# Public neoantigens in breast cancer immunotherapy (Review)

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Received January 17, 2024; Accepted May 15, 2024

DOI: 10.3892/ijmm.2024.5388

**Abstract.** Among women globally, breast cancer is the most prevalent cancer and the leading cause of cancer-related death. Interestingly, though genetic mutations contribute to the disease, <15% of women diagnosed with breast cancer have a family history of the disease, suggesting a prevalence of sporadic genetic mutations in breast cancer development. In the rapidly rising field of cancer genomics, neoantigen-based immunotherapy has come to the fore. The investigation of novel proteins arising from unique somatic mutations or neoantigens have opened a new pathway for both individualized and public cancer treatments. Because they are shared among individuals with similar genetic changes, public neoantigens provide an opportunity for 'off-the-shelf' anticancer therapies, potentially extending the benefits to a wider patient group. The present review aimed to highlight the role of shared or public neoantigens as therapeutic targets for patients with breast cancer, emphasizing common hotspot mutations of certain genes identified in breast cancer. The clinical utilization of public neoantigen-based therapies for breast cancer treatment were also discussed.

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*Key words:* breast cancer, hotspot mutation, immunotherapy, public neoantigen

## 1. Introduction

Breast cancer, the most frequently diagnosed cancer in women globally, accounted for up to 2 million new cases in recent years, making up 25% of all cancer cases in women (1). The complexity of breast cancer is highlighted by its high degree of heterogeneity, with current classifications based on three molecular markers: The estrogen receptor (ER), the progesterone receptor (PR) and the human epidermal growth factor receptor 2 (HER2) (2). Therapeutic approaches and outcomes differ significantly among the various subtypes of breast cancer. The ER-negative (ER-) group, which includes the HER2-positive ($HER2^+$) and triple-negative breast cancer (TNBC) subtypes, is less responsive to neoadjuvant chemotherapy compared with the ER-positive ($ER^+$) group (3,4).

In the past decades, numerous studies utilizing genome sequencing of breast cancers have revealed increasing numbers, up to thousands, of somatic mutations during the onset and progression of cancer (5). However, only a minor proportion of these mutations inherently provide a growth advantage to cancer cells, and are referred to as 'driver mutations' (6). Some of the driver mutations frequently observed in a particular DNA segment are known as 'hotspot mutations' and serve as targets for diagnosis and drug development (7). Finding a 'neoantigen', a tumor-specific antigen present exclusively in tumors, is needed to elicit an antitumor immune response. So far, most studies of neoantigen-targeted immunotherapies have focused on private neoantigens that are patient-specific. Such studies are time-consuming and costly. Neoantigens found in multiple patients with the same type of cancer, namely shared or public neoantigens, could be utilized in a broader spectrum of patients with cancer and aid corresponding cost savings. In the present review, an overview of neoantigens was provided and the public ones in clinical use were highlighted as immunotherapeutic targets in breast cancer treatment.

## 2. Breast cancer incidence, classification and management

Globally, breast cancer is the most common cancer in women and the leading cause of cancer death among women (1). In 2020, the estimated number of new breast cancer cases was 2,261,419, accounting for 12% of all new cancer cases, and the estimated number of deaths was 684,996 individuals, ~7% of all cancer deaths worldwide (1,8). There are several histological subtypes of invasive breast cancer, of which infiltrating ductal carcinoma is the most common, accounting for ~80% of invasive breast cancers, infiltrating lobular carcinoma accounts for 8%, ductal lobular carcinomas make up another 7%, and other less common histologic subtypes comprising medullary, metaplastic, mucinous and papillary carcinomas (9).

Breast cancer is an extremely heterogeneous disease caused by a diversity of genetic alterations that result in various biological and clinical behaviors within cancers themselves and in differences in disease progression and therapeutic response between patients (10,11). Based on the expression profiles of ER, PR, HER2 and Ki-67, four major molecular subtypes have been classified: Luminal A, luminal B, HER-2-enriched and basal-like. Each subtype has a different prognosis and potential therapeutic target (12,13). The luminal subtype is the most common subtype and is characterized by the expression of ER and other genes associated with resemblance to luminal epithelial cells. Cancer type luminal A is characterized by high $ER^+$, $PR^+$, $HER2^-$, and low Ki-67 expression and comprises ~40-50% of all breast cancers, while luminal B, which accounts for 20%, is classified by high $ER^+$, low PR, $HER2^{-/+}$ and high Ki-67 (14). Luminal A cancers present as low grade with a favorable prognosis, whereas luminal B cancers tend to be high-grade with poor prognosis (15,16). The HER2-enriched subtype makes up ~15% of all invasive breast cancer and is characterized by upregulation of HER2 expression and no expression of ER-related genes ($ER^-/PR^-/HER2^-$) (12,17). HER2-enriched tumors tend to manifest a high-grade and more aggressive form with decreased disease-free survival and overall survival (OS) rates compared with subtypes that do not overexpress HER2. However, this subtype responds well to monoclonal antibody-targeted HER2 therapy, which may improve survival outcomes (18). The fourth subtype, the basal-like subtype or TNBC, accounts for 15% of all breast cancer subtypes and has the least favorable prognosis; relapses may occur within five years after diagnosis (19). TNBC is associated with positive expression of cytokeratin 5, 14, and 17 genes in mammary basal cells and high expression of proliferation-related genes, but no expression of ER, PR and HER2 (20).

Management of patients with breast cancer is diverse and varies according to each patient's specific condition. The primary aim of treating early-stage breast cancer is to prevent its progression to invasive breast cancer. This involves a range of management strategies, such as surgery, radiation therapy, and, for suitable patients, adjuvant endocrine therapy to minimize the risk of cancer recurrence (21). Several studies of early-stage breast cancer have demonstrated that incorporating whole breast radiation after surgery reduces the recurrence of breast cancer (22,23). However, this approach does not significantly impact longer-term metastasis-free survival (24). Several drugs targeting the overexpressed or mutated genes have been used in the treatment of breast cancers (25). For example, the US Food and Drug Administration (FDA) has approved the use of tamoxifen to reduce the risk of $ER^+$ breast cancer recurrence (26), and buparlisib has been shown to be safe and efficacious in $HER2^-$, PIK3CA-mutated, advanced, or metastatic breast cancer (27). However, some targeting drugs did not significantly improve progression-free survival (PFS), and their dosing is limited by toxicity, which potentially limits their efficacy (28). Immunotherapy is a newer form of treatment that helps the immune system fight cancer. It is now frequently used in certain subtypes of advanced breast cancer (29). Atezolizumab, a human monoclonal antibody against programmed cell death-ligand 1 (PD-L1), has shown efficacy in enhancing PFS when used in combination with nab-paclitaxel for first-line chemotherapy in patients with metastatic TNBC or in cases of recurrent TNBC that are inoperable (30). The development and early successes in active immunotherapy techniques have led to therapeutic cancer vaccines. The first cancer vaccine, approved by the FDA in 2010, was for treating metastatic prostate cancer (31). However, the availability of specific immunotherapies or cancer vaccines for the treatment of breast cancer remains limited.

## 3. Tumor antigens

Immunotherapy to escalate the host's immune response has become a reputable pillar of cancer treatment, aiming to improve the therapeutic outcome of patients with certain types of hematological and solid tumors (32). Stimulation is achieved by using tumor antigens directly presentable by cancer cells to the host adaptive immune system (33). Strategies for targeting tumor antigens include cancer vaccines, which stimulate the immune system to produce an immune response against specific tumor antigens, and adoptive T-cell therapy, which involves modifying T-cells from a patient's blood to recognize specific tumor antigens and then infusing them back into the patient's body to attack the cancer cells (34,35).

Tumor antigens can be processed and presented with the major histocompatibility complex (MHC) on the cell membrane and recognized by receptors on the cell surface of T-cells to elicit an antitumor immune response (36). The properties of tumor antigens can differ depending on the specific antigen and the type of cancer. Common properties of tumor antigens include: Overexpression-tumor antigens are usually expressed at higher levels in cancer cells than in normal cells, which can make them more detectable by the immune system; mutation-some tumor antigens are mutated versions of normal proteins, which can render cancer cells more distinct from the normal cells and more vulnerable to immune attack; specificity-tumor antigens are specific to certain types of cancer, or even to certain subtypes of cancer which means that therapies targeting these tumor antigens can be less toxic than more broad-spectrum cancer treatments (37); and heterogeneity-when different cancer cells have unique properties and antigens it becomes more difficult to develop effective immunotherapies that target a broad range of tumor cells (38).

There are two main types of tumor antigens: Tumor-associated antigens (TAAs) and tumor-specific antigens (TSAs). TAAs are self proteins that are highly expressed in cancer cells but typically found at minimal levels in normal

cells (39). TAAs naturally arise through genetic amplification or post-translational modification, leading to the aberrant expression of proteins that are then no longer shielded from the MHC-processing machinery and consequently acquire immunogenic properties (40). TAA categories are based on the pattern of expression in normal cells, including overexpressed antigens, oncofetal antigens and cancer-testis antigens. The overexpressed antigens are proteins present in higher levels in cancer cells compared with normal tissues due to amplification of their DNA or mRNA and ultimately protein levels. Examples of overexpressed antigens include HER2/neu in breast cancer or ovarian cancer (41) and mesothelin in brain metastasis tumors (42). Oncofetal antigens are normal proteins that are expressed at high levels during fetal development and increase in adult cancer, but not in normal adult tissues. Examples of these antigens are MART-1/Melan-A in melanoma (43) and carcinoembryonic antigen in colorectal cancer (44). Cancer-testis antigens or tumor germline antigens are proteins that are normally highly expressed only in germ cells of ovaries, testis and placenta but are also expressed in cancer cells. Examples are New York esophageal squamous cell carcinoma 1 (45) and melanoma antigen gene protein (46).

Even though TAAs are great potential targets for cancer immunotherapy, there are several difficulties in their use. The heterogeneity of cancer cells can lead to variability in the mutation and expression of TAAs across different tumor types, stages and patients. This heterogeneity can make it difficult to develop a single immunotherapy effective for all patients with a particular type of cancer (47). TAAs are typically removed from the immune repertoire by central and peripheral tolerance mechanisms, so they are not recognized as foreign by the immune system. This results in an immune tolerance that prevents the immune system from attacking the cancer cells (48). Thus, a cancer vaccine that uses these antigens may not be potent enough. In addition, some normal tissues may share epitopes with or express low levels of TAAs, which can lead to autoimmune reactions and on-target off-tumor toxicities when targeted by immunotherapy. To mitigate this effect, and to further improve the efficacy and safety of TAA-targeted immunotherapies, several approaches have been taken, including selection of only the highly expressed targeting TAA, adjustment of the affinity and avidity of the chimeric receptor in chimeric antigen receptor T-cells to respond only to cells expressing a high level of TAA (49), and the use of immune checkpoint inhibitors (ICIs) in combination with the TAA vaccine (50). Further development of these strategies may strengthen the promise shown by TAAs as targets for cancer immunotherapy.

TSAs are detected as foreign non-self proteins that are presented exclusively on cancer cell surfaces and are particularly potent in eliciting an immune response (51). TSAs primarily originate from mutations in passenger genes, with only a small fraction originating from mutations in driver genes, and are heterogeneous between, and within, patients. The identifying common passenger gene mutations may generate immunogenic TSAs acting as the shared neoantigens among patients. Moreover, the variability of TSAs within individual patients may impact the efficacy of personalized treatments tailored to target specific antigens while minimizing adverse effects associated with non-specific targeting.

Since TSAs are solely expressed in cancer cells, they are ideal targets for cancer immunotherapy. Numerous TSAs are oncovirus antigens, viral proteins derived from oncogenic cancer viruses that have integrated into the genome in cancer cells and induce cell transformation and tumorigenesis (52). These include the E6 and E7 proteins from human papillomavirus that elicit strong antigen-specific T-cell responses in cervical cancer and head and neck cancer (53). Another important group of TSAs comprises neoantigens: A specific type of tumor antigen resulting from genetic and epigenetic aberrations that arise during cancer initiation and progression (54). Multiple types of mutations can give rise to neoantigens when they occur in protein-coding regions and appear as non-synonymous polymorphisms. Well-defined sources of neoantigens are somatic missense mutations that can generate single nucleotide variants (SNVs) and insertions or deletions (INDELs). These may create new open reading frames by frameshifts, as well as other genomic alterations, including gene fusions (55). Frameshift mutations may be more immunogenic than missense mutations due to the lack of similarity to normal protein sequences and an increased probability of generating neoantigens (56). Interestingly, neoantigens derived from gene fusions are now highlighted for immunotherapeutic treatments, especially in cancers that have low tumor mutational burden (TMB) and less immune infiltration (57).

## 4. Genetic mutations associated with breast cancer development

Although inherited genetic mutations are associated with a high risk for breast cancer development, <15% of women with breast cancer come from families with a history of breast cancer diagnosis (58). The majority of breast cancer development in women who do not have a family history of breast cancer may originate from somatic mutations. The important genetic mutations in breast cancer are as follows, and the mechanisms of action are summarized in Fig. 1.

*BRCA1 and BRCA2*. In total, ~20% of women with a family history of breast cancer have mutations in breast cancer susceptibility genes 1 or 2 (*BRCA1* or *BRCA2*) (59). BRCA1 and BRCA2 proteins are tumor suppressors that are crucial for homologous recombinant (HR)-mediated repair of DNA double-strand breaks (DSBs). They protect the integrity of the genome in proliferating cells to prevent tumorigenesis (60). BRCA1 involvement with DSBs requires recruitment of DNA repair protein RAD51 homolog 1 (RAD51) to the sites of DNA damage through interactions with partner and localizer of BRCA2 (PALB2), a stabilizer of the replication fork, and BRCA2 (61). The BRCA mutations impair HR-mediated repair of DSBs, raising the risk of developing inherited and sporadic breast cancer (62,63). Although BRCA mutations can be inherited and are found in a certain breast cancer family histories, Metcalfe *et al* (64) have reported no difference in the incidence of BRCA gene mutation carriers with or without a family history of breast cancer (64).

*PIK3CA*. *PIK3CA* gene encodes the p110α protein isoform, a catalytic subunit of phosphatidylinositol 3-kinase (PI3K) involved in cell proliferation, protein synthesis and DNA

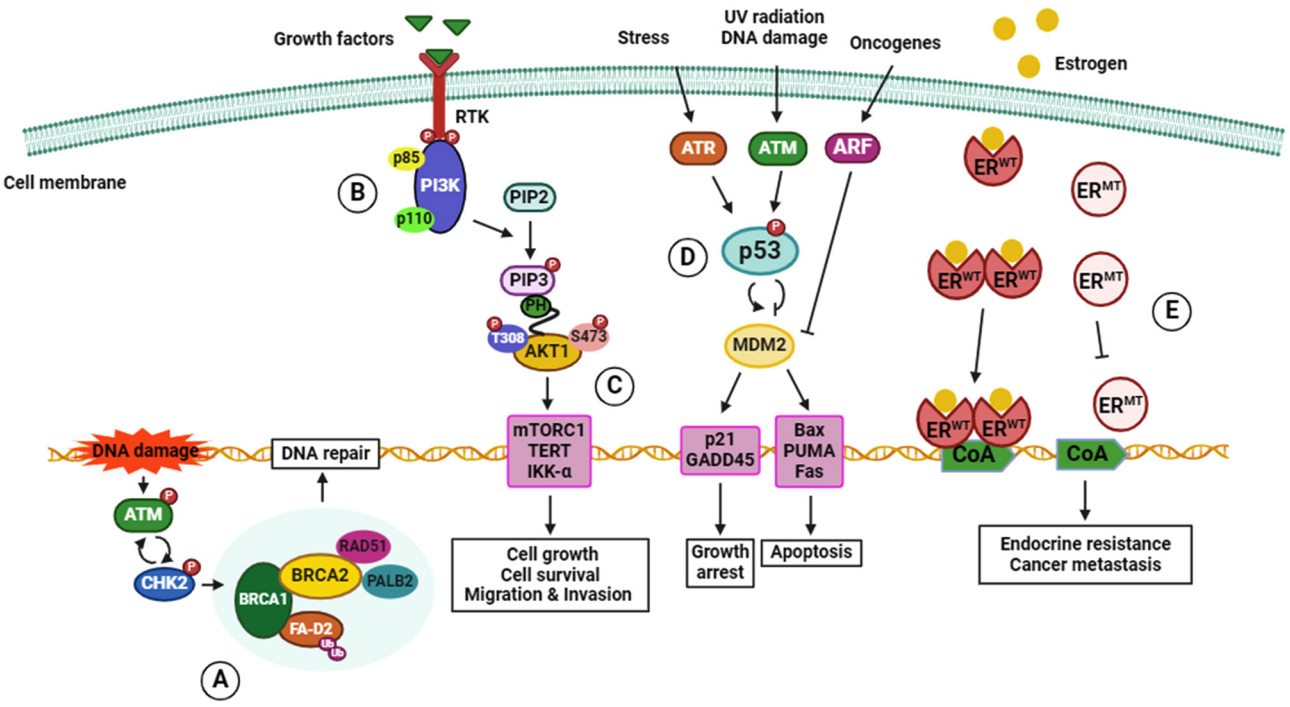


Figure 1. The mechanisms of action of the proteins associated with breast cancer development. (A) The primary recognition molecule for DNA damage is ATM, which phosphorylates CHK2 to activate several proteins, including BRCA1, in response to DNA damage. BRCA1 requires the recruitment of Fanconi anemia complex D2, RAD51, PALB2 and BRCA2 for DNA repair (61,151). (B) The stimulation of the RTK by growth factors initiates the interaction between the receptor and the p85 and p110 subunits, leading to the recruitment of PI3K to activate downstream signaling involved in cancer development (66). (C) For AKT1 activation, PI3K converts PIP2 into PIP3 and phosphorylates PIP3. Then, AKT1 binds to PIP3, exposing activation sites of AKT1 for phosphorylation of serine 473 and threonine 308 to activate downstream molecules, including mTORC1, TERT and IKK-α response for cell growth, cell survival, migration and invasion (66,152). (D) The initiation of p53 pathway signaling through increased ATR or ATM activity causes the phosphorylation of p53, which protects p53 from MDM2-mediated degradation and regulates transcription of numerous target genes involved in growth arrest and apoptosis (68). (E) The mutations in ESR1 primarily occur in the ligand-binding domain of ER, altering the ability of ER to interact with coregulatory proteins (CoA), and, consequently, impacting the effectiveness of hormone therapy. This can lead to the emergence of endocrine resistance and metastasis (74). (Created in BioRender.com). RTK, tyrosine kinase receptor; PI3K, phosphatidylinositol 3-kinase; PIP3, inositol-3,4,5-triphosphate.

repair. In the PI3K signaling pathway, the activation of tyrosine kinase receptors by growth factors leads to the direct interaction of the receptor with the p85 regulatory subunit, and p110 subunit, resulting in the recruitment of PI3K to the membrane (65). The phosphorylation of AKT, downstream mediators, and mTOR activation promote cell growth and inhibit apoptosis (66). Systematically reviewed and compiled evidence from multiple studies indicates that PIK3CA mutation status is associated with shorter PFS and OS in clinical trials involving $HR^+/HER2^-$ breast cancer (67).

*TP53*. *TP53* is a tumor suppressor gene that encodes the p53 protein. This protein is located in the nucleus and binds directly to DNA involved in the control of the cell cycle and cell differentiation, regulation of DNA repair, and cellular senescence in response to radiation and chemotherapy; binding prevents cancer formation (68). TP53 mutations arise mainly in the DNA binding domain, resulting in transcriptional inactivation of the p53 protein in stress responses (69). The TP53 gene mutation occurs in numerous types of cancer, including breast cancer (70). However, the magnitude of activation that arises from p53 mutation, and the specific transactivated targets of mutated p53, vary by cancer cell type (71). Wild-type p53 and its activity can indicate favorable prognoses in cancer (72); it has been indicated that patients with breast cancer with mutant tumor p53 have poorer survival than those with wild-type tumor p53 (70).

*ESR1*. *ESR1*, the ER 1 gene, is the gene that encodes ER, which belongs to the nuclear hormone receptor superfamily. ER functions as a ligand-activated transcription factor and is composed of multiple domains crucial for the binding of hormones and DNA (73). In metastatic breast cancer, mutations of ESR1 mostly occur within the ER ligand-binding domain (LBD) and lead to changes in ER function. This affects the response to hormone therapy and can result in the development of hormone-resistant breast cancer (74). Numerous studies have shown that ESR1 mutations are constitutively active and are resistant to the ER antagonists tamoxifen and fulvestrant (75,76).

*AKT1*. *AKT1*, v-akt murine thymoma viral oncogene homolog 1, belongs to the serine-threonine kinase family and is pivotal in various cellular processes (77). AKT1 is a downstream effector of PI3K, becoming activated by localizing to the membrane after inositol-3,4,5-triphosphate (PIP3) phosphorylation by PI3K. The pleckstrin homology domain of AKT1 binds to PIP3, exposing the activation sites of AKT1 for phosphorylation of serine 473 and threonine 308 (77). The process is often implicated in proliferation, survival and angiogenesis pathways recurrently activated in numerous cancers, including breast cancer (78).

*Other genes*. Other gene mutations are involved with breast cancer development; for example, the phosphatase and tensin homolog (*PTEN*) gene serves as a tumor suppressor

that regulates various biological functions such as cell cycle progression, cell proliferation and apoptosis regulation through negative regulation of PI3K and AKT pathways (79). The alterations or mutations of PTEN promote proliferation, migration and invasion, leading to breast cancer development and metastasis (80). Partner and localizer of BRCA2 (PALB2) plays a role together with BRCA2 in DSB repair (81). Mutations that result in a loss of function in PALB2 are associated with the occurrence of hereditary breast cancer (82). *ATM*, ataxia-telangiectasia mutated, is a tumor suppressor gene that plays a role in repairing damaged DNA by triggering repair mechanisms in response to the DNA damage. Data collected by Gao *et al* (83) indicated that *ATM* missense mutations increase the risk of breast cancer; notably, the V2424G (c. 7271 T>G) missense variant exhibits the highest association with breast cancer in all subtypes (83). In addition, the *CHEK2* gene encodes checkpoint kinase 2 (CHK2) protein, a serine/threonine kinase involved in DSBs repair mechanisms. Failure of kinase functions associated with missense mutation or deletion is found in various types of cancer and frequently in breast cancer (84). The mediator of DNA damage checkpoint 1 gene (MDC1) encodes the MDC1 scaffold protein which plays a crucial role in regulating precise DNA repair in DNA damage response (85). Loss of the MDC1 protein may serve as an indicator of recurrent metastases in patients with breast cancer (86), and mutations in the MDC1 gene have been associated with poor prognoses among patients with breast cancer (87). Hence, mutations of MDC1 may also give rise to plausible neoantigens. Numerous additional mutated genes associated with an increased risk of breast cancer development have been identified and reported (88,89).

## 5. Mutational landscape in breast cancer

The frequency of somatic mutations varies substantially within and between cancer classes, ranging from ~0.001 mutations per megabase (Mut/Mb) to over 400 Mut/Mb (90). TMB indicates the total number of non-synonymous mutations (NSMs) per coding area of a cancer genome and varies widely between individual patients and cancer types; breast cancer exhibits a noticeably lower TMB than other cancers, such as melanoma and lung cancer (54,91). No significant difference in OS or PFS between the high TMB (>5 Mut/Mb) and low TMB (≤5 Mut/Mb) groups have been reported, but high TMB may be associated with more prolonged survival of patients undergoing ICI-based treatment (92). Among breast cancer subtypes, the highest median number of NSMs was found in patients with TNBC (63, range 2-765 NSMs), followed by $HER2^+$ (39, range 1-1206), and the lowest median in $ER^+/PR^+/HER2^-$ patients (32, range 1-2,860) (93). The association between high TMB and NSMs in TNBC indicates that TNBC is the most suitable subtype of breast cancer for high neoantigen loading.

The mutations that provide a clonal selective advantage to cancer cells, are strongly associated with oncogenesis, and play a causative role in the development and progression of cancer are known as 'driver genes' (94). Some driver mutations are inherited in the germline, but the majority of them arise in somatic cells during the lifetime of a patient with cancer, along with numerous 'passenger mutations' that are not linked to cancer development (94). Several driver gene mutations in breast cancer have been reported (95,96). The most frequent or hotspot mutations of the driver genes in breast cancer are summarized in Table I.

*BRCA* mutations include germline and somatic mutations. Germline mutations in *BRCA1* and *BRCA2* genes drive carcinogenesis in ~20% of patients with early-onset breast cancer and only 1-4% of post-menopausal patients (97,98). Somatic mutations in *BRCA* account for 15-30% of all *BRCA1* and *BRCA2* mutations and are found in 3% of all breast cancer cases (99). *BRCA1* mutation is found in 61% of TNBC, while 70% of luminal B subtypes show *BRCA2* mutations (100,101). However, the HER2-enriched subtype exhibits a low percentage of *BRCA1* and *BRCA2* mutations (101,102). The most common mutation type of *BRCA1* is frameshift mutation, whereas missense mutations are the most common in *BRCA2* (103,104). The highest mutation found for *BRCA1* was c.68_69delAG (c.185delAG) followed by c.5266dupC (c.5382insC), whereas the most frequent mutation for *BRCA2* was c.6174delT (c.5946delT).

*PIK3CA* somatic mutations are higher in luminal tumors, ~40% of luminal breast cancers, but they do not independently predict the outcome of endocrine therapy (105,106). Most *PIK3CA* mutations are situated in the helical domains (exon 9) and kinase domain (exon 20), which are located in three hotspot NSMs: $PIK3CA^{E542K}$ (c.70273G>A) and $PIK3CA^{E545K}$ (c.70282G>A) in exon 9 and $PIK3CA^{H1047R}$ (c.86276A>G) in exon 20 (107). Measurements of the frequencies of *PIK3CA* mutations in patients with primary breast cancer revealed that $PIK3CA^{E545K}$ was found in 8.1%, representing 63.6% of the exon 9 mutations, whereas $PIK3CA^{H1047R}$ was detected in 14%, corresponding to 91.7% of the exon 20 mutations (108). The findings were consistent with those of Zhou *et al* (96). Other hotspot mutations of *PIK3CA* in breast cancer have been reported from several studies, such as $PIK3CA^{N345K}$ and $PIK3CA^{C420R}$ in exon4, $PIK3CA^{E726K}$ in exon 13, and $PIK3CA^{H1047L}$ in exon 20 (96,105,108-110).

*TP53* has been observed as the most frequently mutated gene, and accounts for 30-45% of the Cancer Genome Atlas (TCGA) and Guangdong Provincial People's Hospital (GDPH) cohorts (95). Among breast cancer subtypes, HER2-enriched and TNBC tumors contained more *TP53* mutations (60-80%) than luminal A and B subtypes (10-30%) (54,95,96). In total, ~50% of *TP53* mutations were missense mutations, and 50 and 60% of *TP53* mutations were missense mutations (95,111). The most frequent hotspot *TP53* somatic mutation identified in TCGA cohorts by several research groups was $TP53^{R175H}$, while $TP53^{R248Q/W}$ and $TP53^{R273C/H}$ were the most frequent mutation sites in the GDPH cohort (95,111-113). The mutations included classic hot spots, such as $TP53^{G245D}$, $TP53^{R273C}$,and $TP53^{Y220S}$, which have been detected in breast cancer (112,114,115).

Certain patients with $ER^+$ breast cancer have metastatic disease progression due to hormone therapy resistance caused by missense mutations in the LBD of the *ESR1* gene (116,117). In 2013, at least two groups reported that the location is a likely hotspot for activating the LBD ESR1 mutations that mediate clinical resistance to hormone therapy, such as mutations $ESR1^{L536S}$, $ESR1^{Y537S}$ and $ESR1^{D538G}$ (96,118,119). Moreover, a study of circulating tumor DNA in patients with $ER^+$ breast

Table I. The most frequent hotspot mutations of driver genes in breast cancer.

| Gene | Mutation type | cDNA change | Protein change | Frequency (%) | (Refs.) |
|---|---|---|---|---|---|
| *BRCA1* | Frameshift | c.68_69del | p.E23fs | 13.3 | (106) |
| | Frameshift | c.5266dupC | p.Q1756fs | 7.1-0.4 | (101,106) |
| | Non-sense | c.1687C>T | p.Q563X | 1 | (101) |
| *BRCA2* | Frameshift | c.5946delT | p.C315S | 7.3 | (106) |
| | Frameshift | c.4258delG | p.D1420fs | 1.5 | (101) |
| | Frameshift | c.6065c>G | p.S2022X | 0.7 | (101) |
| | Missense | c.7697T>G | p.I249T | 40.0 | (99) |
| | Missense | c.5972C>T | p.T1915M | 20.0 | (99) |
| | Missense | c.8524C>T | p.R2842C | 0.7 | (106) |
| *PIK3CA* | Missense | c.3140A>G | p.H1047R | 45.6-14.0 | (98,107,111,112) |
| | Missense | c.1633G>A | p.E545K | 17.5-1.6 | (98,107,111,112) |
| | Missense | c.1624G>A | p.E542K | 10.7-5.3 | (107,111,112) |
| | Missense | c.1035T>G | p.N345K | 5.5-2.2 | (98,107,111,112) |
| | Missense | c.3140A>T | p.H1047L | 4.4-3.1 | (107,111,112) |
| | Missense | c.2176G>A | p.E726K | 2.9-1.1 | (98,111,112) |
| | Missense | c.1258T>C | p.C420R | 1.9-0.8 | (98,107,112) |
| *TP53* | Missense | c.524G>A | p.R175H | 8.2-1.5 | (97,113,114) |
| | Missense | c.818G>A | p.R273H | 3.6-1.0 | (97,113,114) |
| | Missense | c.659A>G | p.Y220C | 2.6-0.5 | (97,113,114) |
| | Missense | c.742C>T | p.R248W | 2.1-0.6 | (97,113,114) |
| | Missense | c.743G>A | p.R248Q | 1.9-1.5 | (97,113,114) |
| | Missense | c.817C>T | p.R273C | 1.5-0.3 | (97,113,114) |
| *ESR1* | Missense | c.1138G>C | p.E380Q | 58 | (120) |
| | Missense | c.1610A>C | p.Y537S | 8 | (121) |
| | Missense | c.1613A>G | p.D538G | 1.0-7.9 | (98,120,121) |
| *AKT1* | Missense | c.49G>A | p.E17K | 2.5-8.2 | (122,124,125) |

Del, deletion; dup, duplication; fs, frameshift; X, the termination codon which signals the end of translation.

cancer revealed that 58% of $ESR1^{E380Q}$ could be detected in the plasma DNA of metastatic patients (118).

*AKT1* mutations have also been described in breast cancer, especially in the $ER^+$ subtype. The most frequent hotspot mutation was $AKT1^{E17K}$, found in ~3-8% of breast cancer (120-122). Mutations in *MDC1* were found in ~0.6-2.6% of breast cancers (123); however, the information available on its hotspot mutations is limited.

## 6. Neoantigen landscape in breast cancer

Neoantigens are potential targets for vaccine development and adoptive therapy (124,125). Most neoantigens arise from the unique somatic mutations that are typically found in cancers in which there is more than one somatic mutation per Mb. TMB is predictive of neoantigens which result in cancer immunogenicity. Barroso-Sousa *et al* (126) found that patients with breast cancer who have a high mutational load contain a higher neoantigen burden that correlates with increased cytolytic activity and granzyme A (*GZMA*) and perforin 1 (*PRF1*) gene expression. These data support the relationship in breast cancer between TMB and higher neoantigen burden favoring antitumor immune responses.

Narang *et al* (93) reported that the number of potential neoantigens was highest in TNBC (43% of NSMs). TNBC with a high neoantigen load exhibited increased sensitivity to chemotherapeutic drugs, as well as a favorable response to immunotherapy, mainly through the upregulation of program death-1 (PD-1) signaling signature genes during treatment with ICIs (127). Neoantigen prediction tools exist to identify HLA-restricted neoantigens that can bind to HLA class I and class II (128). Ren *et al* (129) predicted distribution across HLA genotypes of neoantigens found in individual patients with breast cancer. The neoantigens restricted to HLA class I were 0 to 1,953 from SNVs, 0 to 17,743 from INDELs, and 0 to 7,255 from gene fusions. For HLA class II-restricted neoantigens, the range was 0 to 3,728 from SNVs, 0 to 45,883 from INDELs, and 0 to 20,383 from gene fusions (129). Interestingly, >1% of neoantigens predicted from their cohort were driver genes. These included *MAP3K1* (746 neoantigens; 167 HLA class I and 579 HLA class II), *TP53* (252 neoantigens; 46 HLA class I, and 206 HLA class II), *GATA3* (58 neoantigens; 8 HLA class I and 50 HLA class II) and *PIK3CA* (37 neoantigens; 6 HLA class I and 31 HLA class II) (129). Notably, the neoantigens were categorized into two types based on the level of

Table II. Clinical trials of personalized immunotherapy with private neoantigens in breast cancer.

| Trial ID (Reference) | BCA subtype | Approaches | Phase | Starting year/Status |
|---|---|---|---|---|
| NCT02348320 (144) | TNBC | Personalized polyepitope DNA vaccine | I | 2015/Completed |
| NCT02427581 (144) | TNBC | Personalized synthetic long peptide vaccine | I | 2015/Recruitment |
| NCT03199040 | TNBC | Neoantigen DNA vaccine alone vs. neoantigen DNA vaccine plus Durvalumab | I | 2019/Active, not recruiting |
| NCT03300843 | All subtypes | Long peptides, tandem minigenes-pulsed DC vaccine | II | 2017/ Terminated |
| NCT03606967 | TNBC | Personalized synthetic long peptide vaccine | II | 2021/Recruiting |
| NCT03970382 | $HR^+$ | Neoantigen targeted TCRs | Ia/Ib | 2019/Suspended |
| NCT04105582 | TNBC | Personalized synthetic long peptide-pulsed dendritic cell | I | 2019/Completed |
| NCT04102436 | All subtypes | Genes encoding TCRs that recognize mutated neoantigens | II | 2019/ Not yet recruiting |
| NCT04879888 | TNBC | Personalized peptide-pulsed autologous dendritic cells | I | 2016/Completed |
| NCT05098210 | $HR^+$, $HER2^-$ | Personalized multi-neoantigen peptide vaccine | I | 2022/Recruiting |
| NCT05269381 | All subtypes | Personalized neoantigen peptide-based vaccine in combination with Pembrolizumab | I | 2022/Recruiting |

From https://clinicaltrials.gov. Completed: the study has ended normally, and participants are no longer being examined or treated. Recruitment: the study is currently recruiting participants. Active, not recruiting: the study is ongoing, and participants are receiving an intervention or being examined, but potential participants are not currently being recruited or enrolled. Terminated: the study was stopped early and will not start again; participants are no longer being examined or treated. Not yet recruiting: the study has yet to start recruiting participants. TNBC, triple-negative breast cancer; HER2, human epidermal growth factor receptor 2.

their individual uniqueness and accessibility: The private and the public neoantigens.

*Private neoantigens in breast cancer.* Private neoantigens are mutated antigens that are unique and diverse among patients (130). Most private neoantigens originate from either passenger mutations or non-recurring driver mutations, which result in tumor heterogeneity with expression of neoantigens unique to individual patients (131). Therefore, therapeutic approaches that target private neoantigens are personalized to suit the unique characteristics of each patient and are referred to as personalized therapy. The authors' group has recently reported personalized neoantigens identified in two patients with breast cancer using whole-genome sequencing and whole-transcriptomic analysis (132). The predicted HLA-binding affinity of these neoantigens was confirmed to ensure the capacity of these neoantigens to activate T-cells with high cytotoxicity against breast cancer cells. However, such approaches are controversial because clinical outcomes in patients with breast cancer vary considerably owing to the variability in the immune status of patients, the cancer surroundings, and the genetic makeup of the cancers themselves (133). Several clinical studies targeting private neoantigens in breast cancer have been reported (Table II) and provide valuable insights into the development of personalized neoantigen-based immunotherapy.

A clinical study of a private neoantigen-based vaccine against TNBC has been reported. Zhang *et al* (134) identified neoantigens in patients with TNBC with distinct HLA phenotypes. *PALB2* (SPVTEIRTDL) and *ROBO3* (RVAGSMSSL) were predicted with HLA-B*07:02, and *PTPRS* (RQLEVPWPYI) and *ZDHHC16* (ALGALTVWL) were predicted with HLA-A*02:01) (134). After stimulating each patient's peripheral blood mononuclear cells with neoantigens, increased neoantigen-specific immune responses were obtained, and there was no cross-reaction with the corresponding wild-type peptides. These neoantigens entered a Phase I clinical trial (NCT02348320) as a neoantigen poly-epitope DNA vaccine applied to patients by electroporation. The presence of T-cells that specifically target neoantigens was induced in both the pre-and post-vaccination phases, and at 36-month follow-up, the median of responding patients was 87.5% [95% confidence interval (CI): 72.7-100%] compared with 49% (95% CI: 36.4-65.9%) of institutional historical control patients (135).

In a clinical trial, NCT04105582, a total of 25 neoantigen peptides from patients with TNBC were administered in autologous dendritic cells (DCs) over 16 weeks. The afore-mentioned study proved the safety and immunogenicity of these vaccines. In another clinical trial, NCT04879888, DC vaccines pulsed with synthetic neoantigen peptides are being tested in nine patients with TNBC. The trial's primary goal is to ensure safety, with a secondary focus on evaluating the vaccine's immunogenicity using interferon-γ (IFNγ) ELISPOT. However, these trials have not examined the stability of the neoantigen peptides. Disis *et al* (136) reported that ~60% of patients with breast cancer who receive a DNA vaccine had persistent DNA at week 16 following vaccination. Currently,

Table III. Shared neoantigens identified in breast cancer and the corresponding HLA alleles.

| Shared neoantigen | HLA allele(s) | Immunogenicity analysis | (Refs.) |
|---|---|---|---|
| BRCA2 reversion (c.5946delT) | HLA-A*0101, HLA-A*3303, HLA-B*3501, HLA-B*4001, HLA-C*0602, HLA-C*1203 | - | (146) |
| PIK3CA$^{H1047R}$ | HLA-A*0201, HLA-A*0301, HLA-A*2402, HLA-A*33:01, HLA-A*33:03, HLA-DRB1*0405 and HLA-DRB1*0901 | Intracellular IFNγ staining, IFNγ ELISA, killing assay, *in vivo* clonal expansion | (147-149) |
| PIK3CA$^{E542K}$ | HLA-B*58:01 | - | (147) |
| PIK3CA$^{E545K}$ | HLA-B*15:17, HLA-B*44:03, HLA-B*57:01, HLA-B*57:02, HLA-B*57:03, HLA-B*57:04, HLA-B*58:01 | - | (147) |
| PIK3CA$^{N345K}$ | HLA-A*02:01, HLA-A*02:02, HLA-A*02:03, HLA-A*02:06, HLA-A*03:01, HLA-A*03:02, HLA-A*11:01, HLA-A*11:02, HLA-A*31:01, HLA-A*68:01 | - | (147) |
| TP53$^{R175H}$ | HLA-A*0201 | Flow cytometric assessment of 4-1BB (CD137), OX40 (CD134), IFNγ ELISPOT assay, TCR sequencing | (150,151) |
| P53$^{Y220C}$ | HLA-A*02:06, HLA-DRB3*02:02 | Flow cytometric assessment of 4-1BB (CD137), OX40 (CD134), IFNγ ELISPOT assay, TCR sequencing | (151,152) |

HLA, human leukocyte antigen.

no trial results have been made available. In another clinical study, Morisaki *et al* (137) identified neoantigens in primary cancer cells from the pleural fluid of a patient with TNBC. In total, 2/10 of predicted neoantigens had a high binding affinity for HLA-A*02:06: *GEMIN2* (AQCPDVLV) and *PARP10* (SISCHVFCL). Both caused a significant increase in the response of IFNγ-producing T-cells to autologous cancer cells observed after inducing cytotoxic T lymphocytes with peptide-pulsed autologous DCs (137). However, the aforementioned study also reported that *AKT1, ARAP3, NOTCH3, PIK3CA* and *SLC35E2* contained identical non-synonymous SNVs without testing immunogenicity. Additionally, there are other ongoing clinical trials to evaluate personalized neoantigen-based immunotherapy in breast cancer (Table II).

*Public neoantigens in breast cancer.* Cancer-driver mutations, which are typically found in oncogenes or specific tumor suppressor genes, often occur in genomic hotspots. These hotspots lead to changes in protein function and are frequently observed across different patients (138). The mutated peptides produced by these genetic alterations, when presented by HLA alleles, can generate neoantigens that are common among individuals with the same genetic changes and HLA types (139). This phenomenon marks these neoantigens as shared among cancer patients, giving rise to 'public' neoantigens (140). Setting aside R157H, other p53 mutants could serve as neoantigens, such as TP53$^{Y220C}$ found in breast cancer. The TP53$^{Y220C}$ neoepitopes, VVPCEPPEV, RNTFRHSVVVPCE and NTFRHSVVVPCEPPE, were recognized by HLA-A*02:01-, HLA-DRB1*04:01 and HLA-DRB3*02:02-expressing tumor-infiltrating lymphocytes (TILs), respectively (141). Consequently, the results of current research support efforts to create off-the-shelf anticancer therapies based on the public or shared neoantigens, which could potentially benefit a broad group of patients with breast cancer.

In breast cancer, most shared target antigens have been reported as TAAs; for example, a Phase I/II clinical trial of autologous DC transfected with the cDNA of mucin (MUC1) in patients with advanced breast cancer (142). The trial demonstrated the feasibility and safety of this vaccine approach and demonstrated immunologic responses in patients who had previously received treatment and had advanced-stage disease (142). *In situ* testing of a HER2-pulsed DC vaccine in 27 patients with HER2/neu-overexpressing ductal carcinoma was well-tolerated and demonstrated reduction or elimination of HER2/neu expression (143).

Previously, a small number of public neoantigens were explored in breast cancer (Table III). Pettitt *et al* (144) analyzed the prediction of an out-of-frame sequence resulting from common founder *BRCA2* reversion (c.5946delT: RENLSRYQMLHYKTQ) that can be presented by numerous HLA class I alleles such as HLA-A*0101, HLA-A*3303, HLA-B*3501, HLA-B*4001, HLA-C*0602 and HLA-C*1203 (144). The results showed a revertant neoepitope sequence that can be presented with a high probability by the MHC class I across the general population and therefore provides an option for targeting public neoantigens (144).

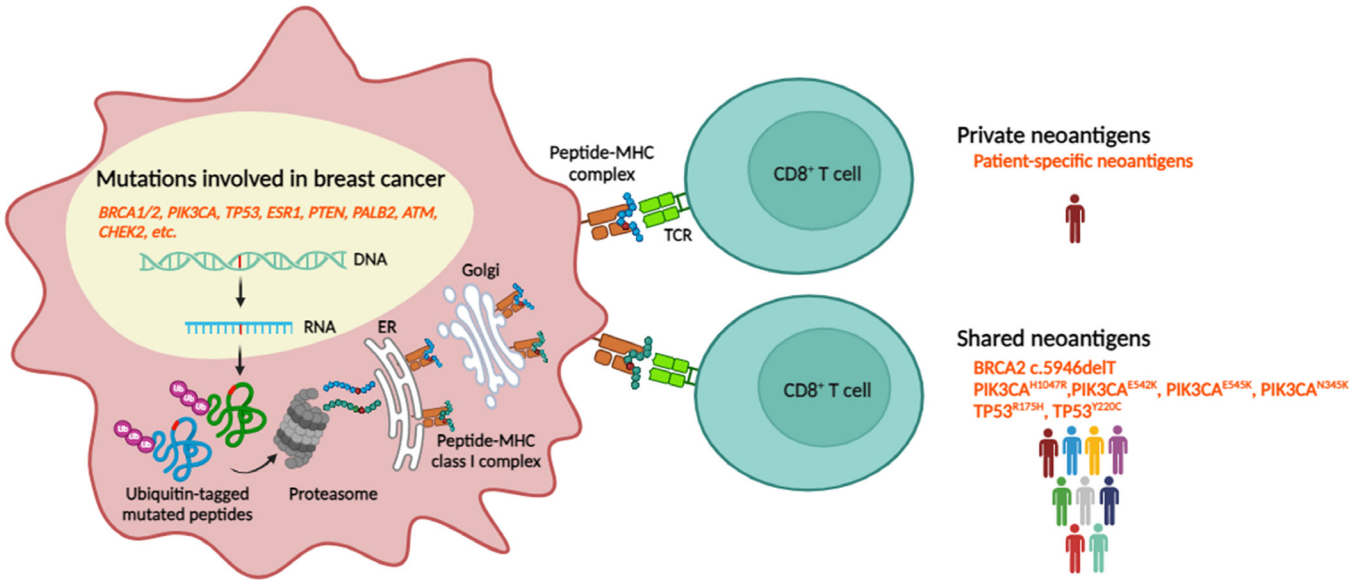


Figure 2. The summarization of the hotspot mutations found in patients with breast cancer, which have been reported to exhibit immunogenicity. These mutations have the potential to provide public neoantigens among patients with breast cancer (Created in BioRender.com).

Ruangapirom *et al* (145) predicted the immunogenicity of common hotspot mutations found in BRCA1-related breast cancer. They predicted elements with high binding affinity for various alleles of MHC class I in peptides of 9-12 amino acids: PIK3CA$^{H1047R}$, PIK3CA$^{E542K}$, PIK3CA$^{E545K}$, PIK3CA$^{N345K}$ and TP53$^{Y220C}$ (145). Although these common neoantigens were predicted based on *in silico* analysis, the findings suggested the potential use of public neoantigen targets in vaccines for the population with breast cancer. Iizumi *et al* (146) provided *in vitro* validation of neoantigens frequently found in breast cancer, such as PIK3CA$^{H1047R}$, that were predicted to bind with HLA-A*0201, HLA-A*2402, HLA-DRB1*0405 and HLA-DRB1*0901. They elicited both CD4$^+$ and CD8$^+$ responses in 16.7% of healthy donors (146). Moreover, Chandran *et al* (147) verified the spontaneous immunogenicity of PIK3CA public neoantigens in eight patients with breast cancer harboring PIK3CA$^{H1047L}$. In total, two of these neoantigens were able to successfully retrieve from a patient a TCR that conferred a response to targets that carried the PIK3CA$^{H1047L}$ mutation. This confirmed that the PIK3CA public neoantigen can trigger an immune response and drive *in vivo* clonal expansion in a subset of patients with breast cancer.

Previous research has indicated that TP53 hotspot mutations can be utilized in a shared neoantigen-based cancer vaccine to treat breast cancers (148,149). Lo *et al* (148) described the identification and characterization of HLA-A*0201-restricted TCRs that react with TP53$^{R175H}$ and recognize various cancer cell lines, including breast cancer endogenously expressing TP53$^{R175H}$. When a patient with the HLA-A*0201 allele and chemorefractory breast cancer was treated by adoptive transfer using TP53$^{R175H}$-TCR-engineered autologous peripheral blood lymphocytes, a significant reduction in subcutaneous tumor deposits occurred, and all skin lesions wholly resolved by day 60 after cell therapy (149). This finding may indicate a suitable shared neoantigen target for the treatment of patients whose cancers convey HLA-A*0201 and contain a TP53$^{R175H}$ mutation (148). Kim *et al* (149) and Zacharakis *et al* (150) reported that patients with breast cancer who received adoptive transfer of *ex vivo*-expanded autologous TILs targeting TP53$^{Y220C}$ exhibited a partial response. There was complete resolution of two right subpectoral lymph nodes, subcutaneous breast nodules, and substantial regression of the primary breast tumor with response durations of 6 months (150). The summary of hotspot mutations recognized as public neoantigens sharing among patients with breast cancer is shown in Fig. 2.

## 7. Clinical utilization of public neoantigens for breast cancer treatment

Despite multiple studies that have identified shared neoantigens in breast cancer and demonstrated their ability to stimulate anti-tumor immune responses *in vitro*, clinical investigations that have focused on exploiting the immunogenicity of the associated shared neoantigens in breast cancer are limited. Ongoing clinical trials testing the immunogenicity of shared neoantigen vaccines are in non-small cell lung, colorectal and pancreatic cancer (Clinical trial NCT03953235). The aforementioned study has been measuring PFS and OS for ~4 years (2019-2023). There remains a crucial necessity for further clinical investigation into the implications and outcomes of these shared neoantigens within breast cancer. The broader application across a diverse spectrum of cancer patients suggests that they may be promising targets for off-the-shelf immunotherapy.

## 8. Conclusion

Cancer mutations are patient-specific, giving neoantigens a highly personalized character. Yet, some neoepitopes are consistently found across multiple patients with cancer, making them shared neoantigens. These common neoantigens hold promise for treating a substantial number of patients

who share several specific HLA types, offering viable targets for off-the-shelf cancer immunotherapies. Current clinical research into breast cancer predominantly investigates personalized neoantigens, whereas the study of shared neoantigens in breast cancer is limited due to the low proportion of neoantigen burden, limited studies of hotspot mutations in breast cancer, and the challenges in locating patients who have both a specific HLA allele and a particular common neoantigen. The true potential of broad HLA-specific public neoantigen prediction remains to be fully explored through clinical trials involving diverse patient groups with various HLA types. This presents a significant challenge for the development of broadly applicable ready-to-use immunotherapies for cancers in general and for patients with breast cancer in particular.

**Acknowledgements**

The authors would like to thank Dr Jan Davies, Faculty of Graduate Studies, Mahidol University, for English editing.

**Funding**

The present study was supported by the Research and Innovation Grant, the National Research Council of Thailand, Ministry of Higher Education, Science, Research and Innovation, Thailand (grant no. R016341038) and the Siriraj Research Grant, Faculty of Medicine Siriraj Hospital, Mahidol University (grant no. R016334002).

**Availability of data and materials**

Not applicable.

**Authors' contributions**

NS designed and wrote the manuscript. CT, PT and PY provided the research direction. NS and CT edited the manuscript. All authors read and approved the final manuscript. Data authentication is not applicable.

**Ethics approval and consent to participate**

Not applicable.

**Patient consent for publication**

Not applicable.

**Competing interests**

The authors declare that they have no competing interests.

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