | Function | Diagnostic Role |
|---|---|---|---|---|
| | | | 1. Determined as a lysosomal aspartyl endopeptidase, decomposing proteins into polypeptide fractions that digest other lysosomal peptide peptidohydrolases and exoproteases [82] 2. Participating in intracellular catabolism within the lysosomes 3. Processing Ags, hormones, and neuropeptides 4. Pro-cathepsin D was also proposed to participate in programmed cell death [83] | 1. A potent prognostic worth was identified for cathepsin D levels in mammary carcinoma and other cancers. 2. Pro-cathepsin D concentrations rose in plasma of metastatic breast carcinoma patients. 3. Cathepsin D *(CTSD)* overexpression was related to a high risk of relapse and demise [84] |
| NES | High molecular weight intermediate filament protein (possessing the shortest head domain (N-terminus) and the most extended tail domain (C-terminus)) [85] | 1q23.1 [52] | A biomarker of neural precursors [86] | 1. Solely expressed in invasive mammary cancer 2. Nestin-positive cancers exhibited high growth rates and *TP53* nuclear expression 3. Lymph-node[+] individuals with nestin[+] tumors had short-term longevity for mammary carcinoma [87] |
| HE4 (also known as WFDC2) | Small secretory protein [88] | 20q13.12 [52] | 1. Functioning as a protease inhibitor 2. Involved in sperm maturation [] | 1. Indicated that *HE4* is expressed in ductal carcinoma of the mammaries. Nevertheless, the serum expression levels and their diagnostic and prognostic worth in mammary carcinoma have yet to be illustrated [89]. 2. Elevated serum concentration of HE4 functions as a new biomarker for diagnosing mammary carcinoma [88] |
| CCNE1 | Protein (50 kDa) [90] | 19q12 [52] | 1. Functioning as regulators of CDK kinases 2. Constituting a complex with and functioning as a regulatory subunit of cyclin-dependent kinase 2 (CDK2), whose activity is needed for cell cycle G1/S transition [90] [] | 1. High cyclin E1 has always been correlated with an inferior prognosis in mammary carcinoma. Overexpression of cyclin E1 was correlated with an augmented peril of mammary carcinoma relapse [91]. 2. A predictor factor for tamoxifen resistance and chromosome instability [92]. |

BC, breast cancer; CEA, carcinoembryonic antigen; CA, cancer antigen; PR, progesterone receptor; ER, estrogen receptor; MUC1, mucin 1; uPA, urokinase-type plasminogen activator; HER-2, human epidermal growth factor receptor 2; PAI-1, plasminogen activator inhibitor 1; TP53, tumor protein P53; CTSD, cathepsin D; NES, nestin; HE4, human epididymis protein 4; WFDC2, WAP four-disulfide core domain 2; CCNE1, cyclin E; EMP, extracellular matrix protein; CDK2, cyclin-dependent kinase 2; MTX, methotrexate, CIS, carcinoma *in situ*.

the cancer-testis (CT) Ag and are the most notable Ags overexpressed in TNBC tumors that are induced by epigenomic alterations [120]. Thus far, over 150 CT Ags have been identified, among which SPANX family member B 1 (SPANXB1), ATPase family AAA domain containing 2 (ATAD2), forkhead box M1 (FOXM1), cancer/testis antigen 1B (CTAG1B), and MAGE family member A (MAGE-A) represent typical attributes of TNBC [120–123]. TNBC is also commonly associated with trophoblast cell-surface antigen 2 (Trop-2), folate receptor alpha (FOLR1), mucin 1 (MUC1), and mesothelin (MSLN) [122,124]. TAAs can be targeted with immunotherapeutic strategies, comprising chimeric antigen receptor (CAR)-T-cell remedies, oncolytic virotherapy, immunoconjugates (such as immunotoxins or drug-conjugated mAbs), naked mAbs, and messenger ribonucleic acid (mRNA) cancer vaccines. The first step in identifying TAAs in resected tumor tissues is molecular subtyping. Then, neoAgs derived from mutant genomes are identified for mRNA vaccine development and administered to patients [125].

The most immunogenic BCs are HER-2^{+} and TNBC subtypes [126]. This group of cancer cells demonstrates a higher mutational burden than hormone receptor-positive cancer cells [127,128]. Research demonstrates that tumor-infiltrating lymphocytes (TILs) are more prevalent in HER-2^{+} BCs and TNBCs than hormone receptor-positive BCs [129–134]. The higher levels of TILs in HER-2^{+} BC and TNBC have been associated with enhanced prognosis and a 15–25% decline in mortality and relapse risk [135,136]. Pathologic complete response (pCR) to neoadjuvant chemotherapy (NACT) is also predicted by lymphocytic infiltration [137–141]. Additionally, TILs are associated with better outcomes following anthracycline-based chemotherapy, indicating the immunogenic part of specific chemotherapy regimens, which may activate pre-existent host immunological responses against cancerous cells [142].

## 3. BC vaccines

Employing the immune system to eliminate malignant cells is a novel treatment strategy. The benefit of active immunotherapies is that they develop a protective impact against neoplastic tissue, readjusting the immune system to an anti-tumor monitoring state [143,144]. In tumor cells, Ag expression is different from healthy cells. The response of the cluster of differentiation (CD) 4^{+} and CD8^{+} T cells is triggered by specialized APCs, such as dendritic cells (DCs) [145], and finally, CD8^{+} T cells travel to the tumor site and eradicate cancer cells [146]. Previous studies have established higher immune infiltration, stromal and intratumoral TILs in TNBC and HER-2^{+} BCs [147–149]. Due to its low antigenicity, immunotherapy is not recommended for ER[+] BCs. According to research findings, several factors are correlated with the low antigenicity of ER[+] BCs, and these factors are associated with diminished neoAg production [150–153]. In recent years, extensive research has been conducted on HER-2 vaccines. Patients with BC have a lower level of humoral immunity to HER-2 (spontaneous Ab production) than others [154]. A series of immunogenic peptides are produced from the receptor molecule HER-2, including peptides from its intracellular, extracellular, and transmembrane domains. These epitopes are AE36 (derived from the intracellular domain), E75 (originated from the extracellular domain), and GP2 (arising from the transmembrane domain) [126]. The
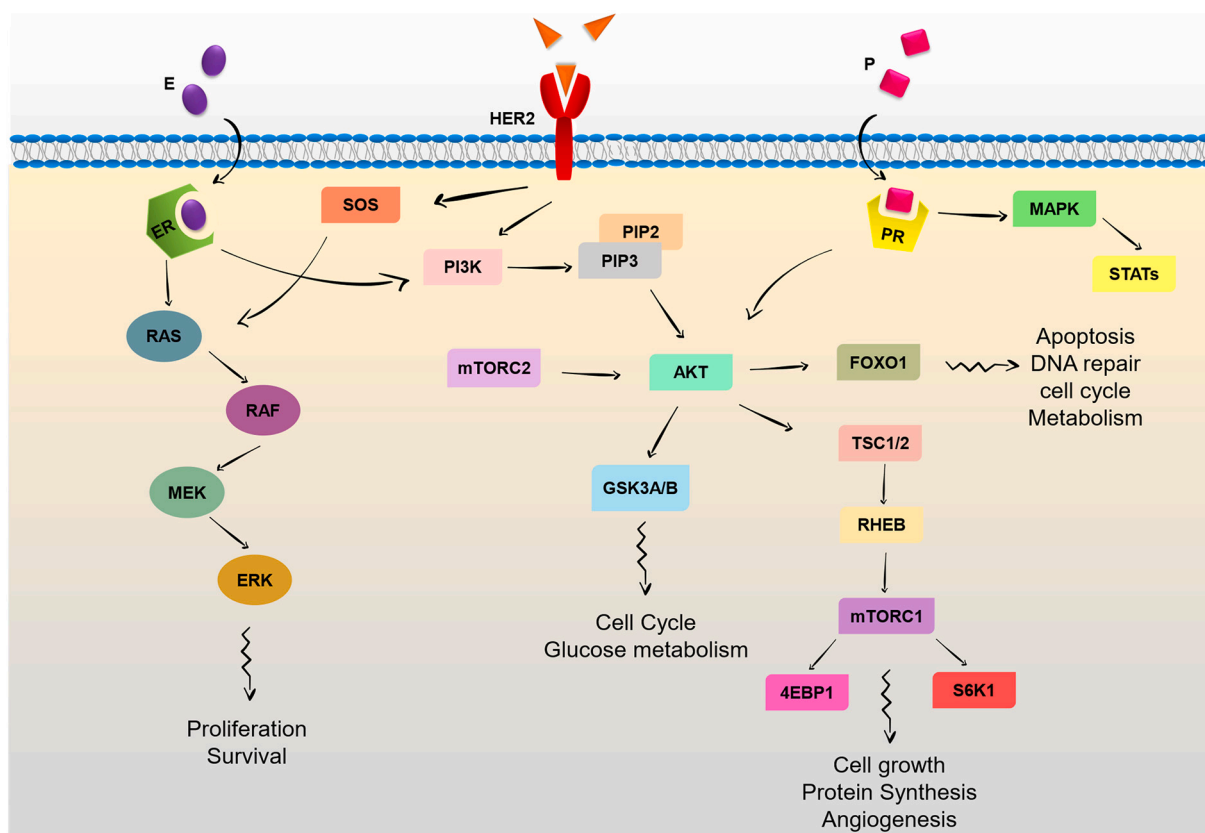
**Fig. 2.** Summary of signaling pathways created by ER, PR, and HER-2 biomarkers and their role in BC progression. ER, estrogen; PR, progesterone receptor; HER-2, human epidermal growth factor receptor 2; BC, breast cancer; AKT, protein kinase B; RAS, rat sarcoma virus; RAF, rapidly accelerated fibrosarcoma; MEK, mitogen-activated protein kinase; ERK, extracellular signal-regulated kinase; PI3K, phosphatidylinositol-3-kinase; PIP2, phosphatidylinositol 4,5-bisphosphate; PIP3, phosphatidylinositol (3,4,5)-trisphosphate; MAPK, mitogen-activated protein kinase; FOXO1, forkhead box protein O 1; STAT, signal transducer and activator of transcription; mTOR, mammalian target of rapamycin; mTORC2, mTOR complex 2; TSC1/2, tuberous sclerosis proteins 1 and 2; RHEB, ras homolog enriched in the brain; 4EBP1, eukaryotic translation initiation factor 4E (eIF4E)-binding protein 1; S6K1, ribosomal protein S6 kinase B; GSK3A/B, glycogen synthase kinase-3 A and B.

polypeptide E75 presents an antigenic determinant with an immuno-dominant cytotoxic T lymphocyte (CTL) response, with high avidity for human leukocyte antigen (HLA)-A2 and HLA-A3. The AE37 polypeptide is a chimeric MHC class II. AE36 is another HER-2-derived polypeptide and could prompt CD8^{+} and CD4^{+} cells. Vaccine immunogenicity is enhanced by LRMK, a four-amino-acid-sequence peptide (accelerates direct charging of MHC class II epitopes to the polypeptide-binding groove), which ultimately leads to enhanced Ag presentation [155,156].

The hypothesis of "immunoediting," including elimination, equilibrium, and escape steps, illustrates the immune system's function in the advancement and evolution of the tumor [157]. In the elimination phase, immunologic cells recognize and annihilate cancerous cells to stop proliferation. In the second step, the equilibrium phase, scant neoplastic cells that escape the elimination phase stay latent, whereas immunologic cells thwart neoplastic cell proliferation. When cancerous cells manage to evade detection and removal, they move on to the escape phase, becoming more aggressive [158]. Activating the CD8^{+} CTLs is the principal constituent of antitumoral immunity, exerting anti-cancer action through the emission of cytokines, for instance, tumor necrosis factor (TNF) and interferon (IFN) [159]. The quantity of CTLs in the tumor microenvironment (TME) and their capability to discriminate TAA significantly inhibit the development and proliferation of cancer [111]. Neoplastic cells can elude the immune system by modifying immunological surface markers, down-regulating the expression of MHC

class I proteins and co-stimulators, and by T cell receptor signaling defects [160]. Other strategies for escaping immune detection comprise activating regulatory pathways, developing immunosuppressant TME by regulatory T cells (Tregs), augmenting myeloid-originated suppressant cells, producing cancer proliferation factors, and interleukin (IL)-10 [158].

TILs comprise T and B lymphoid cells, NK cells, DCs, and macrophages that enwrap cancerous cells [161]. Identifying the number of TILs in the TME and the phenotype of infiltrated cells can foreshadow the immunogenic character of malignancy and enhance prognosis. CD8^{+} CTLs are crucial for cancer cell eradication and are linked to low morbidity in ER^{-}, ER^{+}, and HER-2^{+} malignancies. CD4^{+} T helper (Th) cells are likened to forkhead box P3 (FOXP3) CD4^{+} Treg cells and negatively influence the CTL function. CD4^{+} T cells play specific roles in cancer evolution. Type 1 T helper (Th1) cells are the prevailing subgroup of CD4^{+} T cells in the initial neoplasm phase and are crucial for immune monitoring. Nonetheless, in the progressive stages of malignancy, FOXP3^{+} Treg and Th17 cells are thought to be the most important subsets of CD4^{+} TILs, promoting tumor growth [162]. Polypeptides, proteins, APCs, tumor cell lysates, tumoral cells, deoxyribonucleic acid (DNA), mRNA, and viral vectors are promising approaches for generating cancer vaccines (Fig. 3) [163].
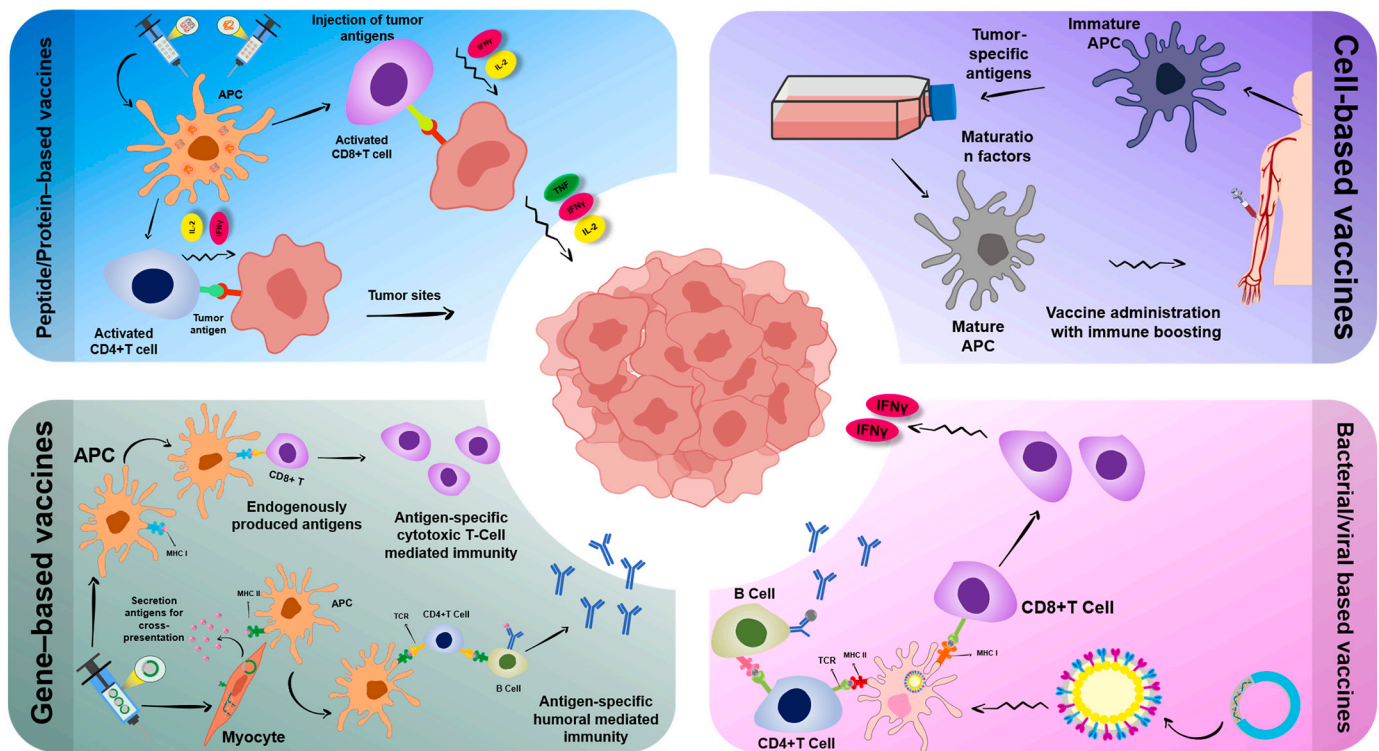
**Fig. 3.** A review of BC vaccines. Cancer vaccine platforms can be gene-, peptide/protein-, bacterial/viral-, or cell-based. BC, breast cancer; APC, antigen-presenting cell; MHC, major histocompatibility complex; CD8, cluster of differentiation 8; IL-4, interleukin 4; TCR, T-cell receptor; IFN-γ, interferon-gamma; TNF, tumor necrosis factor.

### 3.1. Peptide-based vaccines

For nearly 40 years, peptide-based remedial cancer vaccines have been envisioned and developed, but the approach remains appealing for cancer treatment. Contrary to the One advantage of using synthetic polypeptides as vaccines from both immunologic and chemical viewpoints is their versatility. Immunologically, polypeptide vaccines stimulate T-cell reactions more efficiently than complete protein vaccines [144,164,165]. Endosomes transport peptides into the protoplasm, effectively introducing them to MHC molecules more than complete proteins [166]. These vaccines can prompt an immune reaction against malignancies [167–169] and provide several other advantages: easy synthesis, cost-effectiveness, negligible side effects, and safety. Moreover, computational and algorithmic programs can be exploited for filtering amino acid sequences for individuals with MHC class I-restricted polypeptide epitopes of the TAAs. Experimentally, these individuals can be examined for their particular immunologic reactions [170].

E75 is a nine-aminoacid-length peptide originating from the HER-2 receptor and seemingly bound to HLA-A2 to activate CTLs [171–173]. This peptide is the most researched cancer vaccine. Numerous phase I examinations were executed by inoculating the polypeptide and blending it with immunologic adjuvants. Findings demonstrate that the vaccine is secure and can stimulate peptide-specific CTLs. Subsequently, further investigations were assessed by mixing E75 with a granulocyte-macrophage colony-stimulating factor (GM-CSF) in patients with node$^+$ and at-risk node$^-$ BC. Results deduced that the 5-year disease-free survival (DFS) was 89.7% for those administered E75 and 80.2% for placebo takers. In the phase III intervention study, E75 plus the immunoadjuvant GM-CSF vaccine (NeuVax™) was studied in patients with lower *HER-2/neu* gene expression (IHC 1$^+$/2$^+$). This compound exhibited no discrepancy in DFS between placebo and NeuVax™, contributing to the cessation of clinical trials. Notwithstanding, prospective analyses of other pharmaceutical combinations are needed [174].

GP2, a fragment of the HER-2$_{654-662}$ Ag as an immunogenic peptide, is a nine-aminoacid-long polypeptide vaccine. It affixes to the HLA-A2 with less avidity than E75 [175] and triggers CTLs. The phase I intervention study proposed that GP2 plus GM-CSF is safe and mild in lymph node-negative BC patients [72]. The phase II intervention studies were executed in healthy individuals with node$^+$ and at-risk node$^-$ HER-2-expressing cancer (IHC 1$^+$–3$^+$). The findings indicated no remarkable discrepancy in the reoccurrence prevalence between the vaccinated and control groups. Nonetheless, the trials demonstrate that the vaccine is safe to inject.

Additionally, a tendency for therapeutic value was observed in individuals with HER-2-overexpressed malignancies [176]. AE37 is a 15-amino acid-long polypeptide that prompts CD4$^+$ Th lymphoid cells [177]. A study of patients with HER-2-expressed BCs of all phases and IHC of 1$^+$–3$^+$ found that the vaccine had no considerable influence on DFS rates in those with overexpressed HER-2 receptors in the mammary tissue [176]. T cell-based vaccines stimulate immunologic reactions by inserting artificial T cell epitopes into the body. These compounds were aimed at inducing CTLs subsequently. Short peptides were employed to provoke CTLs and Th cells, but nowadays, longer polypeptides are utilized to trigger both. When injected into the patients, these polypeptides attach to the APCs' HLA classes I and II and assemble a polypeptide-HLA compound. When identified by CTLs, this complex is triggered and annihilates cancer cells [178].

Narrow investigations have been conducted into B cell polypeptide vaccines. The triumph of trastuzumab as a pharmaceutical for BC has resulted in an appeal for B cell polypeptide vaccines. The phase I clinical trial was executed by three peptides originating from the HER-2 receptor developed with influenza virosomes in patients with metastatic BC. The results illustrated that the vaccine is safe and is immunogenic in approximately 80% of the patients. The Abs produced by the individuals are similar to those of the existing Ab-based HER-2 therapy pharmaceuticals [178,179]. Another phase I intervention study was performed on two HER-2 B cell epitopes as bonding locations for trastuzumab and

pertuzumab [180]. Using whole proteins (HER-2 intra- or extracellular domains) as vaccines can contain HLA class I and II antigenic determinants, preventing certain HLA limitations. Lengthy peptides or protein-based vaccines can intensely prompt T cells, contributing to a boosted immunologic reaction and excellent T cell activation [164,181].

### 3.2. Protein-based vaccines

Protein-based vaccination, unlike peptide-based vaccines, has not yet been thoroughly investigated. The foremost clinical trial used the HER-2 intracellular domain (a segment of 676–1255 of the entire-length HER-2/neu) to determine whether the vaccination could induce immunostimulation. In this trial, 29 participants with HER-2$^+$ mammary or ovary cancers in recovery following routine treatment were inoculated with various vaccine dosages (25, 150, and 900 μg). Outcomes revealed that the vaccine was well-tolerated, and HER-2 intracellular domain-specified T-cell immunity was acquired in roughly 89% of the patients who finalized the vaccination program. Moreover, almost 82% of the individuals established HER-2/neu-specified immune globulin G (IgG). Additionally, there were no accounts of toxic incidents in grades 2–4 [112].

HER-2/neu helper polypeptide-based vaccines are efficacious in BC cases [182,183]. Hamilton et al. (2012) [184] inspected the antigenicity, safety, and impact of the anti-HER-2 protein. The vaccine dHER-2 is a biosynthetic protein including an extracellular domain (ECD) and a segment of the intracellular domain of HER-2 plus immunostimulant AS15 [185]. The twelve patients registered in the examination with trastuzumab-refractory HER-2-overexpressed metastatic BC acquired the vaccine and oral lapatinib. Consequences illustrated that all individuals attending the research were provoked with the anti-HER-2-specified Ab, and no cases of cardiac toxicity were documented. Statistics also revealed that the overall longevity at 300 days was 92% (confidence interval (CI): 77%–100%), proposing a more longevity advantage in cases with HER-2-overexpressed BCs refractory to trastuzumab [184].

### 3.3. Nucleic acid-based vaccines

DNA vaccines against cancer contain engineered DNA molecules encoding one or more tumor antigens (TAs) or immune modulators [186]. DNA vaccines must get through APCs' cell membranes and migrate to the cytoplasm and nucleus to serve their function. After mRNA is produced, it crosses the membrane into the cytoplasm and is translated into TAAs that CD4$^+$ and CD8$^+$ T cells and B cells can then present these epitopes [187]. APCs activate resident B and T cells, and lymphatic organs such as the spleen and lymph nodes are the final destinations for encoded antigens [188].

#### 3.3.1. DNA-based vaccines

As DNA vaccines blend numerous desired characteristics, mainly clinical usability, genetic immunostimulation employing naked plasmid DNA is of growing fondness in tumor immunology. DNA vaccines: 1) encode multiplex MHC I- and II-restricted antigenic determinants that could be introduced to both CD4$^+$ and CD8$^+$ T cells; 2) preferentially express MHC class I antigen; 3) can prompt both T-cell and humoral immune reactions; 4) comprise cytosine-phosphate diester-guanine (CpG)-rich sequences that are remarkably immunostimulant; 5) can be assembled at relatively low expenditures as a "general" vaccine usable to most persons; 6) and are deemed much less risky compared to viral vectors [189–194]. DNA vaccines were first developed in mice to protect them from later exposure to cancer cells. They were also employed to produce neoplastic models of transgenic founder mice, more likely representing the immune circumstances of people with cancer [189,190,192,193,195].

DNA vaccines can stimulate an antitumoral immune response in patients with BC [196–199]. These vaccines are established on the principle that the gene coding a cancer Ag can commonly be transfected and expressed in an APC. Such Ags are more prepared and introduced to prime a vigorous anti-cancer immune reaction. Selecting or designing a robust episome vector and efficacious targeting systems are essential aspects of DNA-based vaccination. The episome employed through DNA vaccination generally comes from bacterial sources with cytomegalovirus (CMV) or a hybrid SV40–CMV promoter [200,201]. DNA vaccines are developed utilizing various TAAs expressed solely in malignancies or overexpressed by transforming genes. HER-2/neu and mammaglobin-A (Mam-A) (also known as mammaglobin 1, or secretoglobin family 2A member 2) are oncogene proteins overexpressed in BC and are utilized as targeted Ags in devising DNA vaccines. Norell et al. (2010) conducted an intervention study wherein eight advanced/metastatic BC cases were inoculated with a DNA vaccine incorporating the signaling-defective total-length form of HER-2/neu plus a lower dosage of IL-2 and GM-CSF. They noticed robust humoral immune responses following HER2/neu-based vaccination, despite no significant progress in T cell function [202].

#### 3.3.2. RNA-based vaccines

RNA therapy to prevent and treat BC has emerged as an attractive field of medical research. By increasing or decreasing the expression of specific proteins, RNA-based drugs can act as potent drug regulators against cancer cells. Such properties contribute to high specificity and a low risk of off-target effects [203]. An area of nanomedicine relatively new to researchers and medical practitioners is mRNA vaccine immunotherapy, which focuses on developing mRNA vaccines tailored to the individual patient's specific needs [125]. RNA vaccines, on the other side, are founded upon "mRNA synthesized by *in vitro* transcription (IVT) utilizing a bacteriophage RNA-polymerase and template DNA that codes for the target Ag/Ags [204]. When mRNA transcripts are fused to the host, they are translated by APCs, presenting the resulting tumor-specific Ags to T cells, which arouse immunologic reactions [125]. mRNA vaccines can be delivered in three general approaches: 1) transfected into DCs; 2) encapsulated mRNA vaccines; 3) and naked mRNA vaccines [204].

A novel method for delivering vaccines is encapsulated mRNA vaccines using IVT technology. Several methods for encapsulating mRNAs have been introduced, such as using 1,2-dioleoyl-3-trimethylammonium propane (DOTAP), lipid NPs, and nanoemulsions. These carriers enhance cellular uptake and delivery, protect against nuclease degradation, and enhance bioavailability and physical stability [205]. Furthermore, NPs can also be engineered to be fully biodegradable, which could improve the efficiency of vaccine delivery even further. According to research, these NPs are composed of a pH-sensitive poly(b-amino ester) (PBAE) core wrapped in a phosphatide sheath, which effectively delivers mRNA *in vivo* and triggers immunologic responses against cancer [204].

A naked mRNA vaccine exists only in buffer and is not encapsulated in another substance, such as a lipid NP or liposome [204]. While several experiments have displayed that naked mRNA can arouse immunological reactions in a host when applied in animal models, the selection and range of these vaccines have yet to be determined. One important reason for this limitation is the transient protein expression from naked mRNA and its concise extracellular half-time through fast decomposition by ubiquitous RNases [204]. The first limitation may lead to multiple patient visits for repeat treatments because transient protein expression from naked mRNA can reduce the duration of treatment [204]. Liu et al. (2018) recently discovered that the MUC1-based mRNA vaccine stimulates a forceful CTL reaction to TNBC. When combined with ipilimumab, an anti-cytotoxic T lymphocyte-associated protein 4 (CTLA-4) mAB, mRNA vaccines increased T cell immunity more than the sole mRNA vaccine or mABs [124].

## 3.4. Immune cell-based vaccines

Immune cell-based vaccines are patient-specific whole-cell vaccines that use some proficient patient-isolated APCs synthesized *in vitro* to deliver selected cancer Ags. Additionally, it facilitates the return of cells to secondary lymphatic organs, triggering Th1 responses. In addition to peptides and proteins, tumor lysates and mRNAs can also be loaded into APCs (e.g., DCs). In contrast, they can combine with tumor cells or encode tumor Ags by acquiring viruses that provide them with the means to infect them [206].

### 3.4.1. Autologous tumor cell-based vaccine (ATCV)

Most non-cell cancer vaccines are prepared by employing a solitary TAA. Selecting a suitable TAA to optimize the immune processes is a significant trouble in employing vaccine treatments. Cancerous cells separated from the patients prevent the hardships related to Ag choice. The dogma underlying this approach is that a neoplastic cell embodies many TAAs to arouse a potent immune reaction [207].

ATCVs comprise characterized and uncharacterized TAAs to launch an anti-cancer polyclonal reaction [207]. Moreover, since all Ags originate from neoplastic cells at the site of primary invasion, ATCVs are patient-specified, exposing the patients to an entire and personalized Ag repertoire. This attribute is fundamental since each patient with BC incorporates up to 105 mutant genes [208]. Nevertheless, the procedure of devising ATCVs for individuals is complicated and pricey. Accordingly, allogenic neoplastic cell lines can be employed as a substitute for creating cell-based vaccines [209]. Several examinations have resembled ATCVs with irradiated *4T1* murine breast malignant adenoma cells. This resemblance might overvalue vaccine effectiveness since 1) cancerous cell lines are homogeneous, while human BC is remarkably heterogeneous, and 2) fractions of tumoral tissues include non-cancer cells, namely, fibroblasts, lymphoid cells, and endothelial cells [207].

In contrast, *4T1* mammary carcinoma cell lines are highly aggressive, metastatic, and contribute to 100% morbidity 3–5 weeks following vaccination if untreated. Thus, immunotherapies that inhibit *4T1* proliferation need additional care [207]. In preclinical research, weekly immunostimulation with irradiated IL-2-modified *4T1* cells remarkably lessened intuitive lung metastases in rats after footpad injection with parental *4T1* cells [210]. Ostrand-Rosenberg's team indicated that inoculating *4T1* tumor-bearing mice with irradiated MHC class II- or B7.1-transfected *4T1* cells considerably dwindled spontaneous metastasis with no influence on preliminary cancer proliferation [211]. In an associated investigation, adding IL-12 enhanced antineoplastic effectiveness [212]. Furthermore, this research revealed that combining CD4$^+$, CD8$^+$, and NK cells influenced anti-cancer activity. In other examinations by the same team, *4T1* cells were chimerized to express MHC class II, B7.1, and the *Staphylococcal aureus* enterotoxin B (SEB) superantigen. Administrating these Ags as adjuvant immunotherapies after malignancy resection, they found that metastasis diminished and patients' longevity was extended [213].

Two in-progress and three conducted intervention studies have researched the efficacy of ATCVs in BC patients. In a finalized investigation, 121 individuals with BC or ovary cancer were inoculated with autologous BC cells contaminated by the Newcastle disease virus (NDV). The 4-year OS was 96%, authenticating the vaccination efficiency [214]. In another study, 42 patients with BC were inoculated with a vaccine mixture including autologous and allogenic BC cells plus three TAAs blended with GM-CSF and IL-2 [215]. Post-inoculation, a considerable boost in lymphoid cell growth was noticed in 57–100% of the study attendees [215]. In an investigation, Elliott et al. (2013) registered 37 BC patients with depressed immune responses and inoculated them with a whole-cell vaccine including malignant cells plus immunostimulants. It was found that the 10-year OS of inoculated patients with suppressed immune responses increased considerably more following vaccination than that of unvaccinated ones [216]. In the three clinical trials mentioned above, the whole-cell-based vaccination was safe and did not provoke considerable poisonousness [214–216].

Anderson et al. (2022) found that autologous tumor cell vaccines that secrete the GM-CSF could be prepared for patients with metastatic BC by preparing their tumor cells. At least six vaccines were synthesized from harvested tumors in 54% of enrolled patients. On the other hand, the success rate for individuals with stage II–III ailment was considerably lower (39%). Specifically, Anderson et al. (2022) performed this study to harvest treatment-resistant cells following chemotherapy. Nonetheless, the practicability of harvesting viable cancerous cells after treatment may be affected by improvements in neoadjuvant therapies. Autologous GM-CSF-secreting cancer cell-based vaccines are presumably effectual for patients with 1) high-grade hormone receptor-positive, early-stage cancer and 2) those with TNBC with at least 2 cm of residual tumor following neoadjuvant chemotherapy. These vaccines have yet to be thoroughly investigated [217,218]. On the positive side, two cohort studies showed a higher mean GM-CSF yield than the average of previous research on lung cancer and melanoma patients [217,219,220], suggesting minimal toxicity with vaccination among the cohorts (for further information, refer to NCT00317603 and NCT00880464) [217]. A clinical investigation has shown that ATCVs are ostensibly influential and safe vaccines for BC cases. Nevertheless, the increased variation of the vaccine and the intricate fabrication procedure are detriments to administering ATCVs [207].

### 3.4.2. DC-based vaccines

Primary research demonstrated that BC-infiltrating DCs were recognized in more than 40% of individuals with early and progressive BC [221]. DCs have indicated that they can provide a memory response to cancer Ags and suppress cancer proliferation in BC patients [222,223]. Gong et al. (2000) reported that autologous CTLs could decompose cancerous cells after fusing DCs with BC cells [224]. Moreover, when DCs containing allogeneic BC cells were activated, they triggered CTLs, which led to the destruction of the targeted cells [225]. A study with HER-2$^+$ rats was undertaken to increase the antigenicity of HER-2. Mice were sensitized with DCs expressing the lymphocyte antigen 75 (Ly75 or DEC205) receptor, and a significant quantity of T and B immune cells were noticed despite the scant quantity of HER-2 protein [226]. Scientists have sought to conquer resistance to trastuzumab (an anti-HER-2 mAB) by delivering ovalbumin (OVA)-specific exosome vaccines. This vaccine comprised DC-released exosomes (EXOOVA) regulated *via* CD4$^+$ T cells (OVA-TEXO). The experiments have resulted in protective immunity in these mice [227]. In addition, HER-2-adenovirus-transduced DCs were tested to prevent the proliferation of BC-infiltrating DCs in HER-2-transgenic rats [228]. Using the immune cytokine of IL-2 and an Ab against phosphatidylserine, researchers assessed the ability of a whole-cell BC vaccine to impede the development of malignancy in mice. Eighty percent of the rats survived tumor-free, and their splenocytes' specific cytotoxicity increased significantly more than controls [229].

One study investigated whether HER-2- or MUC-pulsed DCs could be used to vaccinate individuals with metastatic BC and heavily pretreated aggressive ovary cancer. Brossart et al. (2000) collected samples from ten patients with metastatic BC and severely pretreated advanced ovary cancer [230]. A total of ten patients, even those who had been highly pretreated with chemotherapy, saw no side effects and showed an improvement in their immune responses, supporting the notion that peptide-based DC vaccination can also effectively eradicate residual disease following severe or even high-dose chemotherapy. A potential limitation of DC vaccines is their low immunogenicity and the relatively few Ags that have been identified. One tactic is the fusion of autologous malignant cells with DCs. Using patient-derived tumor cells and autologous DCs, Avigan et al. (2004) showed that individuals with metastatic breast and renal cancer manifested clinically significant immunological anti-tumor reactions. These responses could be sustained without significant side effects [231]. Patients with ER$^-$/PR$^-$ BC also demonstrated similar results [232].

The studies showed that 58% of the subjects experienced specific delayed type IV hypersensitivity reactions after cell activation. This research suggests that tumoral lysate-pulsed DCs supply a vast reservoir of active Ags in stimulating anti-BC responses in patients. Immunostimulants such as cytokine adjuvants (e.g., IL-12 and IL-2) would help DC vaccines' efficiency. Six individuals with metastatic renal cancer and four with metastatic BC were enrolled in a phase I/II intervention study examining the DC vaccine and IL-2 [233]. The individuals involved in the experiment were treated twice with autologous tumor lysates stimulated with low-dose IL-2 from mature DCs. Although everyone who received the vaccination experienced a tolerable response, it is worth remarking that only one individual with renal cancer who received the vaccine accomplished stable disease outcomes.

Serody and Svane (2004-2018) conducted various studies to determine how DC immunization can work synergistically with other remedies, such as chemotherapeutics (e.g., vinorelbine or cyclophosphamide) or targeted therapies. Combining one or more remedial approaches with different mechanisms of action might allow for a more robust and specific immune response that could later be used against the cancerous cells to stop their growth. Three clinical trials (NCT00088985, NCT00266110, and NCT00978913) are in phase I and II studies evaluating these combination therapies' efficacy and noxiousness [234].

### 3.5. Bacterial/viral-based vaccines

Viral particles are innately immunogenic, and their genetic substances can transfer any transgene for their expression within the host cells. Infection and expression of the transgene can be achieved by various recombinant viruses in immune cells, such as APCs, particularly DCs [235]. In addition, tumor Ags are exposed more readily to the immune system, resulting in high numbers and avidity of CTLs that target cancerous cells with the Ags expressed by the vaccine vector [236]. According to reports thus far, recombinant viruses are easier to produce, distribute, and control than other immunotherapy strategies. The outcome may have been achieved due to understanding individual virus characteristics with their unique virtues and disadvantages, which determines the utility of a particular therapeutic approach [237]. Given viruses' ability to naturally infect human cells, they can deliver vaccines successfully as they elicit host T-cell reactions and humoral immunity against them [238–240]. Virus-mediated oncolysis can kill tumor cells directly through direct or indirect activity, such as triggering immune responses by expressing Ags specific to the tumor [241]. Oncolytic viral administration excites antiviral and anti-tumor immunity. Adaptive and innate immune responses to viruses may determine how effective oncolytic viral therapy is. When cancer cells become infected by oncolytic viruses, they produce viral Ags on their exterior, distinguished by $CD4^+$ and $CD8^+$ T cells, which destroy cancerous cells. Innate immunity also uses NK cells to target cancer cells [242].

Recent BC treatment approaches and research have studied the oncolytic effects of adenoviruses. Replication-defective adenoviruses lack specificity to target tumor cells. Therefore, their therapeutic value is limited. If the gene accountable for viral growth/replication is placed underneath promoters specified for a tumor or tissue, then the specificity of the therapy will be increased. Adenoviruses' replication is controlled by a gene located downstream of the E2F-1 promoter, as E2F-1 expression is significantly higher in BC tissues than in healthy ones [243,244]. In a recent experiment, Yan et al. (2019) indicated that recombinant adenovirus vectors containing the E2F-1 promoter and the immune regulator IL-15 can be used for replication-selective virotherapy [245]. While activating oncogenes in normal cells and facilitating linker-insertion mutageneses are retroviral vectors' safety concerns, they have valuable properties such as high-molecular-weight transgenes and long-term transgene expression.

McCrudden and McCarthy (2014) reported that recombinant retroviral vectors could express transgenes in malignant cells [246]. Enzymes metabolize recombinant retroviruses into active toxic metabolites by cancer cells, a process called gene-directed enzyme prodrug therapy (GDEPT) [247]. A product of the recombinant retroviral vector MetXia-P450 is active phosphoramide mustard and acrolein, produced during the cyclophosphamide monohydrate (sendoxan) metabolism. T47D BC cells sensitized to cyclophosphamide by the retroviral vector MetXia-P450 decreased tumor measures in MDA-MB-231 breast tumor heterograft models [248,249]. In addition to Rexin-G™, several other cancers, such as BC, can be treated with retroviral vector-based vaccines that promote replication-incompetence [250]. An engineered human cyclin G1 transgene encoded by Rexin-G™ can induce apoptosis and prevent angiogenesis by affecting the expression of the cyclin G1 [250,251].

Virus vector-DC vaccines, such as virus vector-CAR-T, are another potential strategy for delivering vaccines via virus-mediated delivery. In order to guide the immune system towards immunity or tolerance, DCs can be targeted for transgene expression. A transcriptional approach may be adopted by refocusing the tropism of the virus vector or retargeting DC-specific promoters [252]. Enhanced DC maturation is the main benefit of genetically modified DCs using viral vectors [253]. Because recombinant adenoviral vectors are highly effective in inducing humoral and cellular immunity, they have frequently been used to transmit tumor Ags to DCs [254]. Using an adenovirus-transduced human DC for an up-regulation of CD83 and a down-regulation of CD14, researchers characterized the mature DC phenotype while down-regulating the production of IL-10 [254].

Chen et al. (2001) showed concomitant stimulation of protective and remedial immunity against an HER-2/neu-overexpressing breast tumor cell line by the adenovirus type 5 (Ad5)-expressing HER-2/neu gene on DCs [255]. Transfecting DCs also increased the protection with Ad5 and IL-12. Oncolytic viruses became highly relevant to recent research on cancer treatment by synergistically directing/targeting CAR-modified T cells on tumors [256]. CAR-modified T cells have previously promised to treat patients with hematological malignancies. Recent studies have demonstrated that viral vectors with synergistic effects enhance tumor-specific T cells' functions to selectively deliver gene-based vaccines/therapeutic transgenes to solid TME [257]. Using the inverted cytokine receptor 4/7ICR in transferring the suppressive IL4 signal, Bajgain et al. (2018) reported that CAR-modified T cells targeting the transmembrane glycoprotein Mucin 1 (MUC1) boosted the antitumoral impact at the tumor site of a BC model in vivo [258].

Besides viruses, bacteria can also be used to make vaccines. In addition to conventional anticancer therapies, live tumor-targeting bacteria can also be used as a complementary therapy to enhance clinical outcomes [259]. As a result of their natural motile ability, bacteria have been of particular desire because they tend to distance themselves from the microvasculature and incorporate themselves into hypoxic areas of the tumor, which ultimately leads to their proliferation within the tumor cells [260,261]. When drugs are delivered directly to tumors via bacteria, they enhance specific cancer-targeting remedies and reduce the risk of adverse effects [262]. Furthermore, bacteria can also generate therapeutic molecules within the tumor on-site. In the same way, bacteria can travel from place to place to produce drugs [263].

Kim et al. (2009) demonstrated that various immunization approaches with LM-LLO-Mage-b$_{311-660}$ had different influences in a highly aggressive mouse model of metastatic BC. Three preventive or three therapeutic immunizations were superior to the combined approach. In addition, these findings unequivocally suggest that Listeria-specific CTLs are involved in the cytolysis of tumor cells. Reduced efficiencies may be due to separate actions related to vaccine-provoked immune responses or direct killing. By combining immediate eradication and immune reactions against highly immunogenic Ags instead of weak TAA, this dual mechanism against cancerous cells has not been identified earlier. It can be used to eradicate BC effectively [264].

## 4. Nanotechnology in BC vaccination

We have previously shown that various categories of nanomaterials are currently used to accurately detect various tumor markers, such as HER-2, CA125, CA15-3, MUC1, and CA19-9 [265]. A subunit vaccine typically generates a short-term immune response with weak immunogenicity. To tackle this challenge, scientists have created novel formulations that serve as carriers for vaccine subunits. NPs facilitate Ag delivery and presentation by APCs [266,267]. Recent advances in nanotechnology have made it possible to develop nanomedicines and vaccines. NPs synthesized from biocompatible materials have been widely explored in experimental and clinical trials to overcome the difficulties of immunotherapy against cancer [268,269]. Fig. 4 summarizes different NPs that have been used to modulate immune responses.

As another example shown in Fig. 5, core-shell gold nanocage (AuNC@MnO2, AM) NPs have been designed to enhance photodynamic therapy (PDT) in a murine model of metastatic TNBC. The TME's acidic pH degrades the NP's outer shell, allowing these particles to release large amounts of oxygen into the tumor. Oxygen-boosted PDT, on the other hand, triggers cancer cell immunogenic cell death (ICD)[270].

Nanovaccines are one of the hottest research topics in cancer immunotherapy [271,272]. Nonetheless, most reported nanovaccines

involve complicated synthesis and modification processes. Additionally, they present several technical and manufacturing challenges. They might be developed with simple synthesis, inexpensive manufacture, scalable production, and clinically realizable translation [272]. Nanovaccines offer some benefits versus subunit vaccinations: 1) Ags encapsulated in NPs can increase Ag stability and prevent degradation; 2) co-encapsulating adjuvants and Ags in nanovaccines can co-deliver them, ultimately increasing vaccine antigenicity and remedial effectivity; 3) nanovaccines can be effortlessly phagocytized and prepared by APCs; 4) NPs fabricated for cytoplasmic Ag delivery can generate cross-presentation, thereby enhancing Ag escape from the endosome and improving CTL responses (critical for cancer immunotherapy); 5) modifying the surface of NPs with targeting ligand allows them to be directed to lymphatic tissues and APCs for accurate immunomodulatory therapy; 6) eventually, the polyvalent Ag presentation on the exterior of nanovaccines permits cross-linking of B cell receptors, resulting in heightened humoral immune responses [266,267].

### 4.1. Use of nanocarriers in BC vaccines

Based on the working mechanisms of nanotechnology, nanocarriers can serve as effective vaccine carriers. Macrophages and DCs can capture particles smaller than 10 nm. This property enhances Ag' cellular
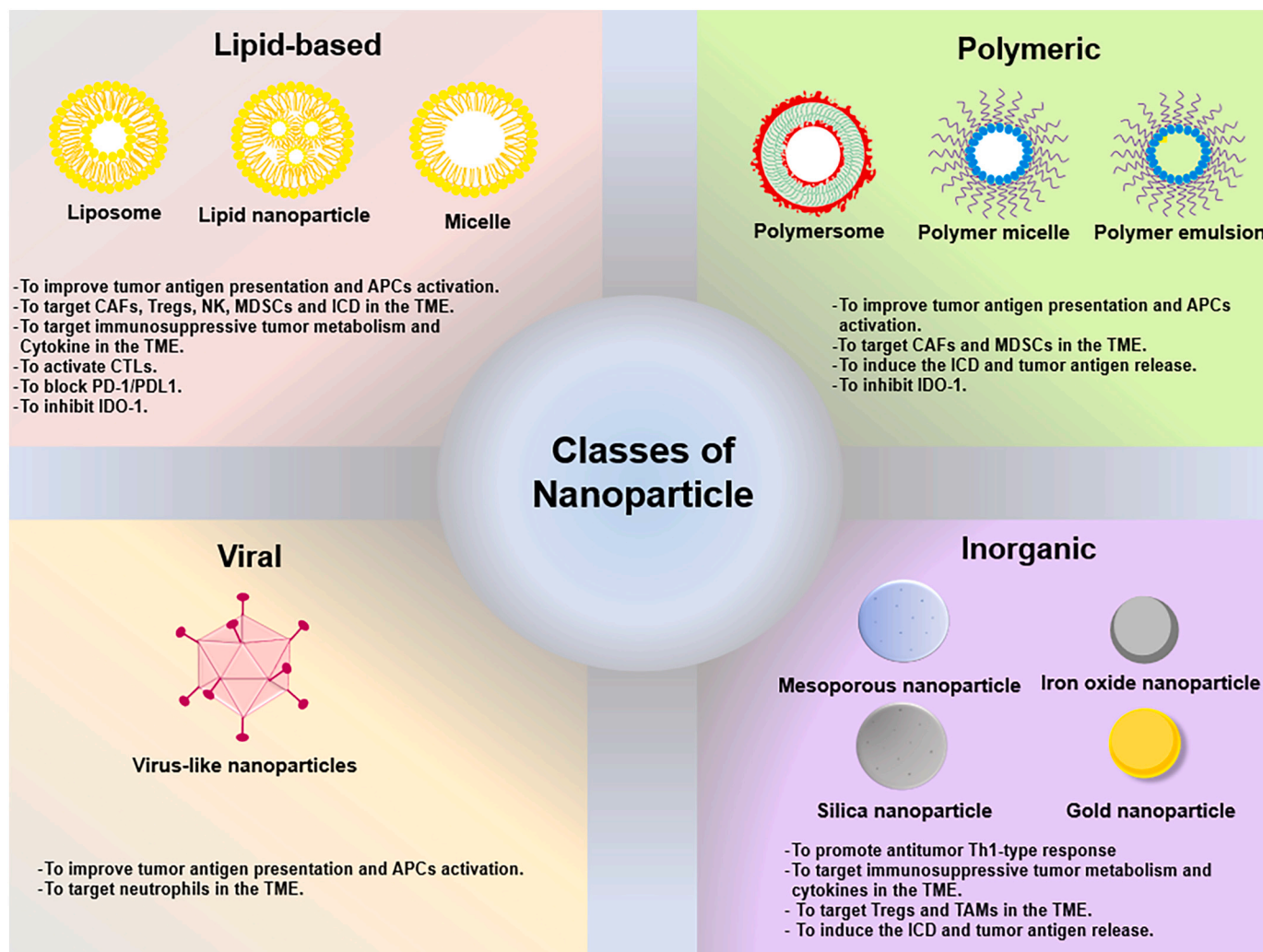


**Fig. 4.** Classifications of different NPs and their mechanisms of action toward modulation of immune responses. NP, nanoparticle; PD-L1, programmed cell death ligand 1; anti-PDL-1, programmed cell death protein 1; CTLs, cytotoxic T lymphocytes; NK, natural killer; Tregs, regulatory T cells; TAM, tumor-associated macrophage; IDO-1, indoleamine 2,3-dioxygenase 1; APC, antigen-presenting cell; ICD, immunogenic cell death; MDSC, myeloid-derived suppressor cell; TME, tumor microenvironment; CAF, carcinoma-associated fibroblast.
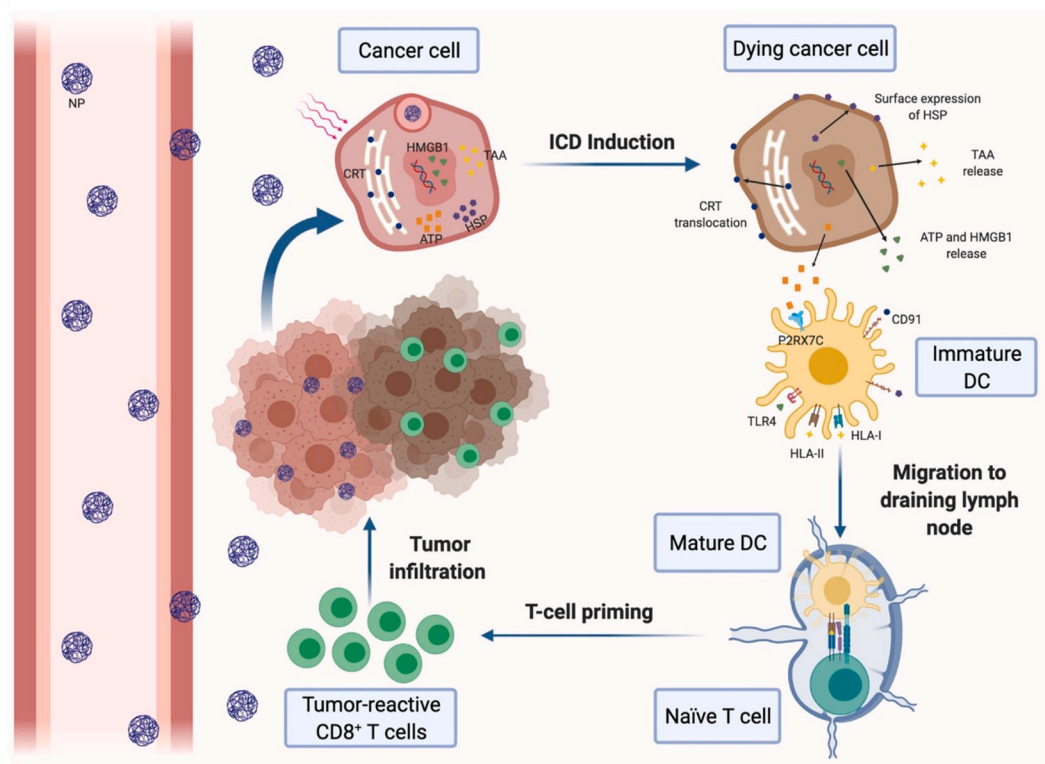
**Fig. 5.** Induction of immunogenic cell death (ICD). Reprinted from [270] under the terms and conditions of the Creative Commons Attribution (CC BY) license (https://creativecommons.org/licenses/by/4.0/).

uptake, improving their recognition and presentation [273]. Vaccines delivered *via* the oral or mucosal route may be protected from degradation by solid nanocarriers and enable entry into gastrointestinal and mucosal lymph nodes [274]. Nanocarriers altered by surface modifications might aid in targeting Ag delivery. The immune system's vast array of surface receptors, such as the mannose-fucose receptor (MRC1 protein), scavenger receptors (acetyl-LDL receptor), and toll-like receptors (TLRs), initiate immunological responses [275]. Transporting Ags and adjuvants with nanostructures wrapped with immune cell-targeting compounds will allow prophylactic vaccines to induce specific and selective immune responses by targeting overexpressed receptors [276–279].

### 4.1.1. Liposomal-based vaccines

Liposomes are made up of bilayer phospholipids and cholesterol as their primary building blocks. They protect vesicles from degradation by providing a solid framework [280]. Since the lipid in liposomes is similar to that in the cell membrane, they could get into the cell more easily and function with the reticular and endothelial systems, providing a targeted immune response function [281]. Liposome formulae for vaccine delivery systems have been extensively researched since liposomes were first reported to function as immunological adjuvants [279,282].

Many benefits have been reported for liposomes: 1) in comparison to conventional drug delivery methods, these systems were less toxic and allowed higher doses of drugs to be administered [283]; 2) depending on the intended application, they can be synthesized in various sizes, compositions, and lipid loads [283–285]; 3) it is possible to use logic to design liposomal delivery systems that work best for delivering specific Ags [286]; 4) when liposomes are used, Ags can be encased in a hydrophilic core, trapped in a hydrophobic bilayer, attached to the exterior with an electric charge, absorbed, or held in place by changing the acyl chains. The bioavailability and therapeutic window are improved due to liposomes' ability to delay Ag degradation by enzymes and to augment

absorption rates *via* biological membranes (the lowest and highest dose of the pharmaceutical that can treat the disease efficaciously while manifesting minimum noxiousness) [287]; 5) liposomes could target a specific area of the body [288,289]; and finally, 6) releasing Ags into endosomes, neoplasms, and inflammatory tissues can be better induced [290].

The encapsulated Ag could be more effectively targeted by applying targeted liposomes, where the liposomes' surface contains moieties that can recognize target immune cells and attach to them, resulting in internalization of the liposomes [291,292]. Liposomal cancer vaccine delivery has many advantages, comprising a favorable immune response, increased Ag delivery to specific tissues without toxicity [283], and improved APC uptake by altering the number of molecules exposed on the liposome surface. Stability over time is a significant issue for liposomes, but freeze-drying and carbohydrate attachment may be solutions. As a result, it is challenging to evaluate the impact of a single composition's parameters (e.g., surface charge or lamellarity) on the immune reaction without modifying other criteria (e.g., lipid composition and method of preparation) [287]. Lipopolymers may be tuned according to Ag properties to maximize their immunogenicity, including their size, composition of lipids, and structure. The phospholipid bilayers that compose liposomes allow them to load and deliver both hydrophobic and hydrophilic molecules; adjuvants and Ags may be delivered simultaneously because of these properties. Liposome surfaces can be easily modified using a lipid bilayer of functionally active lipids [279]. The potentiating effects of BC vaccines have also been reported with liposomal NPs containing peptides [270]. Importantly, anti-HER-2 nanoliposomal vaccines are easily synthesized and very efficient at stimulating HER-2-specific CTL immune responses. Besides, advancements in the nanoliposomal vaccines, such as adding either TLR ligands or other immunostimulants, might even further enhance the effectiveness of these vaccines [156].

In one study, Talesh et al. (2016) encapsulated multi-epitope P5 polypeptide in nanoliposomes containing DOTAP—a potent

immunostimulant that enhanced Th1 and CTL responses. This combination activated DCs, cholesterol (Chol), and poly(I:C). Tumor-bearing mice were given the formulation three times every two weeks. Because of their cationic liposomal structure, nanoliposomes containing P5 were introduced into APCs' cytosol. The nanoformulation also enabled T lymphocytes to produce IFN-γ, reducing tumor growth in mice and preventing tumor regression [293]. In this regard, Shariat et al. (2014) also designed liposomes of P5 carriers to release peptides into APC cytosols, especially in DCs, with dioleoyl-phosphatidylethanolamine (DOPE). Also, the liposomes provided monophosphoryl lipid A (MPL), which stimulated TLR4 and caused co-stimulatory compounds and inflammatory cytokines to be produced by DCs. This approach enhanced the provision of P5 to CD8$^+$ lymphocytes by APCs. Using Lip-DOPE-P5-MPL compound, the TUBO tumor-bearing mice were subcutaneously inoculated three times, and remarkable CTL responses were generated against the P5 Ag and escalated IFN-γ production by CD8$^+$ T cells. Treatment with Lip-DOPE-P5-MPL precluded cancer proliferation and increased survival in mice, as MPL and DOPE synergistically enhance vaccination efficiency [294].

Zamani et al. (2019) reported that CTL-specific peptide P5 was effective in mice subjected to peptides containing rat her-2/Neu proteins and pan HLA-DR (PADRE) peptide (an epitope on CD4$^+$ Th cells), as well as MPL, which is a co-stimulatory lipid that activates TLR4. CD8$^+$ T cell immunity was then observed in the TUBO-bearing mice inoculated with liposomal P5 polypeptide, PADRE, and MPL. Additionally, the anti-tumor efficacy of this formulation was superior to a liposomal vaccine containing only P5 in *BALB/c* mice overexpressing HER-2 protein. A study on mice inoculated with the Lip-P5-integrated PADRE-MPL combination found significant increases in producing IFN-γ, CD8$^+$ T cell numbers, and survival. Accordingly, Lip-P5-integrated PADRE-MPL, following more validation, can generate robust CTL anti-tumor immune responses useful for the remedy of HER-2$^+$ BC [295].

In another investigation, Zamani et al. (2020) demonstrated that some alteration to a polypeptide-based vaccine could affect the immunogenicity of the vaccine and its anti-tumor efficacy. TAA peptides were presented in a DOPE-encompassing liposome alongside PADRE, and two short peptides were linked to produce a single lengthy multi-epitope polypeptide. The liposomal presentation of polypeptides improved the immunogenicity of peptides. The PADRE was the second modification, and the linkage of the peptides was the final modification. When administrated non-liposomally, E75-AE36 did not exhibit the desired enhancements. PADRE improved the properties of both mixed and linked immunogenic polypeptides. In contrast, the immunogenic and anti-tumor effects were significantly improved when both lengthy peptide and PADRE were given as liposomes. The group injections of non-liposomal short peptides, long peptides, short peptide + PADRE, and long peptide + PADRE experienced no significant differences. In contrast, the group injections of liposomal peptides showed an improved anti-tumor response by adding PADRE to the long peptide. Generally, the results suggest that liposomal formulations might optimize the immune response of peptide-based vaccines. For example, when combined, a liposomal formulation of long multi-epitope peptides and PADRE can elicit more powerful immunological responses [296].

Rastakhiz et al. (2019) executed research to evaluate the antitumoral and immunomodulation of the liposomal vaccine. The vaccine consists of the P5 HER-2 peptide—a neu-originated polypeptide attached to the exterior of high-temperature nanoliposomes-DOPE, and MPL adjuvant in the HER-2/neu overexpressed BC model. Results showed that interferon-γ and CTL reactions were the highest when tumor-bearing mice were immunologically sensitized to Lip/DOPE/MPL/P5, leading to the smallest tumor measure and most prolonged survival time. Lip/DOPE/MPL/P5 formulation has shown promising results for inducing a robust Ag-specific immune response against BC [297].

Arab et al. (2018) generated a vaccine delivery system to improve anti-tumor immunity against the E75 peptide. The system included an efficacious vaccine/adjuvant delivery system by binding the polypeptide to the exterior of liposomes comprising definite phosphatides (distearoyl phosphatidylcholine (DSPC) and distearoyl phosphoglycerol (DSPG) with high temperature and DOPE). Using enzyme-linked immuno spot (ELISpot) assay and flow cytometry analyses, the results suggested that mice inoculated with DSPC/DSPG/Chol/DOPE/E75 generated considerably more significant levels of Ag-specified IFN-γ from CD8$^+$ T cells and stimulated CTL anticancer immunologic reactions in the TUBO tumor-bearing mice, inhibiting cancer progression and increasing survival. Consequently, the liposomes containing DSPC/DSPG/Chol/DOPE are appropriate candidates to prevent and treat HER-2$^+$ BC [298]. Additionally, in another study, Zamani et al. (2020) demonstrated that Lip-Pep and Lip-doxorubicin (DOX) induced tumor infiltration with TILs and NK cells, enhanced IFN-γ excretion, and diminished myeloid-derived suppressor cells (MDSCs) and CD25+FOXP3+Treg populations in the TME more effectively than E75 and DOX alone. Furthermore, Lip-Pep+Lip-DOX-treated mice experienced significantly weakened cancer proliferation rates and lessened survival than the untreated ones [299].

A nanoliposomal vaccine delivery system based on P435 HER-2/neu-originated polypeptide conjugated to maleimide-polyethylene glycol (PEG)2000-DSPE was designed by Farzad et al. (2019). This immunoadjuvant consisted of nanoliposomes prepared from DSPC/DSPG/Chol/DOPE and MPL. The anti-tumor efficiency of these formulae was tested by immunizing tumor-bearing *BALB/c* mice and studying the immunologic responses induced by administering the ELISpot test and flow cytometry analyses. Interestingly, findings deduced that the Lip + POPE + P535 blend led to the smallest tumor measure and the most lasting survival in a TUBO tumor-bearing mouse, making it an ideal candidate for developing safe and effective vaccines against HER-2$^+$ BC [300].

A recent investigation by Barati et al. (2017) has exhibited that the AE36 peptide can be incorporated into nanoliposomes that contain the DOTAP, DOPE, and cholesterol (DDC) or DD, along with the CpG motifs. TUBO breast tumors' prevention and treatment models showed that liposomal nanoformulations raised IL-4 and IFN-γ production, decreased tumor scale, and long-term survival [301].

In recent research in 2020, Wallis et al. showed the potential of a liposomal-based vaccination that three-dimensionally separates target and helper polypeptides to induce a fast, high-titer, isotype-interchanged, humoral immune response against HER-2, changing the function of pre-existent non-cognate CD4$^+$ T helper cells. Subsequently, it was revealed that these Abs might trigger cell death in an HER-2-overexpressing cell line *in vitro*. A liposomal system consisting of spatially divided HER-2 polypeptides to trigger B cells and OVA 323339 peptide to promote non-cognate T cell activity was employed to create Abs against the epitope of the HER-2 targeted by pertuzumab [302]. This investigation is deemed a novel advancement in liposomal vaccination.

Mohammadadian et al. (2021) studied immunotherapy by co-delivery of liposome-coupled immune checkpoint molecule lymphocyte activation gene 3 fused to the Fc portion of IgG molecule (LAG3-Ig) as an adjuvant, and P5 tumor Ag in a TUBO-bearing murine model. In contrast to free LAG3-Ig, the liposomes-conjugated one significantly increased the maturation of DCs by exerting immunostimulatory effects through polyvalent binding to MHC class II. More efficiently than locally injected soluble LAG3-Ig plus P5, LAG3-Ig-P5 immunoliposomes triggered preservative anti-cancer responses. Following immunoliposome treatment, it was indicated that the proportion of CD4$^+$ and CD8$^+$ T cells in the spleen increased, and these effector cells infiltrated the tumor site more quickly and pronouncedly. Last but not least, LAG3-Ig-P5-immunoliposomes' induction of anti-tumor immunity resulted in higher tumor shrinkage and longer life in treated animals than soluble immunotherapy [303].

Naghibi et al. (2020) used a nanoliposome containing 1,2-distearoyl-sn-glycero-3-phosphocholine (DSPC): 1,2-distearoyl-sn-glycero-3-phosphoglycerol (DSPG): cholesterol with/without

dioleylphosphatidylethanolamine (DOPE) delivery vehicle. To accomplish this goal, the researchers conjugated the P5+435 peptide to maleimide-PEG2000-DSPE and then attached it to the exterior of nanoliposomes. The Lip-DOPE-P5+435-vaccinated animals exhibited the largest number of IFN-generating CTLs with the best cytotoxic function, considerably shrinking tumor measures and prolonging the longevity in the TUBO murine model. As a result, liposomes with high transition temperature phospholipids such as DSPC are more durable and accessible to the immune system during extended *in vivo* circulation [304].

### 4.1.2. Polymeric vaccines

The biocompatibility, biodegradability, and mucoadhesive properties of natural polymers like chitosan (CS) and alginate make them excellent candidates for developing particle-based vaccine delivery vehicles [305]. Synthetic polymers that are typically employed in biomedical utilities comprise poly(lactic acid) (PLA), poly(glycolic acid) (PGA), and poly(lactic-co-glycolic acid) (PLGA) [306]. Additionally, poly(ethylene imine) (PEI), poly(N,N-cystaminebis(acrylamide)-co-4-amino-1-butanol) (pABOL), poly(ε-caprolactone) (PCL), and poly(anhydride) (PAN) are other synthetic polymers applied for developing vaccines [307]. Polymeric nanostructures, which can imitate pathogen biophysical and biochemical features thanks to their formula, could be modularized to elicit powerful and protective immune responses by replicating these signals in vaccination [307]. Vaccine delivery techniques based on polymeric structures include micelles, nanogels, polymersomes, and core-shell NPs. Ags can be incorporated inside the polymer or exhibited on its surface, leading to various Ag-loaded polymer shapes and measurements [307]. When used as a carrier in cancer vaccinations, polymer NPs have numerous advantages: 1) their ability to efficiently deliver Ags, proteins, and drugs to the target region; 2) they have exhibited less cytotoxicity and can preserve the Ags or medications supplied by mucosal administration from degradation under unfavorable conditions; 3) increased and robust immune responses can be induced by the absorption of such NPs by APCs; and finally, 4) using these NPs, the vaccine's Ag can remain active for longer [308].

Polymer NPs' size and shape control Ag distribution to the MHC II loading pathway and charge; thus, consideration should be given to these properties when using polymer NPs as a carrier. Diverse immune responses could be elicited by delivering Ags *via* varied particle sizes [309–314]. For example, nano-sized particles (20, 40, 49, 67, 93, 101, and 123 nm) provoke significant T-cell reactions. Particles with a diameter of 40–49 nm produce the most Ag-specific Th1 cytokine (IFN), whereas particles with a diameter of 93–123 nm primarily produce Th2 cytokine (IL-4) [309]. By altering the form of polymeric particles, Th cell responses can be elicited. Polystyrene spheres elicited more Th1 responses than rod-shaped polystyrene particles, as evaluated by the IgG1: IgG2a ratio, but rod-shaped polystyrene particles prompted more Th2 responses [313]. The Th response induction appears to be influenced by the polymeric NP's surface charge. An anionic immune response was more balanced than a Th1/Th2 response when exposed to cationic particles [315,316].

Notwithstanding the fast progress of polyester-based particle vaccinations, there are still limitations to their broad usage. PLGA degrades to lactic acid, and the concentration of glycolic acid in the PLGA particles can drop dramatically due to this degradation. Microparticles, which have a bigger volume and a deeper inner core than NPs, attain a pH of 1.5 [317] and exacerbate the local acidification [318]. In other words, low pH has a deleterious effect on Ag structure, leading to aggregation [319], which diminishes APCs' ability to take up Ags and inhibits immunological responses [320]. Encapsulated Ags can also be damaged by exposure to high temperatures during organic solvent elimination and incompatibility with excipients during their removal in the polyester particle production process [321]. Developing particles with high homogeneity and batch-to-batch repeatability is arduous when creating particles with numerous surface functions and expanding from

laboratory to industry. In addition, it should be emphasized that harmful organic chemicals must be removed from PLA/PLGA particles before they can be considered safe to be utilized in medicine. The necessity to create the specific Ags to be integrated into the PLA/PLGA particles further complicates manufacturing [306].

Polymers can produce Ag nanocarriers that APCs can encompass because of their nanometer scale. Further, adjuvants can enhance and modulate the immune response on either side of the polymer, whether it is a self-adjuvant or not [322]. Nanovaccines for BC were made using polymeric NPs containing different peptides [270]. Zupančič et al. (2018) tested the polymeric NP in the HER-2+ orthotopic BC model as a nanovaccine. These polymeric NPs combine the HER-2 peptides MHC class I and II with CpG and MPL (EntrapNP). As a result of the internalization of EntrapNP by DCs and cross-presentation of exogenous Ags *via* the MHC pathway, EntrapNP was well absorbed and internalized. After three doses of the nanovaccine and a specific immune reaction against neoplastic cells, tumor infiltration was increased with TILs (primarily cytotoxic memory-T cells). Tumor growth was significantly delayed, and metastatic lesions were lower in treated mice [323]. APCs, mainly DCs, eagerly endocytosed PLGA NPs containing the CpG-covered tag and then presented. Tumor Ag encapsulated in a membrane lysate from *4T1* cells. Finally, these CpG-NP-tag enhanced DCs' maturity and awakening, induced a positive response of the tumor-specific CTLs, and inhibited the progression of breast carcinogenesis and angiogenesis *in vivo* [324].

In another experiment, Campbell et al. (2015) employed PLGA-NPs to block Hp91, an immunostimulatory polypeptide from high mobility group box 1 (HMGB1). Laboratory tests have shown that this nanovaccine strongly activates DCs more than free peptides. By combining NPS PLGA HP91 with the free HER-2 peptide, researchers could activate the CTL response specifically against HER-2, barricade cancer proliferation, and extend the survival of the HER-2 peptide [325].

According to Hu et al. (2021), the *Physalis mottle virus* (PhMV) can be a potential nanovaccine against HER-2+ BC. This study explored two formulations: 1) a virus-like particle (VLP) presenting the CH401 Ag, particularly the mice-originated CH401 epitope, was generated and named PhMV-CH401. Copper-free click chemistry then bound the polypeptide epitope to exterior-exposed Lys side chains on the PhMV VLP; 2) a VLP presenting the CH401 Ag on its exterior and enriched with a TLR9 receptor agonist, particularly an artificial oligodeoxyribonucleotide (ODN) including unmethylated CpG motifs, was designed and named CpG-PhMV-CH401. CpG-ODN was added as an immunostimulant because it activates innate immunity *via* TLR9 signaling, human plasmacytoid DCs (pDCs), and B cells [326,327].

Using nanopharmaceuticals and epitope-based NPs, Liu et al. (2020) proposed that direct programming can be accomplished *in vivo* through simultaneous delivery. His research contributed to developing a heat-sensitive hydrogel. This compound consisted of polymer NPs containing curcumin (nanomedicine) and a nanopaque vaccine capable of covering primary tumors and treating cancerous cells that remain after surgery *via* stable delivery of nanotherapy. As a result of their ability to provoke the ICD of remaining cancer cells, curcumin NPs prompt the recruitment and maturity of DCs and promote tumor antigenicity. The E75 peptide and CpG-ODN were set together in a polymeric NP to enhance the antitumor T-cell response. Accordingly, the infiltration of CD8+ T cells in the recurred cancer escalated after inserting the hydrogel into the postoperative *4T1* mammary carcinoma model, and the systemic immune response turned synergistic. This approach attenuated tumor recurrence and pulmonary metastasis [328].

A new approach to developing biomimicry cytomembrane nanovaccines (CCMP@R837) is presented by Xiao et al. (2021). These researchers used NPs made up of antigenic cancer cell membrane (CCM) caps and imiquimod (1-isobutyl-1H-imidazo(4,5-c)quinolin-4-amine (R-837)) as an immunoadjuvant to stimulate immunity. Using the CCMP@R837 system, the same researchers rendered bone marrow-derived DCs mature. Nevertheless, they displayed a significantly

improved anticancer response against BC *4T1* cells *in vitro*. In addition, after three times of immunological sensitization with CCMP@R837 in *BALB/c* mice, an immune memory was established. When CCMP@R837-immunized *BALB/c* mice developed tumors, they showed suppressed growth and prolonged survival (75% remained alive for more than 50 days). This nanovaccine raised CD8^{+} T cells and lessened regulatory T cells in the tumor to achieve long-term antitumor immunity. Conversely, memory T lymphocytes in the spleen were augmented [329].

Moreover, Zhou et al. (2020) studied the antitumoral function of a neoAg-loaded nanovaccine in a *BALB/c* mouse bearing *4T1* mammary carcinoma to indicate the possible generality of the nanovaccines for tumor relapse. Notably, the *4T1* breast carcinoma has low antigenicity and a dense tumor burden, preventing the influx of CTLs into the tumor [330–332]. By the same preparation technique of OVA-loaded nanovaccines, *4T1* tumor neoAg SHRSCSHQTSAPSPKALAHNGTPRNAI (M32) was substituted for OVA. After vaccinating the *BALB/c* mice with the nanovaccines containing M32-loaded, the *4T1* tumor-bearing mice were treated twice, one week apart, with the M32-loaded nanovaccines. Even though the Man-PDPM@M32 nanovaccine significantly slowed *4T1* tumor proliferation and prolonged survival, the tumor was not annihilated. The tumor also recurred after the antitumor research, which could be elucidated chiefly by the immune suppressive TME in *4T1* tumors [333].

Glaffig et al. (2014) generated a new synthetically effective vaccine using biocompatible hyperbranched polyglycerol as a polymer carrier. The globular composition of the macromolecular carrier guarantees that the MUC1 glycopeptide B-cell and tetanus toxoid T-cell epitope peptides are presented on the exterior of these nano-sized combinations. The P2 Th-cell epitope- and the tumor-associated MUC1 glycopeptide Ag-based vaccination in mice resulted in substantial immunological responses and IgG isotype Abs. Due to the dendritic carrier form, the Ag is delivered to the immune system in ideal multiple displays. The entirely synthetic and water-soluble vaccination stimulated Ab production, which detects human BC cells. Their findings provided evidence for the presentation of Ags on hyperbranched polyglycerols as nanocarriers, enabling the use of existing methods for the production of entirely synthetic anticancer vaccines, which is a promising future development in the field of synthetic vaccine research [334].

### 4.1.3. Virus-like particles (VLPs)

VLPs form a robust and compliant platform that harnesses the antigenicity of viruses without harm, as VLPs cannot infect nor replicate due to the lack of the viral genome [335]. VLPs have incredibly repetitive structures identified as potent geometric pathogen-associated structural patterns (PASP). These patterns contribute to the efficacious cross-linking of B cell receptors and recruit innate humoral immune system elements such as natural Abs and complements, further promoting immune responses [336–338]. VLPs incorporated with innate stimuli enhance these Ags' immunogenicity [339–341]. Tumor-specific Ags are included through genetic fusion or chemical/peptide linkage, enabling immunization against peptides, peptide strings, or whole proteins [342]. A VLP ranges from 20–200 nm, a convenient measure for draining into lymph nodes [337,343,344]. Some VLPs congregate around RNA fragments (noninfectious or replication competent) during the expression process in host cells. VLPs can also be separated and recongregated with different TLR-ligands, namely, CpG-ODN (TLR-9 ligand), polyGlu, and single-stranded RNA (TLR 7/8 ligand) or double-stranded RNA (TLR-3 ligand) [345–349]. Overall, VLPs have been widely applied as vaccines due to these positive attributes.

VLP exposed loops allow peptides to be put into them to emerge from the surface and be more readily recognized by the immune system. Short peptides with comparatively basic structures can be modularized inside exposed loops, but those with more complicated compounds need further platform engineering [350]. The 20aaa-helix of the influenza HA2 subunit on the Flock House virus (FHV) platform shows that the structural features of complex peptides can be maintained by incorporating epitope scaffolds into VLPs' exposed loops [351]. Although this technique involves extensive structural understanding of the peptide of interest and an appropriate scaffold fragment, it is a viable option. Hepatitis B core antigens (HBcAgs) that are introduced into the immunodominant loops require to have their N and C termini placed closely together to preserve the VLP integrity [352], a restriction for the number of used Ags. The SplitCore technology was developed to facilitate the modularization of Ags with a more sophisticated structure [352]. The HBcAg can be divided into two pieces inside the immunodominant c/e1 loop, both of which can generate VLPs when co-expressed. Prior to co-expression, Ags that would have been structurally incompatible can be modularized by fusing them to the c/e1 termini of either segment [353].

The ability to mount several antigenic epitopes on the surface of VLPs by displaying whole protein domains makes these epitopes take on their native shape. Despite their vast dimensions, steric hindrances might lead to poor VLP assembly [354]. Consequently, various methods have been devised to modularize principal Ags. Glycine-rich connectors were designed to surround the Ag in the central c/e1 loop of the HBcAg platform, permitting spatial detachment and inserting proteins up to 238 amino acids long [355]. Ag-specific optimum lengths must be obtained empirically, despite their effectiveness. Steric hindrance may also be alleviated in some cases of VLP Ag level reduction [353]. These approaches can help VLP assembly; however, it is plausible that adding Ag mass meanwhile diminishing Ag number leads to lesser antigenicity [356]. This exchange is ineluctable when the Ag mass grows around the carrier, necessitating a grasp of the optimization domain. It is also possible for non-aqueous components of vaccines to intervene with the HA Ag's agarose gel diffusion [357], imposing a tremendous challenge for measuring VLPs in unpurified samples taken at different production and purification steps.

Patel et al. (2015) delivered HER-2 into BC-bearing mice with glycosylphosphatidylinositol (GPI)-anchored proteins *via* influenza virus-derived lipopolysaccharide. Th1 strongly influenced immune responses- and Th2-types following vaccination, and IgG specific to HER-2 was significantly increased. In contrast, GPI-HER-2 stimulated weak Th2-type responses after vaccination. Additionally, vaccinated mice demonstrated protection against HER-2-expressing tumors [358]. Palladini et al. (2018) also exhibited that VLPs produced robust and lasting anti-HER-2 CTL responses in a murine model. This kind of NP contributed to the prolonged survival of mice administered the VLPs and prevented spontaneous tumor growth and development [359].

In 2021, Hu and colleagues employed a plant VLP to develop a HER2-specific vaccine and studied the efficacy of nanoformulation *in vivo*. They used infusion encapsulation methods as well as the copper-free click chemistry to fabricate VLPs displaying the HER2-derived CH401 peptide epitope in the presence or absence of TLR9 agonists encapsulated in the interior cavity of VLPs. Afterward, the prepared nanovaccine was subcutaneously injected into *BALB/c* mice, and blood serum was collected for further assessment. Their findings revealed that adding the CpG adjuvant was not associated with changes in immune priming, while the two developed nanovaccines (PhMV-CH401 and CpG-PhMV-CH401) strongly provoked the mice's immune system. This was evidenced by either an increase in the titer of HER-2-specific immunoglobulins or increased anti-proliferative activity of anti-sera to *DDHER2* murine BC cells. Moreover, PhMV-based anti-HER2 vaccine PhMV-CH401 markedly decreased the proliferation of malignant cells, leading to increased survival of the vaccinated versus naive *BALB/c* mice. Altogether, their results supported the idea that VLPs derived from PhMV can be a promising platform for the efficacious development of BC vaccines (Fig. 6) [326].

In a similar study, Cai et al. (2019) evaluated the efficacy of a heterologous prime-boost strategy utilizing three diverse VLPs to provide HER-2-specific epitopes (CH401) to HER-2^{+} BC. The three VLPs were founded on Cowpea mosaic virus (CPMV), Cowpea chlorotic mottle virus (CCMV), and Sesbania mosaic virus (SeMV). To allow the immune
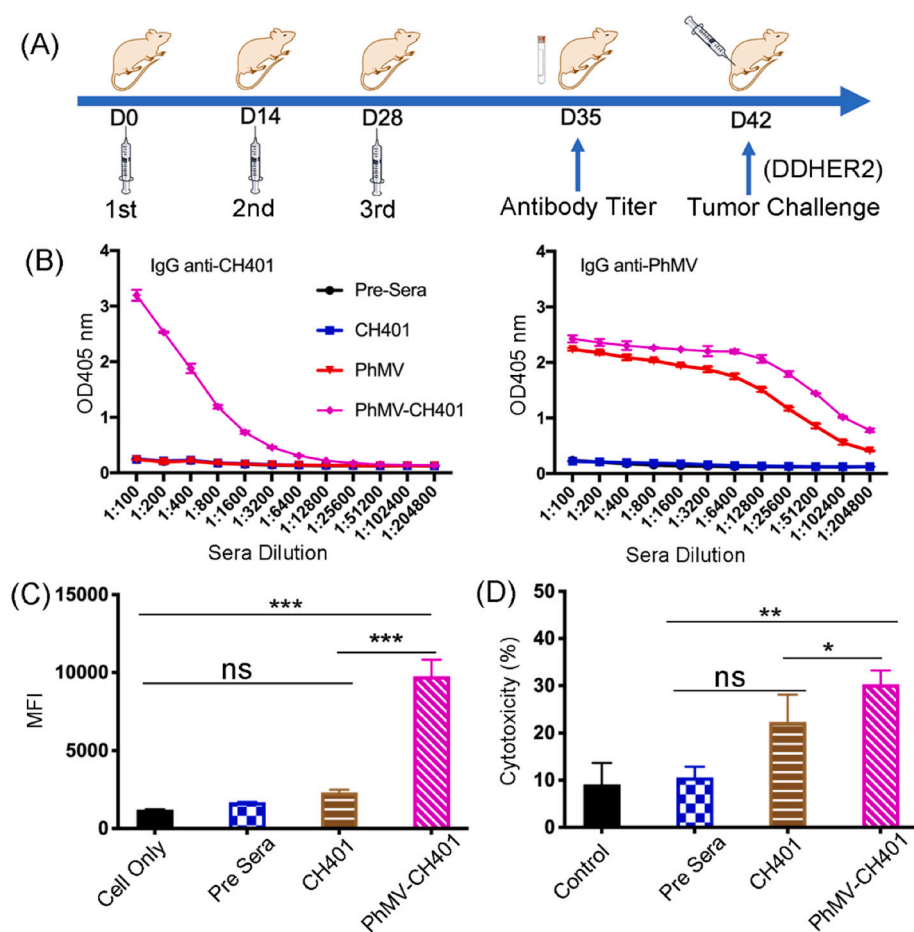
**Fig. 6.** Immunological assessments of *Physalis mottle virus* (PhMV)-derived nanovaccines. A. Schedule of immunization using *BALB/c* mice. B. Enzyme-linked immunosorbent assay (ELISA) to detect CH401 peptide and native PhMV in serum samples. C. Flow cytometry analysis for determination of sera binding to mice BC cells (columns represent the mean fluorescence intensity and standard variation of the three independent studies. D. Evaluation of sera toxicity against *DDHER2* cells using MTT colorimetric assay (* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$). Reprinted from [326] under the terms and conditions of the Creative Commons Attribution (CC BY) license (https://creativecommons.org/licenses/by/4.0/).

response to focus on one epitope simultaneously, the same scientists injected nanovaccine *in vivo* sequentially only once. CH401-specific immunoglobulin titers were higher with this vaccination regimen, and a Th1-dominated response was more potent and cytotoxic toward cancer cells than repeated vaccinations. It was demonstrated that the heterologous prime-booster lessened cancer proliferation and improved survival in treated mice more dramatically than convectional vaccination, demonstrating that novel vaccination approaches can enhance cancer-prevention success rates [360].

Tegerstedt et al. (2005) merged a 683-amino acid length domain derived from the HER-2 to the murine polyomavirus (MPyV) [361]. MPyV-VLPs were viral DNA-free and got into the cells like natural viruses [362]. Since the MPyV receptor is widely distributed on most cells in various species, including mice and humans, they might also be able to transfer molecules coupled to VLPs into cells [363]. The Her2(1-683) PyVLPs vaccine immunized mice against autochthonous BCs and HER-2-transfected mammary carcinomas. As HER-2-specific reactions were seen in ELISpot assays, a cellular immune response was likely the cause of the protection induced by the Her2(1-683)PyVLPs vaccine [361].

In recent research, Rolih et al. (2020) developed a VLP-based vaccine (AX09) to suppress *de novo* metastasis and extend the longevity of patients with metastatic BC. This bacteriophage MS2 VLP-based vaccine displayed the third extracellular domain of the xCT (ECD3) transporter on its exterior and was applied for treating metastatic BC-bearing mice. As a result of a significant oligoclonal Ab response, the xCT function was neutralized, impairing BC cells' proliferation and metastasis [364].

Nika et al. (2019) have demonstrated that budded VLPs derived from *Sf9* insect cells are an effective substrate for producing complex cell surface proteins. To evaluate the effectiveness of Ag-displaying VLPs as active cancer vaccines, they immunized *BALB/c* mice with insect cell

and mammalian-like glycosylated HER-2 VLPs plus two diverse immunostimulants and challenged them with HER-2$^+$ mammary carcinoma cells. Compared to mammalian-like glycosylated HER-2 VLPs, mice immunized with insect ones produced higher HER-2-specific Ab titers and effector functions. Besides, administering insect cell glycosylated HER-2 VLPs resulted in a protective response in mice implanted with HER-2$^+$ BC cells. Surprisingly, no protection was seen in mice injected with Poly(I:C). This study indicated that Ag-displayed VLPs generated in *Sf9* insect cells elicited powerful and long-lasting immune responses *in vivo* [365].

### 4.1.4. Lipid/calcium/phosphate (LCP) NPs

Calcium phosphate (CaP), a naturally biocompatible and biodegradable substance, is a reputed non-viral vector for *in vitro* gene transfection. The interaction between calcium ion and nucleic acid phosphate group allows for effective and comprehensive nucleic acid encapsulation [366,367]. More significantly, as an acid-sensitive material, CaP could promptly dissolve in the acidic endosomal or lysosomal environment and deliver its contents into the cytoplasm [368,369], rendering the matrix suitable for acid-stimulated pharmaceutical release. Despite these benefits, the out-of-control fast CaP precipitation contributes to poor colloidal stability, thus fluctuating drug release and resulting in common therapy outcomes [370]. Lipids and polymers have been employed to prevent the CaP precipitate from aggregating for manufacturing nano-sized and colloidal stable CaP NPs.

A decade ago, Huang's lab creatively developed lipid-coated calcium phosphate (LCP) NPs in which the synthesis of CaP was limited to a nano-sized region, enwrapped with lipids such as dioleoylphosphatydic acid (DOPA) to preclude aggregation [369,371]. Such lipid-coated CaP NPs are then sheathed with a second layer of lipids containing 1,2-

distearoryl-snglycero-3-phosphoethanolamine-N-[methoxy(polyethyleneglycol-2000) ammonium salt (DSPE-PEG) whose distal end is bound with a targeting ligand such as anisamide for fast and specific cellular internalization.

Combining the benefits of liposome and CaP, the LCP platform permits extended circulation, pathologic tissue/cell targeting, and endosome escape, providing a versatile transmitter for mono- and co-delivery of macromolecules, small-molecule medicines, and theranostic agents. Notably, these LCP NPs are safe since $Ca^{2+}$ pumps can rapidly reduce their cytosolic concentration on the plasma and mitochondrial membranes [372]. Due to its 1) small size, 2) well-PEGylated lipid exterior, and 3) slight negative surface charge, 25 nm LCP was capable of penetrating tissues, entering the lymphatic system, and accumulating in lymph nodes *via* lymphatic drainage. Moreover, utilizing a *4T1* BC model with lymph node metastasis, the capacity of intravenously injected 111In-LCP to visualise an enlarged, tumor-laden sentinel lymph node was demonstrated [373].

Liu et al. (2018) delivered mRNA encoding MUC1 to lymph nodes using an LCP modified with mannose. In an orthotopic TNBC model, the therapeutic efficiency was evaluated post-vaccination with the mRNA-loaded NPs. An mAB against CTLA-4 plus mRNA vaccine was shown to boost the antitumoral immune response by focusing on the regulatory pathways within T cells. When combined with the vaccine and anti-CTLA-4 mAB, immunotherapy could escalate the anti-cancer immune response more than the sole vaccine or mAB. In addition, findings point to a potential CTLA-4 inhibitor plus NP-based mRNA vaccine to treat TNBC [124].

### 4.1.5. Chitosan-derived nanostructures

The biocompatibility, low immunogenicity, and low toxicity of chitosan (CS), a natural cationic polysaccharide, have drawn much interest [374,375]. As an added benefit, the cationic characteristics of chitosan have been shown to intensify NP ingestion by DCs through static charge contact with the cells' surface and promote clathrin-mediated endocytosis, thereby increasing DC absorption [376].

Despite the many advantages of CS, it also has a few drawbacks. CS is a biopolymer with a broad spectrum of utility. However, its use in drug delivery is limited because of its water-insoluble nature. CS is soluble in acidic environments and moderately at neutral pH levels (6.8–7.4), limiting its use for drug delivery [377]. The solubility of CS is also influenced by its high molecular weight. Due to this issue, CS cannot be combined with other natural active substances that are difficult to absorb and dissipate in circulation [378]. As a result, functional groups like $C_6$–OH and $C_2$–$NH_2$ reactive with CS are chemically modified to produce their derivatives. In contrast to native CS, these derivatives have a decreased molecular weight, increased water solubility, and improved drug binding effectiveness [379]. CS is a potentially appealing option and its capacity for mucosal delivery of numerous Ags, from peptides to plasmids and mRNA, is a different approach for creating therapeutic vaccines, especially for BC [380–382].

Jadidi-Niaragh et al. (2016) observed positive effects of CD73-small interfering ribonucleic acid (siRNA) encapsulated into chitosan lactate (ChLa) NPs on inhibiting the expression of the *CD73 (NT5E)* gene on *4T1* mammary carcinoma cells *in vitro*. In order to produce ChLa NPs, tripolyphosphate (TPP) was used to ionic gelate ChLa. The siRNA-loaded NPs had a polydispersive index of less than 0.3 and a zeta potential of about 13. ChLa NPs with a Ch of 50 kDa demonstrate the best characteristics concerning the physicochemical properties, as they can encapsulate large amounts of siRNA. In addition to binding with siRNA, synthesized NPs also protect against serum and heparin decomposition and enhance transfection. According to flow cytometry, NPs transfected with Ch-plasmids expressing green fluorescent protein (pEGFP) showed low toxicity in 72-hour cell culture but transfected efficiently in *4T1* cells, with a 53.6% transfection rate. As measured by flow cytometry and quantitative reverse transcription-polymerase chain reaction (qRT-PCR), CD73-siRNA-loaded ChLa NPs could repress *CD73* gene expression efficiently. Consequently, CD73-siRNA-loaded ChLa NPs could be deemed a promising way to treat malignancy; nonetheless, additional *in vivo* studies are needed [383].

Liu et al. (2013) synthesized alginic acid-coated CS NPs (A.C.NPs) as an oral delivery transmitter for an asparaginyl endopeptidase DNA vaccine. A.C.NPs preserved DNA more efficiently from decomposition in acidic solution (pH 1.5) than sole C.NPs. Moreover, distribution analyses showed that A.C.NPs tended to aggregate and constitute micrometer compounds at pH<2.7 while spreading into NPs at higher pH levels. Orthotopic *4T1* BC-bearing mice received the vaccine orally. This research showed increased active CTLs (CD3$^+$/CD8$^+$/CD25$^+$), and tumor measures were remarkably smaller [382].

### 4.2. Immunostimulatory nano-adjuvants

Cancer vaccines containing immune adjuvants can arouse the immune system, enhance the immune responses induced by Ags and direct the specific elicited immune responses [384,385]. These adjuvant properties are essential for subunit Ags, which generally are weakly immunogenic [266].

#### 4.2.1. Porous silicon microparticles (pSiMPs)

pSiMPs are biocompatible and water-soluble microparticles that could be employed to deliver chemotherapeutic drugs and remedial small inhibitory RNA and microRNA molecules [386–391]. Because of their small size, there are only a few delivery options for pSiMPs. There have been pSiMPs used to convey payloads such as weakly soluble hydrophobic small molecule pharmaceuticals [392–394] and proteins (e. g., insulin, serum opsonin proteins, bovine serum albumin, glucagon-like peptide 1) [395–397]. Besides loading and releasing these therapeutic payloads, pSiMPs have also been investigated for their bioactivity and biocompatibility [398].

A therapeutic cancer vaccine can be enhanced using pSiMPs that induce type I interferon expression. The TRIF/MAVS pathways are involved in this process, independent of the TLR on the cell surface or the endosomes. Furthermore, pSiMPs have nanometer-sized pores capable of storing Ags and releasing them over time. Shen et al. (2016) developed a nano-DC vaccine comprised of bone marrow-derived DCs loaded with HER-2-loaded pSiMPs. The nanovaccine developed Ag-specified CD8$^+$ T cells in murine models of HER-2$^+$ BC, facilitating the transition from Th2 to Th1 in the TME to promote anti-cancer function [399].

Meraz et al. (2014) used porous silicon (pSi) NPs in conjunction with DOX-loaded NPs (DOX-NPs) intravenously in an immunocompetent BC murine model. MPL-adsorbed pSi microparticles (PSM) have been discovered to promote Th1 polarization in tumors and have anti-tumor activities independent of and additional to those caused by DOX-NPs. The study indicated that injecting MPL-PSM into mice with *4T1* tumors diminished proliferation and induced a Th1 bias in the TME. When MPL-PSM was injected into the tumors of mice that had previously been given DOX-NPs, the number of CTLs, F4/80+ macrophages, and DCs was elevated even more. A decrease in CD204$^+$ macrophage numbers, a marker of tumor aggressiveness, was seen following injection of DOX-NPs; this impact was amplified by adding MPL-pSi [400]. Ultimately, the polyvalent presentation of MPL by PSM allows a bias toward Th1 polarization, making it an attractive immunostimulant for combination immunotherapy and later uses in vaccine design.

#### 4.2.2. Selenium nanoparticles (SeNPs)

SeNPs prompt cellular and humoral components of the immune system and provoke proinflammatory cytokines. NPs can excite the release of IFN-γ from splenocytes, and colloidal particles encourage the Ag presentation to the reticuloendothelial system [401]. Selenium (Se) is an integral part of various selenoenzymes like glutathione peroxidases (GPxs), thioredoxin reductases (TXNRDs), and deiodinases (DIO), which are needed for multiplex biochemical reactions, including the

physiological antioxidant defense system [402]. It has unique anti-oxidant and pro-oxidant impacts relying on the dose, duration, and oxidation state [403]. The developed SeNPs markedly diminished the Se-associated acute toxicity death up to four times in a rodent model [404].

Additionally, high-Se-associated liver damage is noticeably diminished using SeNPs, as evident from the biomarkers of hepatotoxicity [405]. SeNPs showed good bioavailability and biological function than inorganic and organic Se compounds. However, low cellular absorption is the chief challenge of SeNPs. Efforts have been made to conquer this constraint by conjugating targeting ligands on NP's exterior [406,407]. Surface decoration of SeNPs with various carriers and ligands might be a profitable strategy [408] in order to enhance the selectivity and efficacy of the pharmaceuticals and, simultaneously, reduce toxicity [409].

In another experiment, IFN and IL-12, two Th1 cytokines, were upregulated in BC cells following the administration of SeNPs. The delayed-type hypersensitivity reactions of the treated mice were much higher than those of the untreated ones [410]. In the same way, SeNP-enriched *Lactobacillus plantarum* can generate an effective immune response by increasing IFN, TNF, and IL-2 levels and NK cell activity [408]. Another study found that supplementing with SeNPs raised the TNF-α and Th1 cytokine levels [411]. These investigations illuminated the efficiency of SeNPs as an adjuvant in BC vaccines. Applications of nanomaterials in the designing BC vaccines are summarized in Table 2.

According to Yazdi et al. (2015), SeNPs are immunomodulating when used in formulating a tumor-associated Ag-based vaccine. There was a considerable escalation in serum IFN-γ, IL-2, and IL-12 levels and a significant decrease in transforming growth factor β (TGF-β) in SeNPs vaccine-injected mice. Furthermore, there was a reduced tumor volume, more potent delayed-type hypersensitivity responses, and an overall higher survival rate than the control and tumor lysate vaccine groups. Based on these findings, SeNPs can seemingly be used as an immunos-timulant in a vaccine to elicit vigorous immune reactions against BC [412].

### 4.3. Nanomedicine for overcoming the immune escape in BC

TME components help cancerous cells evade the immune system. The external immune escape mechanisms comprise four principal aspects: lack of immune cells, the presence of immunoinhibitory cells (such as type 2 macrophages and Tregs), high concentrations of immunoinhibitory cytokines (such as IL-10 and TGF-β), and fibrosis [413]. Mammary carcinomas are lowly immunogenic, except for TNBC and HER-2$^+$ subtypes. Compared with other cancers, the burden of nonsynonymous DNA mutations in BC is proportionately poor; therefore, the MHC molecules display lower numbers of neoepitopes (mutant cancer Ags) than the effector immune cells. Accordingly, the antigenicity of BCs is poor, and the anti-tumor T-cell reactivity is modest [414]. Additionally, most BC subtypes manifest low TILs because of the immunosuppressive TME, which is considered an inferior prognosis [130,415–417].

NPs can defeat physical and biological barriers by providing immunomodulatory therapy. Therefore, nanomedicine can be used to increase the effectiveness of immunotherapy as an ideal approach to overcome the immune evasion mechanisms prompted by cancer cells [418]. Cancerous cells use mechanisms to escape the immune system. These mechanisms limit innate and adaptive immune responses and influence cancer progression [419,420]. For example, cancer cells disrupt the activity of DCs and deliver Ags. Many studies have shown that by using NPs, the tumor Ags can be better presented by DCs, and the maturity and triggering of DCs can be improved, which were previously hindered in the TME [124,298,360,421]. DCs presenting tumor Ags HLA-II interact with the T-cell receptor (TCR) to activate T cells.

Malignant cells typically overexpress the inhibitory programmed death ligand 1 (PD-L1) on their membrane; thereby, n inactivating T cells in the tumor site and suppressing the antitumoral immune

responses [422,423]. PD-L1 overexpression in neoplastic cells has been discovered in various types of BC, comprising small-cell breast carcinomas, basal tumors, and inflammatory BCs [424,425]. Encapsulation of PD-L1 [426] and PD-1 siRNA [427] in NPs has been examined in primary BC models. Wu et al. (2019) studied the use of two inorganic NPs—layered double hydroxide (LDH) and lipid-coated calcium phosphate (LCP)—for PD-1 and PD-L1 siRNA delivery, indicating that LCPs had better cellular absorption and gene delivery. Conversely to polymer NPs, lipid ones commonly utilize ionizable or cationic lipids, such as DOTAP, employed in these LCP NPs, which helps endosomal evade and release negatively charged material. Such attributions using cholesterol and PEG to enhance NP stability make lipid NPs a preferential delivery platform for nucleic acids [428].

Core-shell gold nanocage@manganese dioxide (AuNC@MnO2, AM) NPs have been synthesized in another experiment to augment oxygen levels in the TME, enhancing the PDT in a metastatic TNBC murine model. The acidic pH of the TME decomposes the nanoshell, and NPs massively release oxygen in the tumor site. In turn, oxygen-boosted PDT stimulates tumor cells' ICD, followed by the liberation of damage-associated molecular patterns (DAMPs) and their subsequent presentation by mature DCs to effector immune cells. Thus, the nanoplatform combined with PDT in this model induced a systemic antitumor immune response, destroyed primary tumors, and prevented cancer metastases [429].

A study by Navarro-Ocón et al. showed that NPs could enhance T-cell priming and block the interaction between CD86-CD80/CTLA-4 and PD-1 and PD-L1 on DCs, thus preventing T-cell inactivation by DCs [270]. Carcinoma-associated fibroblasts (CAFs) play a substantial part in BC among stromal factors. By secreting diverse soluble immunomodulatory factors, such as IL-1 and TGF-β, CAFs interfere in immune escape. In mammary cancers, roughly 80% of stromal fibroblasts have the CAF phenotype [430,431]. The suppression of tumor CAFs seems to be a more appealing approach. In this regard, a novel puerarin nanoemulsion (nanoPue) was synthesized to downregulate reactive oxygen species (ROS) production in activated CAFs. ROS are engaged in multiple pro-fibrogenic pathways and are essential for CAFs' activation. Therefore, nanoPue manifested a potent ability to inactivate CAFs in the TME. Thus, collagen deposition in the tumor site was diminished, and tumor penetrability was increased, which enhanced the chemotherapy efficacy in the desmoplastic TNBC model and prompted a two-fold increase in tumor infiltration with CTLs and lessened tumor measures. Moreover, remodeling of TME improved the efficacy of PD-L1 blockade therapy in this model. Therefore, nanoPue could be an adjuvant therapy for chemotherapeutic agents and immunotherapies in highly desmoplastic tumors, such as TNBC [432].

MDSCs are highly accumulated in cancer sites, where they can repress the activation and proliferation of CTLs and stimulate Treg cells. Nanomedicine-mediated depletion of MDSCs in the TME could be a novel approach in cancer immunotherapy. Compared with free DOX, treatment with the DOX-polyglycerol-nanodiamond conjugate (Nano-DOX) has reduced therapeutic robustness to a greater extent but outweighs some advantages over the free drug. This nanodiamond was applied to treat *4T1* breast tumor-bearing mice and indicated better tolerance and less noxiousness than standard DOX without chemo-resistance in the *4T1* cells, a chief problem of free chemotherapeutic agents. Besides, nano-DOX downregulated tumor-derived granulocyte-colony-stimulating factor (G-CSF) and suppressed tumor infiltration through MDSCs. Ultimately, it released DAMPs by *4T1* cells and the subsequent activation of M1 macrophages, DCs, and CD4$^+$ and CD8$^+$ T cells in the tumor. Together, these findings lighten the possibility that chemotherapeutic drugs in nano-forms acquire new, improved properties, and these nanomedicines, combined with immunotherapy, might provide a more effective treatment of cancer [433].

The presence of specific T cells infiltrating tumors is enhanced when NPs are applied. The infiltrated T cells distinguish cancerous cells by interacting with tumor HLA-I/peptides. However, tumor cells often

**Table 2**

Summary of the application of nanotechnology-based materials in the development of BC vaccines.

| Role | Formulation | Experiment Type | Results | Reference |
|---|---|---|---|---|
| Nanoadjuvants | BMDCs loaded with HER2-loaded pSiMPs | Murine models of HER-2$^+$ BC, *in vivo* | • Activated and increased Ag-specific CD8$^+$ T cells<br>• Advanced a Th2-to-Th1 transition in the TME to boost anti-tumor activity | Shen et al. [399] |
| | pSiMPs | *BALB/c* (6–8 weeks) mice, | • Injection of MPL-pSi microparticle to *4T1* tumor-bearing mice reduced tumor proliferation and provoked a Th1 bias in the TME. | Meraz et al. [400] |
| | Tumor-associated Ag-based vaccine with SeNPs | 4T1 Breast Murine Cancer, *in vivo* | • Remarkably raised the level of serum IFN-γ, IL-2, IL-12<br>• Lessened TGF-β<br>• Lowered tumor volume and caused more prolonged survival<br>• More potent DTH responses | Yazdi et al. [412] |
| Nanocarrier | CpG-NP-Tag | BC *BALB/c* mice model, *4T1* murine mammary carcinoma cell line, *in vitro* and *in vivo* | • Higher CD80/86 expression<br>• Enhanced IL-12 secretion levels<br>• Demonstrated attenuation of tumor growth and angiogenesis<br>• Potent cytotoxic T-lymphocyte responses | Kokate et al. [324] |
| | VLPs (PhMV) | DDHER2 murine model of HER-2$^+$ BC, BC female *BALB/c* mice model, *in vitro* and *in vivo* | • High titers of HER-2-specific Abs<br>• Increased toxicity of antisera to DDHER2 cancer cells | Hu et al. [326] |
| | Nanoliposomes containing DOTAP | TUBO tumor mice model, *in vivo* | • Strong antitumor responses<br>• Slow tumor growth | Talesh et al. [293] |
| | P5 HER-2/neu-derived polypeptide conjugated to Maleimide-PEG2000-DSPE | *BALB/c* mice and in TUBO tumor mice model, *in vivo* | • High CTL responses<br>• Smallest tumor measure and the most prolonged survival in a mice model of TUBO tumor | Shariat et al. [294] |
| | Liposomal formulations composed of DSPC: DSPG: Chol: DOPE containing both AE36 and E75 peptides | HER-2þ TUBO-tumoured mice, *in vivo* | • Excellent stimulation of CD4$^+$ and CD8$^+$ T cells responses<br>• Boosted IFN-γ | Zamani et al. [296] |
| | Nano-liposomal vaccine containing P5 peptide, a CTL-specific peptide derivative of rat HER-2/neu protein, PADRE peptide, a universal CD4$^+$ T helper cell epitope, and MPL, a toll-like receptor 4 ligand | Mice bearing HER-2$^+$ tumors, *in vivo* | • Enhanced anti-tumor impacts against cells overexpressing HER-2 in *BALB/c* mice | Zamani et al. [295] |
| | Lip-Pep and Lip-DOX | TUBO/BC-bearing *BALB/c* mice *in vivo* | • Tumor infiltration with TILs and NK cells<br>• Enhanced IFN-γ<br>• Reduced MDSCs and CD25+FOXP3+ Treg populations in the TME<br>• Decreased tumor growth and increased survival | Zamani et al. [299] |
| | Lip/DOPE/MPL/P5 | *BALB/c* mice bearing TUBO carcinoma | • IFN-γ and CTL responses were the highest<br>• Smallest tumor size and longest survival time. | Rastakhir et al. [297] |
| | Liposomes consisting of DSPC/DSPG/cholesterol (Chol)/DOPE (15/2/3/5 molar ratio) | *BALB/c* mice TUBO tumor model, *in vivo* | • Promoted the Ag-specific IFN-γ response of CD8$^+$ T cells<br>• Developed CTL antitumor responses | Arab et al. [298] |
| | Liposomes composed of DOTAP, DOPE, and DDC or DD | *BALB/c* mice model of HER-2-overexpressing BC | • Therapeutic (DD+pG) and prophylactic (DDC+CpG) influences<br>• Decreased the size of tumors | Barati et al. [301] |
| | Liposomal-based (DSPC/DSPG/Chol/DOPE) | Tumor-bearing *BALB/c* mice, *in vivo* | • Smallest tumor size and the longest survival | Farzad et al. [300] |
| | PLGA-NPs | HER-2/neu transgenic mice | • Robust activation of DCs<br>• Increased activation of HER-2-specific T cells<br>• Delayed tumor development<br>• Prolonged survival | Campbell et al. [325] |
| | The coordinated delivery of Ag and two adjuvants (Monophosphoryl lipid A, oligodeoxynucleotide CpG) by NPs | *B16.MO5* melanoma tumor-bearing mice | • Induced a 3-fold escalation in cytotoxic memory-T cells<br>• 5-fold production in IFN-γ cytokine<br>• Increased lymphocyte count over 50% in the TME<br>• The number of lymphocytes at the tumor site doubled | Zupančič et al. [323] |
| | Influenza VLPs+GPI-HER-2 | *D2F2* murine BC cell line | • Protein transfer of HER-2 did not modify the immunogenicity of viral proteins expressed on the VLPs | Patel et al. [358] |
| | VLPs | *MAMBO89* cell line, established from a mammary carcinoma of *huHER-2* transgenic mouse | • Dwindled spontaneous development of mammary carcinomas by 50%–100%<br>• prohibited the proliferation of HER-2$^+$ tumors implanted | Palladini et al. [359] |
| | VLPs (CCMV, CPMV, and SeMV) | | • Higher titers of HER-2-specific Abs | Cai et al. [360] |

*(continued on next page)*

**Table 2** (*continued*)

| Role | Formulation | Experiment Type | Results | Reference |
|---|---|---|---|---|
| | | *In vitro* cultures of BMDCs harvested from *BALB/c* mice | • Increasing the toxicity of the antisera toward tumor cells<br>• Induced a Th1-predominant response<br>• Lessened tumor growth<br>• Enhanced survival in mice | |
| | CD73-siRNA encapsulated into ChLa NPs | *4T1* breast tumor cells, *in vitro* | • Suppress the expression of *CD73*<br>• Protect siRNA against serum and heparin degradation | Jadidi-Niaragh et al. [383] |
| | mRNA Encoding MUC1 and HA Tag | TNBC *4T1* cell line arising from a spontaneous mammary carcinoma in a *BALB/c* mouse | • Potent, Ag-specific cytotoxic T lymphocyte response<br>• Combination immunotherapy of the vaccine and anti-CTLA-4 monoclonal Ab could considerably promote anti-tumor immune responses | Liu et al. [124] |
| | Spatially separated HER-2 peptide to activate B cells and ovalbumin peptide 323339 (OVA) | Human lung carcinoma cells *(A549)*, female *BALB/c* and *FVB/n* mice | • The effects were removed by the absence of pre-existing OVA immunity in the mice or OVA323−339 peptide in the liposomes.<br>• Generated Abs were subsequently demonstrated to stimulate cell death of an HER-2- overexpressing cell line *in vitro* | Wallis et al. [302] |
| | Liposomal formulations conjugated with P5 peptide and LAG3-Ig | Female *BALB/c* mice (4-6 weeks old; weight range of 18–20 g), | • LAG3-Ig-P5-immunoliposomes effectively prompted protective anti-tumor responses more than locally injected soluble LAG3-Ig+P5<br>• The higher percentage of $CD4^+$ and $CD8^+$ T cells in the spleen and more rapid and prolonged infiltration of these effector cells into the tumor site were seen following immunoliposome therapy | Mohammadadian H et al. [303]. |
| | Lip-DOPE-P5+435 | *BALB/c* mice (female, 4–6 weeks), *CT26* murine colon carcinoma cell line (rHER-2⁻) | • Mice vaccinated with Lip-DOPE-P5+435 formulation had the highest IFN-γ-producing CTLs with the highest cytotoxic activity, consequently leading to significantly smallest tumor size and prolonged survival rate in the TUBO mice model | Naghibi et al. [304] |
| | The MUC1 glycopeptide B-cell epitope and tetanus toxoid T-cell epitope peptide are presented on the surface of hyperbranched polyglycerol as a polymeric carrier. | *BALB/c* mice (6–10 weeks), | • The vaccine, comprising the P2 Th epitope and the tumor-associated MUC1 glyco-peptide Ag, led to substantial immunological responses and IgG isotype Abs | Glaffig et al. [334]. |
| | VLPs, containing a fusion protein between MPyV VP2 and the extracellular and transmembrane domain of HER-2, Her-2₁₋₆₈₃PyVLPs | Female *BALB/c* mice; *D2F2*, a murine mammary carcinoma cell line, and *D2F2/E2*, obtained by transfection of *D2F2* with a Her2 expressing plasmid | • The protection elicited by Her-2₁₋₆₈₃PyVLPs vaccination was most likely due to a cellular immune response; because a Her-2-specific response was shown in ELISpot assays, whereas Abs against Her-2 were not detected in any of the two models | Tegerstedt et al. [361] |
| | Bacteriophage MS2 VLP to display an extracellular loop of xCT (AX09) | *MDA-MB-231* cells, *4T1* cells, Female *BALB/c* mice | • AX09 in several *MBC* mouse models showed that it was well-tolerated and evoked a robust Ab response against xCT | Rolih et al. [364] |
| | VLPs produced in *Sf*9 insect cells | Female *BALB/c* mice (6-8 weeks), *SK-BR-3 (ATCC HTB-30)*, human mammary gland cancer cell line expressing human HER-2, *TUBO* cells (mouse mammary tumor cells), *Sf*9 insect cells *(ATCC CRL-1711)*. | • Higher HER-2-specific Ab titers and effector functions were induced in mice vaccinated with insect cell glycosylated HER-2 VLPs compared to mammalian-like glycosylated counterparts.<br>• Insect cell glycosylated HER-2 VLPs elicited a protective effect in mice grafted with HER-2⁺ mammary carcinoma cells | Nika et al. [365] |
| | A.C.NPs as an oral delivery carrier for a legumain DNA vaccine | Female *BALB/c* mice, | • Legumain DNA vaccine carried with A.C. NPs exhibits a similar, if not better, impact on suppressing tumor proliferation and long survival of tumor-burdened animals compared with both attenuated *S. typhi*-based vaccine and vaccine carried by C. NPs. | Liu et al. [382] |

Ab/Abs, antibody/antibodies; ChLa, chitosan-lactate; BMDC, bone-marrow-derived dendritic cell; DC, dendritic cell; HER-2, human epidermal growth factor receptor 2; CD8, cluster of differentiation 8; Th2, T helper 2; BC, breast cancer; TME, tumor microenvironment; pDNA, plasmid deoxyribonucleic acid; GM-CSF, granulocyte macrophage colony-stimulating factor; TGF-β, transforming growth factor β; CD4, cluster of differentiation 4; MHC, major histocompatibility complex; PLGA-NP, Poly (D,L-lactic-co-glycolic) acid nanoparticle; ICR, inverted cytokine receptor; PEG, polyethylene glycol; DTH, delayed type hypersensitivity; GPI, glycosylphosphatidylinositol; PhMV, Physalis mottle virus; CpG, cytosine-phosphate-guanine; DSPC, distearoylphosphocholine; DSPG, distearoyl phosphoglycerol; DOPE, dioleoylphosphatidylethanolamine; CTL, cytotoxic T lymphocyte; MDSC, myeloid-derived suppressor cell; PADRE, Pan HLA-DR; MPL, monophosphoryl lipid A; DOTAP, 1,2-dioleoyl-3-trimethylammonium propane; LM, *Listeria monocytogenes*; LLO, listeriolysin O; LM-LLO-Mage-b₃₁₁₋₆₆₀, amino acid fragments 311 to 660 of TAA Mage-b; NSG, NOD scid gamma; Ad, adenovirus; ROS, reactive oxygen species; IL-4, interleukin 4; TIM3, T cell immunoglobulin domain and mucin domain 3; PD-1, programmed cell death protein 1; VLP, virus-like particle; IFN-γ, interferon-gamma; NK, natural killer; CD25, cluster of differentiation 25; FOXP3, forkhead box P3; CD73, cluster of differentiation 73; siRNA, small interfering ribonucleic acid; AdmIL-12, adenoviral vector-mediated murine interleukin 12, ATCV, autologous tumor cell-based vaccine; AUTOC, autologous cancer cells; ALLOC, allogeneic breast cancer *MCF-7* cells; IL-2, interleukin 2; NDV, Newcastle disease virus; ATV-NDV,

autologous tumor cell vaccines-Newcastle disease virus-infected; pSiMPs, porous silicon microparticles; TNBC, triple-negative breast cancer; mRNA, messenger ribonucleic acid; Lip, liposome; A.C.NP, alginic acid-coated chitosan nanoparticle; C.NP, chitosan nanoparticle; *S. typhi, Salmonella typhi*; pSi, porous silicon.
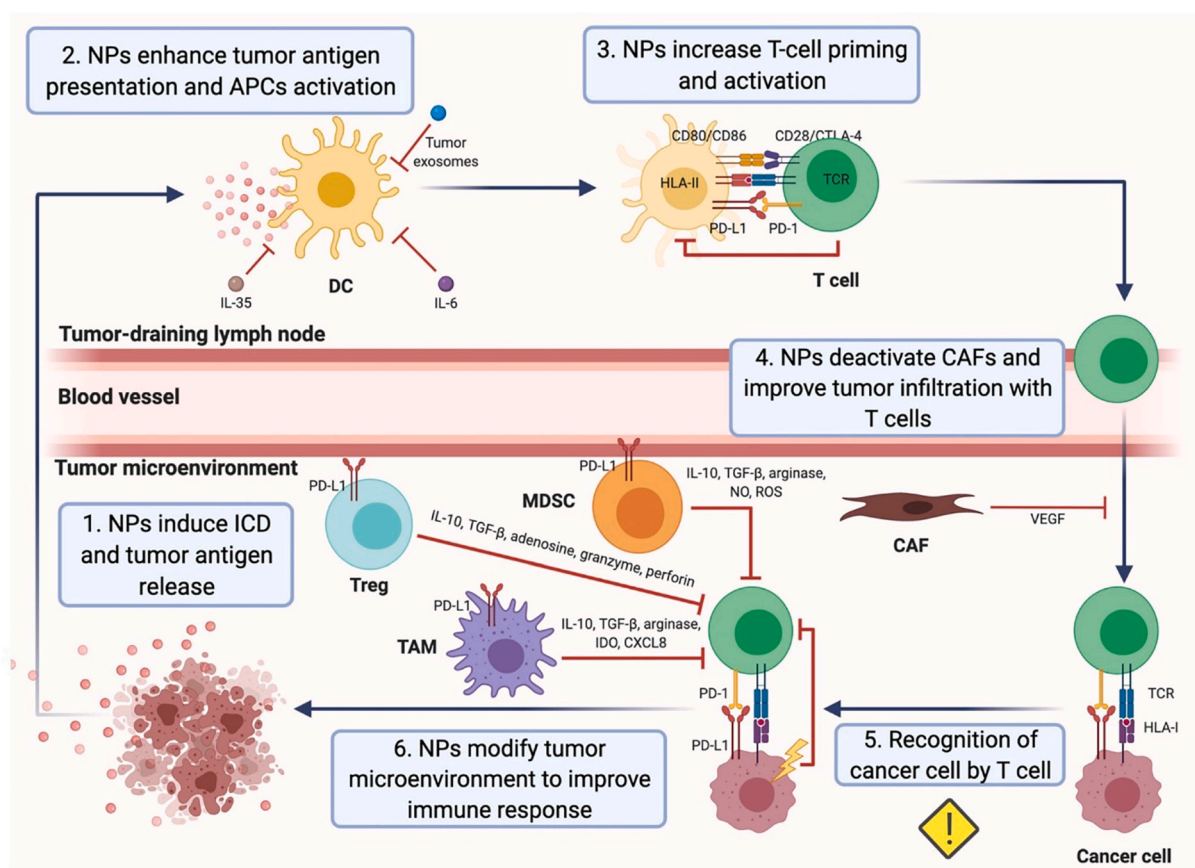


**Fig. 7.** Strategies for overcoming immune escape mechanisms based on nanoparticles (NPs). NP-based approaches can produce a robust antitumor T-cell response to inhibit breast tumor growth by enhancing several immune evasion mechanisms. It was initially demonstrated that NPs loaded with various drugs can trigger immunogenic cell death (ICD) in BC cells. Reprinted from [270] under the terms and conditions of the Creative Commons Attribution (CC BY) license (https://cre ativecommons.org/licenses/by/4.0/).

display modifications in the HLA-I expression, complicating recognizing them. However, NP-based strategies are not yet available to overcome this immune escape mechanism in BC. The TME also contains tumor cells and immunosuppressive cells that inhibit or promote the activation or inactivation of T-cells. Many NP-based therapeutic strategies aim to inactivate or reduce immunosuppressive immune cells such as MDSCs, Tregs, and tumor-associated macrophages (TAMs) in the TME to enhance antitumoral T-cell responses. Likewise, inhibiting indoleamine 2,3-dioxygenase (IDO), IL-10 production, and PD-1/PD-L1 interaction results in increased effector cell activity (Fig. 7) [270]. To enhance the remedial efficacy of NP-mediated cancer immunotherapy, employing several nanomedicine-based strategies to concurrently conquer different mechanisms of immune escape in cancer should also be considered. For example, the antitumoral T-cell reaction is modified by several immunosuppressive cells and cytokines within the TME and through the interaction between the immune checkpoint molecules expressed by cancerous and T cells. Thus, combining diverse nanotherapies to target different tumor immunosuppression factors simultaneously will have synergistic impacts and provoke more robust antitumoral T-cell responses. Accordingly, tumor growth inhibition and the remedial efficacy of multiplex treatments might be more significant [270].

Immunomedicine interacts with the immune cells and activates the immune system against disease-causing factors. Cancer immunotherapy is challenging in targeting malignant cells, lowering the adverse effects, and improving therapeutic outcomes. For instance, the anti-PDL-1

results in virtually 25% therapeutic efficacy but has several side effects. Therefore, the nanocarrier's unique features are ideal for delivering mAbs and pharmaceuticals to neoplasia. However, designing and developing stable nanocarrier systems to deliver immunomedicines has been enhanced. Induction of oxidative stress, free radicals' development, and damage in the protein, nucleic acids, lipids, other components, and major organs were significant difficulties in engineering the nanoplatform systems [434]. Thus, when designing nanocarrier systems, biocompatibility is vital. This attribute does not lead to any remarkable adverse effect on the human body while posing a high level of targeting efficiency and potential drug release at cancer sites. In the preclinical selection and assessment of efficacy in immune nanomedicine, employing an appropriate model is crucial due to intratumoral molecular heterogeneity [435].

## 5. Clinical Trials

NeuVax™, developed by Mittendorf et al. (2016), is a tumor vaccine containing HER-2-derived polypeptides and eradicates HER-2$^+$ BC cells. In the phase III clinical trial, this vaccine was blended with Leukine® (GM-CSF) and compared to GM-CSF (NCT01479244) for disease-free survival. Furthermore, in three clinical investigations, the NeuVax™ and trastuzumab and/or GM-CSF vaccine compounds were in phase II (NCT02297698, NCT02636582, and NCT01570036, respectively). In these experiments, a notable clinical advantage in the treatment was

observed with trastuzumab. In another research, AVX901 containing trastuzumab to target HER-2 was demonstrated to be safe in phase I (NCT01526473) and proceeded to the phase II clinical trial (NCT03632941) with pembrolizumab [436].

DC vaccination (DCV) against HER-2$^-$ BC is a highly challenging research field. Recent research (NCT02018458) inspected the safety and practicability of blending cyclin B1/Wilms tumor Ag (WT1)/CEF (Ag)-loaded DCV with preoperative chemotherapy in TNBCs [437], authenticating the safety of autologous DC vaccine during treatment in these patients [437]. Eighty-three patients with HER-2$^-$ BC with untreated stage II-III participated: 39 patients from the NCT01431196 clinical trial inoculated with combined neoadjuvant chemotherapy (NACT) with autologous DCV and 44 of them from a control group solely injected with the same NACT. The benefit of DCV was remarkable in the PD-L1 negative tumors possessing a basal immune-appropriate milieu. The PD-L1 expression levels hint at a more repressed niche where DCVs cannot arouse Ag provision and cytotoxic function. The PD-L1 positive population responds more highly to both NACT±DCV than the PD-L1-negative group, even though the profit seems higher in the NACT alone cohort [438]. Phase II investigations are now ongoing, and intriguingly, one of them is assessing a DC-based vaccine (DC1) vs. a plasmid-based DNA vaccine (WOKVAC) in individuals with residual disease following neoadjuvant chemotherapy for HER-2$^+$ BC (NCT03384914). Rudimentary outcomes are awaited in early 2023 [439].

After releasing positive consequences from the phase I clinical trial, researchers conducted a phase II trial (NCT00524277) to scrutinize the advantages of the AE37 + GM-CSF vaccine in precluding the relapse rate in node$^+$ and risky node$^-$ BC cases. Individuals merely injected with AE37 or GM-CSF did not exhibit a remarkable discrepancy in recurrence rate and 5-year DFS. However, findings indicate the privilege of the AE37 + GM-CSF vaccine in reducing the relapse rate in individuals with TNBC, which requires more clinical examination [440]. The synergistic impact of trastuzumab and vaccines was inspected in phase I/II clinical trials. Findings indicated extended and vigorous T-cell reactions with low noxiousness [441]. A further step in the HER-2 vaccination field is the development of HER-2 vaccine-primed autologous T cells for remedial infusion, which were reported to be practicable and well-tolerated in initial phase I trials [442]. Furthermore, phase II trials investigated the NeuVax™ (E75 + GM-CSF) vaccine plus trastuzumab in patients with high-risk HER-2$^+$ BC (NCT02297698). Moreover, a blend of the DC1 vaccine and two mAbs against HER-2, trastuzumab, and pertuzumab, is being assessed for HER-2$^+$ DCIS (NCT02336984). Ongoing clinical trials of BC vaccines are summarized in Table 3.

## 6. Challenges and future directions

Several decades have been devoted to developing therapeutic cancer vaccines. Clinical trials are underway for many vaccine candidates to treat BC, and several preclinical studies are ongoing. The biology of some vaccination candidates promises a potential remedy for BC in the advanced stages of clinical trials. A cancer vaccine called NeuVax™ contains peptides derived from HER-2 that target the expression of HER-2 on BC cells (NCT01479244) [174]. The phase III clinical trial for this vaccine derived from the E75 peptide has been completed. However, the FDA has not yet approved any vaccine for BC treatment. Although tumor vaccines have demonstrated some promising treatments, they have not delivered remarkable clinical advantages to immunotherapies like PD-L1 inhibitors.

Consequently, immune checkpoint inhibitors and antiangiogenic pharmaceuticals have been suggested as combination therapies [443]. There is a significant challenge in developing whole cell-based vaccines, particularly those derived from allogeneic tumor cells, as cell lines may not accurately provide the actual Ag repertoire of the tumor [170]. However, peptide-based vaccines also have some disadvantages in addition to their benefits. Peptide-based vaccines can only produce an

**Table 3**

Ongoing clinical trials of BC vaccines based on the information available at https://clinicaltrials.gov/.

| Clinical Trial Identifier | Recruitment Status, Number of participants | Interventions | Last Update Posted | Clinical Phase |
|---|---|---|---|---|
| NCT02018458 | Completed, N = 10 | LA TNBC: DC vaccine+Preop chemo ER$^+$/HER-2$^-$ BC:DC vaccine+Preop chemo | 2021 | Phase I Phase II |
| NCT02063724 | Active, not recruiting, N = 15 | HER-2-pulsed Dendritic Cell Vaccine | 2022 | Phase I |
| NCT00524277 | Completed, N = 456 | GP2 peptide + GM-CSF vaccine GM-CSF (sargramostim) AE37 + GM-CSF vaccine | 2020 | Phase II |
| NCT00807781 | Completed, N = 15 | Mammaglobin-A DNA vaccine | 2015 | Phase I |
| NCT01431196 | Completed, N = 29 | Autologous dendritic cell vaccination | 2016 | Phase II |
| NCT02348320 | Completed, N = 18 | Personalized polyepitope DNA vaccine | 2020 | Phase I |
| NCT03384914 | Recruiting, N = 110 | DC1 Vaccine WOKVAC Vaccine | 2022 | Phase II |
| NCT00304096 | Completed, N = 12 | synthetic BC peptides-tetanus toxoid-Montanide ISA-51 vaccine | 2013 | Phase I |
| NCT00892567 | Completed, N = 9 | 9 Peptides from HER-2/neu, CEA, & CTA | 2016 | Phase I |
| NCT02297698 | Completed, N = 100 | NeuVax™ vaccine Drug: Trastuzumab Drug: GM-CSF | 2022 | Phase II |
| NCT03014076 | Completed, N = 30 | GP2 peptide + GM-CSF vaccine plus trastuzumab Drug: Trastuzumab | 2017 | Phase I |
| NCT00266110 | Completed, N = 17 | Sargramostim therapeutic autologous dendritic cells trastuzumab Drug: vinorelbine ditartrate | 2018 | Phase II |
| NCT02336984 | Withdrawn (PI left Abramson Cancer Center and study never opened at Moffitt Cancer Center), N = 0 | HER-2-pulsed DC1 Drug: trastuzumab Drug: pertuzumab | 2021 | Phase I Phase II |
| NCT00978913 | Completed, N = 31 | DC vaccine | 2015 | Phase I |
| NCT02593227 | Completed, N = 80 | Low dose FR# vaccine Drug: Cyclophosphamide High dose FR# vaccine | 2021 | Phase I |
| NCT00573495 | Completed, N = 11 | hTERT/Survivin Multi-Peptide Vaccine | 2016 | Phase I |
| NCT01390064 | Completed, N = 6 | Vaccination with Mimotope P10s-PADRE/ MONTANIDE ISA 51 VG | 2019 | Phase I |
| NCT00088985 | Terminated (Funding unavailable), N = 56 | Biological: therapeutic autologous dendritic cells | 2017 | Phase II |

(*continued on next page*)

Table 3 (*continued*)

| Clinical Trial Identifier | Recruitment Status, Number of participants | Interventions | Last Update Posted | Clinical Phase |
|---|---|---|---|---|
| NCT00266110 | Completed, N = 17 | Biological: trastuzumab Drug: vinorelbine ditartrate Biological: sargramostim Biological: therapeutic autologous dendritic cells Biological: trastuzumab Drug: vinorelbine ditartrate | 2018 | Phase II |
| NCT00952692 | Completed, N = 12 | Biological: dHER2 + AS15 ASCI Drug: Lapatinib | 2021 | Phase I Phase II |
| NCT00317603 | Completed, N = 15 | Biological: Autologous, Lethally Irradiated BC Cells | 2022 | Phase I |
| NCT00880464 | Completed, N = 8 | Biological: Autologous, Lethally Irradiated BC Cells | 2022 | Phase I |

effective immune response with the proper adjuvant. A limited immunological reaction against tumoral cells results from the immune system's focus on a few epitopes. Other constraints are secondary structure, enzymatic stability, short half-time, and high eradication paces [35,444,445]. Polyvalent synthetic long peptides (SLPs) containing MHC class I and II antigenic determinants have been administered to boost the excitement of both CD8 and CD4 T cells [165,170].

Since most cancer cell mutations are specific to each patient, personalized medicine holds great promise in the clinic. While genetically altered cancer cells also raise cross-presentation and elicit an immune reaction against them, the engineering process is time-taking and pricey, making it an impractical option for patients with advanced cancer. Furthermore, DC maturation is inhibited by the absence of danger signals, such as TLR agonists. The immune priming and tumor suppression efficacy of several phase III trials of whole cell-based vaccines are low [446]. Using cell-based Ags, cytokines, and other immune stimulatory signals, new material science, and nanotechnology can escalate the efficacy of autologous cancer cell-based vaccines [267].

Drug development has been drawn to chemically defined subunit vaccines as they are easily manufactured and usually harmless. Although subunit vaccines frequently induce short-term immunity, their immunogenicity is weak. Several pharmaceutical engineering approaches have been performed to develop subunit vaccines with delivery carriers (e.g., micro/nanoparticles) to facilitate Ag provision by APCs [267]. In order to arouse immunologic responses in patients with malignancy, the activated Ag-specific DC vaccines can be inoculated. Ineffectively absorbing tumor Ags causes this method to be time-consuming and expensive, severely limiting its clinical effectiveness. Creating artificial APCs (aAPCs) is a possible solution to this challenge. The aAPC comprises an antigenic peptide embedded in MHC and co-stimulators binding to or triggering T cells [41,266,447].

DC-originated exosomes are enriched with receptors and structures needed for Ag provision and T cell provocation [448]. In addition, they can deliver exogenous vaccines to patients with cancer, which is beneficial. The development of nanomedicines based on exosomes presents several challenges, including the cost and time of manufacturing them, especially at clinically significant scales [266]. Even though neoAgs can be identified using technology, the process still presents difficulties and takes months to accomplish. Most somatic mutation products have indiscernible antigenicity, which might preclude wide use in clinics [266]. In part, nanovaccines may untangle these issues by elevating

vaccine delivery and thus enhancing the antigenicity of neoAgs. This technology can escalate the percentage of somatic mutations, qualifying for neoAg vaccines and hopefully expanding the population to benefit from neoAg vaccine-based immunotherapy. The disulfide conjugated peptide neoAgs were delivered to draining lymph nodes using synthetic high-density lipoprotein nanodiscs, a clinically harmless compound [449]. Plasmid DNA (pDNA) vaccines contain genetically engineered DNA sequences designed to trigger the expression of proteins from a specific pathogen when introduced into an organism. Using a simple vaccine formulation with naked pDNA appears attractive but results in low transfection efficiency. Clinically tested approaches include cationic lipid-based materials and surface-active polymers to avoid this limitation [450].

In most of the cases examined, the results and outcomes indicated that polymer-based vaccine platforms would be successful for further development. In contrast, polymer nanomedicines make up most of the market, but there are few polymer nanovaccines [307,451–454]. To advance the application of polymer-based nanovaccines, researchers should gather from different fields to develop novel vaccine platforms for infectious diseases. This collaboration could overcome polymer-based nanovaccines, which is the first step. Other important issues include: (a) the synthesis of novel polymers with low nanotoxicity and low antigenicity is a limitation that must be addressed further to develop polymer therapeutics [307,451–454]. (b) All nanomedicines under development must be thoroughly analyzed using specialized techniques regarding physicochemical properties and morphological properties. Preclinical studies for this formulation are more expensive than for other formulations [307,451–454]. (c) The pharmaceutical industry should develop or change the equipment required for developing and quality-controlling polymer-based nanovaccines. The design and devise of polymer-based nanovaccines require the skills of polymer scientists in pharmaceutical companies [307,451–454]. Finally, many gray areas in the regulatory landscape affect nanoformulations, presenting an additional issue for developing a vaccine dossier. Despite these limitations, the development of polymer-based nanovaccines has slowed down, indicating trouble and a chance for the strong teamwork of scientists to conquer constraints [307,451–457].

Nanovaccines that use mRNA for cancer have shown great promise in clinical trials. However, mRNA-based vaccines present many challenges. Firstly, Ag-encoding mRNAs must be devoured by APCs before being degraded by extracellular ribonucleases. Secondly, mRNA must evade the acidic endolysosomes to translate into the cytosol post-ingestion. In order to promote intracellular delivery of mRNAs into APCs, it is critical to devise delivery systems such as nanocarriers that preserve mRNA from decomposition. Recent years have witnessed an exponential boost in the encapsulation of mRNA vaccines in nanocarriers used in immunotherapy. For instance, liposomes efficiently increased the delivery of mRNA nanovaccines to the spleen and DCs [41,266,458,459]. Vaccines generally do not stimulate the desired immune response, and sometimes carriers do not deliver vaccines correctly to the recipient. As a result of nanoscience, NPs can be designed with different configurations, shapes, sizes, and surface properties in nanomedicine [41].

Novel vaccines are an alternate method of delivering Ags and activating different elements of a person's immune system while being biocompatible. Since nanovaccines are tiny, they can elicit multiple immune responses through different mechanisms. Additional ingredients may be added to nanovaccines to enhance their immunogenicity or stability *in vivo*. Nanovaccines pose challenges when it comes to sterility and toxicity. Despite nanovaccines being relatively new, their safety profile has not been thoroughly investigated. Therefore, research on the toxicity of nanovaccines is crucial [450]. As cancer drugs are expensive and the survival rate is considerably low, remedial vaccines hold great promise in the future of cancer treatment. Advances in each relevant field of science and technology have continued to benefit the field of nanovaccines. A number of the new nanovaccine approaches could profoundly impact cancer therapy.

## 7. Conclusion

In recent years, identifying molecular phenotypes of BC has prompted many prospects for vaccine development. The TNBC and HER-$2^+$ phenotypes are often used to target tumor cells in BC by the vaccine. For instance, a series of immunogenic polypeptides are produced as peptide-based vaccines from the HER-2 receptor molecule, including peptides from the intracellular, extracellular, and transmembrane domains. Despite many promising results, no BC vaccine has yet been approved by the FDA. In addition, it has not shown significant results and more clinical benefits compared to other immunotherapies. The success of tumor vaccines requires a comprehension of the TME, dealing with the mechanisms by which the tumor escapes from the immune system, and using immunogenic adjuvants in the vaccine. Advanced technologies such as nanotechnology can augment the efficacy of vaccines. NPs employed as carriers and immunizing adjuvants in vaccination are related to the induction of anticancer cells and increased detection of malignant cells. Furthermore, NPs can overcome many physical hindrances in the TME and preclude the immune system's evasion mechanisms due to their tiny size. The issue of developing safe nanovaccines with minimal side effects is an intriguing and practical one that could be usefully explored in future research.

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## Data availability statement

This article's data sharing is not applicable as no new data was created or analyzed in this study.

## CRediT authorship contribution statement

**Fatemeh Davodabadi:** Writing – original draft. **Mohammad Sarhadi:** Writing – original draft. **Javad Arabpour:** Writing – original draft. **Saman Sargazi:** Conceptualization, Writing – review & editing, Supervision. **Abbas Rahdar:** Writing – review & editing, Supervision. **Ana M. Díez-Pascual:** Supervision.

## Declaration of Competing Interest

The authors declare no conflict of interest.

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