Toll-like receptor 3 ligands for breast cancer therapies (Review)

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Abstract. Breast cancer is the most common cause of cancer worldwide and is the leading cause of mortality for women across most of the world. Immunotherapy is a burgeoning area of cancer treatment, including for breast cancer; these are therapies that harness the power of the immune system to clear cancerous cells. Toll-like receptor 3 (TLR3) is an RNA receptor found in the endosome, and ligands that bind to TLR3 are currently being tested for their efficacy as breast cancer immunotherapeutics. The current review introduces TLR3 and the role of this receptor in breast cancer, and summarizes data on the potential use of TLR3 ligands, mainly polyinosinic:polycytidylic acid and its derivatives, as breast cancer monotherapies or, more commonly, as combination therapies with chemotherapies, other immunotherapies and cancer vaccines. The current state of TLR3 ligand breast cancer therapy research is summarized by reporting on past and current clinical trials, and notable preliminary *in vitro* studies are discussed. In conclusion, TLR3 ligands have robust potential in anticancer applications as innate immune stimulants, and further studies combined with innovative technologies, such as nanoparticles, may contribute to their success.

Contents

1. Introduction

Breast cancer is the most common cancer across the world and despite increased awareness and screening, it is still one of the leading causes of mortality in females (1). There is an obvious need for improved breast cancer therapeutics. In comparison to chemotherapy and radiation therapy for cancer treatment, immunotherapy is still in its infancy, but is a rapidly expanding repertoire of anticancer therapies. Immunotherapies help the patient's own immune system to fight tumour cells (2). There are several different types of cancer immunotherapies, including, but not limited to, checkpoint inhibitors, cytokines, immunomodulators, cancer vaccines, and monoclonal antibodies (3).

Recent evidence has suggested a link between certain immune system receptors and cancer development. Toll-like receptors (TLRs) are a family of pattern recognition receptors (PRRs) that play important roles in innate immunity (4). TLRs recognize cognate ligands including pathogen-associated molecular patterns (PAMPs) and damage-associated molecular patterns (DAMPs). Recognition of PAMPs or DAMPs triggers signalling cascades that culminate in the expression of proinflammatory cytokines and/or type I interferon (IFN) pathways, Fig. 1 (4). TLRs can be expressed on a variety of immune cells, such as dendritic cells and macrophages, and non-immune cells, such as fibroblasts and epithelial cells (5). Certain cancer cells have been found to have dysregulated expression of TLRs compared to normal cells, suggesting the potential application of TLR ligands as cancer therapies (6). Despite their important roles in innate immunity, and the established link between immunity and cancer establishment and progression, there are only three TLR-agonists that have been approved for anti-cancer usages (7). The role of TLRs in cancer has been previously reviewed for multiple types of cancer (8).

TLR3, the focus of this review, binds to nucleic acids, specifically double stranded (ds)RNA (9). Binding to long dsRNA in the endosome leads to dimerization and activation of TLR3, recruiting the adaptor protein a toll/interleukin (IL)-1) receptor (TIR)-domain-containing adapter-inducing interferon-β (TRIF; also known as TICAM-1) protein. TRIF initiates downstream pathways which lead to activation of transcription factors including interferon-regulatory factors (IRF3/7), activator protein 1 (AP-1), and Nuclear Factor kappa B (NF-kB), culminating in type I IFN/IFN-stimulated genes, and inflammatory cytokines, Fig. 1 (4). TLR3 is characterized by extracellular leucine-rich repeats (LRRs), a transmembrane domain, and a cytoplasmic tail with a TIR domain (9). TLR3 is highly expressed in endosomes of antigen-presenting cells (APCs), epithelial cells, and other cells with expression varying depending on the tissue and cell-type (10). TLR3 is

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also expressed on endosomal membranes of myeloid dendritic cells, endothelial cells, keratinocytes, and some cancer cells (11,12). TLR3 activation can occur in cancerous cells or surrounding cells within the tumour microenvironment, including immune cells, and activation can have a variety of outcomes, Fig. 1. While there is variability in responses, general anticancer effects of treatment with TLR3-activators include production of anticancer cytokines, such as IFN-α, IL-12, IL-21, and the induction of apoptosis, possibly through intrinsic and extrinsic pathways (9,10,12-14) The cytokines produced through TLR3 stimulation induce the activation of tumour-suppressive macrophages and neutrophils (15).

On the other hand, TLR3 activation can promote tumour recurrence, metastasis, and cell proliferation through the production of pro-tumour cytokines, such as IL-6, and machinery involved in other hallmarks of cancer, such as production of hypoxia-include factor 1α to improve resistance to hypoxia, and secretion of vascular endothelia growth factor to support angiogenesis (16-17). To further highlight the duality of this receptor, pro- and antitumour effects have been seen within the same cell line depending on the delivery mode of the TLR3-ligand, in two breast cancer cell lines surface stimulation has resulted in protumoural effects whereas cytoplasmic stimulation has been antitumoural (16). In human lung cancer cells, TLR3 activation leads to the production of cytokines that enhance migration, and in human melanoma cells TLR-agonist mediated effects were improved when one of the induced cytokines was blocked, demonstrating the complexity of the induced proteins from TLR3 activation (18,19). In breast cancer, TLR3 expression can be increased or decreased compared to normal cells, and the function of TLR3 in various cancerous cells differs. TLR3 activation can induce apoptosis in some tumour cells, while inducing tumour cell proliferation in other cells (10). In a large association study, it was found that two single-nucleotide polymorphisms were susceptibility variants within TLR3, and these variants were associated with larger tumour size (20).

There are currently no TLR3-based therapies approved for any cancers, however the preclinical data suggest that these therapies may prove to be novel treatments or may enhance existing therapies (7). As TLR3 activation can hinder or promote cancer development, despite appearing paradoxical, there is the potential for TLR-agonist or -antagonists as breast cancer therapies (21). In this review we will summarize the current TLR3-dependent breast cancer immunotherapies, Table I, and associated clinical trials, Table II, to provide perspective on the TLR3-dependent therapy pipeline and to help guide further research into breast cancer therapeutics (22-34).

2. TLR3 agonist therapies

TLR3 has the capability, when activated, to induce apoptosis and recruit immune cells to attack tumour cells, therefore TLR3 agonists could have anticancer capabilities (21). A main consideration is the administration of the therapy. With systemic administration of TLR3 agonists, there is potential for chronic inflammation and concerns regarding therapeutic doses reaching the tumour cells (34). Multiple TLR3 agonists have been explored as potential cancer treatments and cancer vaccine adjuvants, Table I, many of which have reached phase 1 and 2 clinical trials, Table II (35). TLR3 agonists are strong immunomodulators that activate adaptive and innate immune responses and help promote the recruitment of cluster of differentiation (CD)8^+ T lymphocytes, natural killer (NK) cells, and dendritic cell maturation, Table I (36). They also promote the production of anti-tumour Th1 (type 1 T helper) cytokines (28). TLR3 agonists have been explored as monotherapies or combination therapies with existing treatments, with many *in vitro* studies elucidating monotherapies, and most clinical trials focused on combination therapies with standard chemotherapeutics or other immunotherapies (37-39). Combination therapies have the potential to increase efficacy of the standard dosage or maintain efficacy while reducing the dosage of conventional treatments, decreasing negative side effects (39).

Poly (I:C). The largest body of dsRNA research in breast cancer relies on polyinosinic:polycytidylic acid (poly (I:C)) and poly (I:C) derivatives. Poly (I:C) is a synthetic dsRNA that has no sequence variation and has a high affinity for mammalian TLR3 compared to other dsRNA molecules (40). In many cells, including in breast cancer, poly (I:C) binds and activates pathways which lead to the activation of transcription factors (e.g., NF-kB, IRF3/7, AP-1) that promote the production of proinflammatory cytokines, type I IFNs, as well as costimulatory molecules (41). Not only can poly (I:C) activate innate immune responses, it can additionally help in the activation of long-lasting T cell immunity (41). By directly or indirectly recruiting leukocytes to the tumour microenvironment, the activation of TLR3 can aid in tumour lysis and induction of apoptosis by NK cells as well as cytotoxic T cells (41). Breast cancer cell apoptosis can be induced by poly (I:C) and can be achieved in a TLR3-dependent fashion (12). Poly (I:C) can also activate myeloid dendritic cells using similar pathways that involve TRIF and type I IFNs, which promotes NK cells to attack major histocompatibility complex (MHC) class I negative tumours (12).

The activation of TLR3 with poly (I:C) leads to downstream pathways that produce STAT1 (signal transducer and activator of transcription 1) phosphorylation, production of TRIF-dependent IFN-β, NF-κB activation, and cytokines that are pro-apoptotic, Fig. 1 (21). In mice, poly (I:C) complexed with polyethylenimine was delivered via intratumoural injection into 4T1 tumours; the treatment was effective at reducing tumour size (42). Nanoparticles have been employed in many poly (I:C) studies to increase delivery and efficacy in breast cancer cells, some examples include, but are not limited to, mesoporous silica, mannosylated poly lactic-co-glycolic acid, magnetic dendrimers, liposome-silica hybrids (43-46). While poly (I:C) is common in *in vitro* studies, it has issues with stability and toxicity in clinical trials, and as such most therapies involve modified versions of the dsRNA, such as Poly $IC_{12}U$ (also known as Rintatolimod, Ampligen or IPH 3102) or the poly ICLC (also known as Hiltonol) (47,48). Poly $IC_{12}U$ is a poly (I:C) derivative whereby an unpaired uracil/guanine introduction results in mismatched dsRNA that shows reduced toxicity; poly ICLC is a poly (I:C) derivative stabilized with the poly-l-lysine and carboxymethylcellulose (47,48). Poly (I:C)-based therapies are frequently tested as adjuvants or

Table I. A summary of TLR3 agonist and antagonist therapies and their proposed mechanisms.

Therapy	Composition	Mechanism
Polyinosinic: polycytidylic acid (Poly (I:C))	Synthetic dsRNA, TLR3 agonist	- Activation of NK cells - Induction of type I interferons - Cytotoxicity - Dendritic cell maturation
Poly ICLC (Hiltonol)	Stabilized with poly-lysine, TLR3 agonist	- Activation and infiltration of CTLs and NK cells
Poly $IC_{12}U$ (Rintatolimod, IPH 3102, Ampligen)	Modified Poly (I:C) with cytidine replaced by uridine, TLR3 agonist	- Activation of CTLs and NK cells - Converts M2-type macrophages to M1-type macrophage - Induction of type I interferons - Cytotoxicity
Polyadenylic-polyuridylic acid (Poly(A:U)	Synthetic dsRNA, TLR3 agonist	- Induction of type I interferons - Cytotoxicity
C10 (Phenylmethimazole)	TLR3 inhibitor	- Blocks IL-6

CTL, cytotoxic T cells; NK, natural killer cells; dsRNA, double-stranded ribonucleic acid; TLR3, toll-like receptor 3; IL-6, interleukin-6.

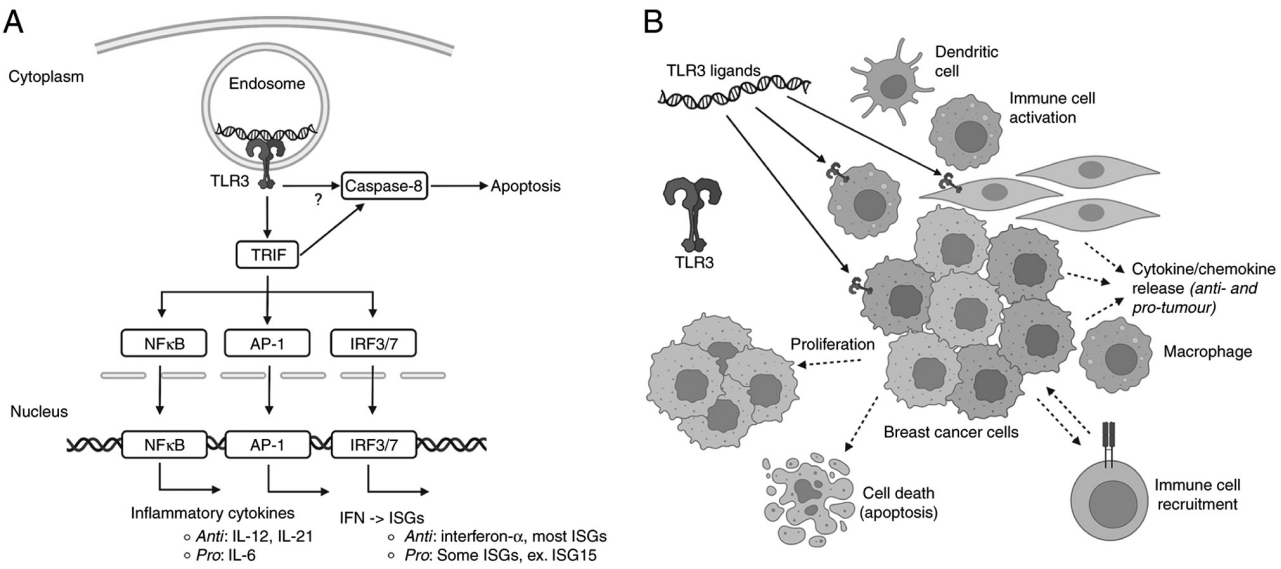

Figure 1. TLR3 ligands can be sensed by cancerous cells or cells in the tumour microenvironment. (A) Activation of TLR3 can trigger multiple pathways (B) that lead to diverse outcomes. Created with BioRender.com. TLR, Toll-like receptor; TRIF, TIR-domain-containing adapter-inducing interferon-β; IFN, interferon; IRF3/7, interferon-regulatory factors; AP-1, activator protein 1; NF-κB, nuclear factor κB; IL, interleukin.

combination therapies, currently there is only one clinical therapy with poly ICLC as a standalone treatment, Table II.

Poly (I:C) and derivatives: combination therapies. The combination therapy of poly (I:C) with chemotherapeutics has shown synergistic effects on cytotoxicity and inhibitory tumour growth effects (49). This increase in efficacy may help decrease some of the side effects that come with higher doses of chemotherapy treatments (49). Several studies have shown synergy between poly (I:C) and doxorubicin in breast cancer cells, a combination of poly (I:C) and doxorubicin delivered with iron oxide nanoparticle, mesoporous silica nanoparticle, and magnetic dendrimer nanoparticle induced higher levels of apoptosis; in the case of the iron oxide nanoparticle tumour apoptosis was caused through direct killing, dendritic cell-initiate and cytotoxic T cell-mediated responses (43-54). Poly (I:C) can synergistically improve the efficacy of the chemotherapy gemcitabine in breast cancer mouse models (51). While there are a multitude of preliminary studies on these combinations there are fewer clinic trials into these combinations, as seen by bolded co-interventions in Table II, and a greater effort into combination poly (I:C)/derivatives and immunotherapies.

Additionally, poly (I:C) improved the efficacy of other drugs not traditionally used for cancer treatments, such as retinoic acid and ferumoxytol (41,52). Poly (I:C) combination therapies allow for potential improvement upon existing therapies, decreasing doses to improve patient experience, and to

Table II. A summary of clinical trials using TLR3 ligands.

A, Poly ICLC (Hiltonol)

Clinical trial number	Combination treatments	Phase	Status
NCT00986609	- MUC-1 peptide vaccine	Early phase 1	Completed
NCT02643303	- Durvalumab[a] - Tremelimumab[a]	Phase 1 Phase 2	Completed
NCT02826434	- PVX-410 vaccine - Durvalumab[a]	Phase 1	Active (not recruiting)
NCT03362060	- Pembrolizumab[a] - PVX-410 vaccine - Montanide (adjuvant)	Phase 1	Active (not recruiting)
NCT05098210	- Neoantigen peptide vaccine - Nivolumab[a]	Phase 1	Recruiting
NCT03789097	- Pembrolizumab[a] - Flt3 ligand vaccine - Radiation	Phase 1 Phase 2	Recruiting
NCT05098210	- Neoantigen peptide vaccine - Nivolumab[a]	Phase 1	Recruiting
NCT03606967	- Carboplatin[b] - Durvalumab[a] - Gemcitabine hydrochloride[b] - Nab-paclitaxel[b] - Personalized synthetic long peptide vaccine - Tremelimumab[a]	Phase 2	Recruiting
NCT03606967	- Nab-paclitaxel[b] - Durvalumab - Tremelimumab[a] - Neoantigen vaccine	Phase 2	Recruiting
NCT04116320	- Echopulse device - Standard of care PD-1 therapy[a]	Phase 1	Recruiting
NCT01532960	- 9 peptides from Her-1/neu, CEA, CTA - Peptide-TET	Phase 1	Terminated (Futility for immune responses to the vaccine, component of study drug was in short supply)
NCT02427581	- Personalized synthetic long peptide vaccine	Phase 1	Withdrawn (drugs not available)
NCT04616248	- CDX01140 (anti-CD40 agonist mAb)[a] - Radiation therapy - Recombinant Flt3 ligand[a]	Phase 1	Withdrawn (implementation issues)
NCT02427581	- Personalized synthetic long peptide vaccine	Phase 1	Withdrawn (drugs not available)
NCT02661100	- Pembrolizumab[a] - CDX1401[a]	Phase 1 Phase 2	Withdrawn (drug unavailable)
NCT01984892		Phase 2	Terminated (low enrollment)

B, Poly $IC_{12}U$ (Rintatolimod, IPH 3102, Ampligen)

Clinical trial number	Combination treatments	Phase	Status
NCT01355393	- HER-2/neu peptide vaccine - Sargramostim[a]	Phase 1 Phase 2	Completed
NCT03599453	- Chemokine modulation therapy - Celecoxib - Recombinant interferon alfa-2b - Pembrolizumab	Early phase 1	Active (not recruiting)

Table II. Continued.

B, Poly $IC_{12}U$ (Rintatolimod, IPH 3102, Ampligen)

Clinical trial number	Combination treatments	Phase	Status
NCT04081389	- Celecoxib - Cyclophosphamide[b] - Doxorubicin hydrochloride[b] - Paclitaxel[b] - Recombinant Interferon Alfa-2b	Phase 1	Suspended (analyzing data)

C, Poly (A:U)

Clinical trial number	Combination treatments	Phase	Status
NCT01355393	- HER-2/neu peptide vaccine - Sargramostim[a]	Phase 1 Phase 2	Completed
NCT03599453	- Chemokine modulation therapy - Celecoxib - Recombinant interferon alfa-2b - Pembrolizumab	Early phase 1	Active (not recruiting)
NCT04081389	- Celecoxib - Cyclophosphamide[b] - Doxorubicin hydrochloride[b] - Paclitaxel[b] - Recombinant Interferon alpha-2b	Phase 1	Suspended (analyzing data)

MUC-1, Mucin 1; Flt3L, Fms-related tyrosine kinase 3 ligand; PD-1 programmed cell death protein 1; Her-1/neu, human epidermal growth factor receptor 1; CEA, carcinoembryonic antigen; CTA, cancer testis antigens; anti-CD40 agonist mAb, anti-cluster of differentiation 40 agonist monoclonal antibody. [a]Immunotherapies; [b]chemotherapies.

repurpose non-traditional drugs into cancer therapies. There are limited published results from the clinical trials in Table II. The results from (NCT02643303) have been reported and the combination treatment of intratumoural tremelimumab and poly ICLC combined with systemic durvalumab demonstrated clinical responsiveness and induced an immune response mediated by increased $CD8^+$ T cells (and increased cytotoxicity, activation, and proliferation), $CD20^+$ B cells, mature dendritic cells, macrophages, and $CD56^+$ NK cells (53).

Poly (I:C) and derivatives: cancer vaccine adjuvants. Poly (I:C), as a clear inducer of the innate immune response, is an adjuvant candidate. To this end there are several clinical trials that have explored poly (I:C) derivatives in combination with PVX-410 (Oncopep Inc. human leukocyte antigen A2-restricted multi-peptide cancer vaccine), MUC-1 (mucin 1), Her-2 (human epidermal growth factor receptor 2), Flt3L (Fms-like tyrosine kinase 3 ligand), and neoantigen peptide vaccines, Table II. In a phase 1 trial (NCT02826434), although full results have not yet been published, the combination of the PVX-410 vaccine with durvalumab and poly ICLC was tolerated and induced antigen-specific T cell expansion and activation, this response persisted for six months in some patients (54). A novel triple negative breast cancer (TNBC) vaccine has been developed to stimulate antigen-specific T cell responses using a multi-peptide anti-cancer vaccine. CD4+ T cells are activated which in turn leads to dendritic cell generated immune responses (55). A peptide vaccine, with a tetanus helper, was tested as a combination therapy with Poly ICLC in a clinical setting on individuals with various stages of breast cancer (55). While an increased immune response was observed in response to several of the peptides in the vaccine, response levels were lower than expected in comparison to similar peptide vaccines tested on breast cancer patients. An increase in T cell responses was observed in some of the patients, however these results were lower than expected. Although the results of this combination therapy were not as significant as had been hoped, this study proved that the combination of Poly-ICLC with multi-peptide cancer vaccines is safe for use in a clinical setting (55). In another model of TNBC poly (I:C) significantly enhanced the benefits of an anti-PD-1 therapy, prolonging metastasis-free survival (56). A MUC-1 glycopeptide including a T-cell epitope from polio virus was combined with poly (I:C) and induced approximately 6 times as much IgG antibody compared to those without poly (I:C) in mice (57). This antibody response was able to respond to aberrantly glycosylated MUC-1 on MCF-7 breast cancer cells (57).

Poly (A:U). Polyadenylic:polyuridylic acid (poly (A:U)) is another synthetic, non-variable sequence dsRNA. Poly (A:U)

has been tested for adjuvant activity in breast cancer (58). A randomized clinical trial of 194 breast cancer patients showed that adjuvant treatment with dsRNA was found to be associated with a reduced risk of breast cancer metastatic relapse in patients with TLR3-positive cancers (58) Data on this therapeutic efficacy shows that dsRNA can mediate its therapeutic effects on tumour cells that express TLR3 (58). Since certain breast cancer cells express TLR3, this dsRNA adjuvant treatment can lead to the recruitment of immune cells to the tumours, ultimately leading to apoptosis of tumour cells.

Poly (A:U) is recognized by only TLR3 whereas the poly (I:C) agonist is recognized by TLR3 in the endosome, and cytosolic receptors such as RIG-I and MDA-5 (melanoma differentiation-associated protein 5) (19). Poly (I:C) has been found to enhance antigen-specific CD8+ T cell responses and helps in antigen presentation and antigen cross-presentation by dendritic cells (57). Effector CD8+ T cells and NK cells also help increase the IFN-γ release (19). In mice, it was shown that poly (A:U) treatment promoted Th1-immune responses as well as enhanced antibody production (19). Poly (A:U), was not able to trigger a potent immunostimulatory effect on its own, but if it was combined with a vaccine or chemotherapy, the treatment was able to trigger a T-cell dependent and TRIF-dependent response (19). A randomized trial completed in 1980 administered Poly (A:U) as a combination therapy with conventional treatment (surgery alone or surgery plus cobalt therapy) on patients with operable breast cancer. Survival time was found to be significantly higher in the combination therapy group compared to those who received conventional therapy alone, and incidence of relapse, particularly in patients with lymph node disease, was lower in the combination group (59).

3. TLR antagonists

Most research conducted with TLR3, and breast cancer have found positive effects against tumour cells, however, there is some evidence that TLR3 expression can promote carcinogenesis and resistance to antitumor drugs. Studies on breast cancer have found a supportive role of TLR3 in their metastasis and increased tumour growth (10). There are some cells in tumour masses that can aid in the stimulation of tumour growth and development (60). For example, dendritic cells, macrophages and myeloid-derived suppressor cells (MDSCs) may have a role in tumour neoangiogenesis by inhibiting some immune responses against the tumour (60).

C10 Phenylmethimazole. Many of the immunotherapies studied have promoted TLR3 activation, some inhibit the signalling pathways. In breast cancer an agonist that is in the earlier stages of exploration is the TLR3 inhibitor phenylmethimazole (C10), delivered in combination with tamoxifen (33). In breast cancer cells, MCF-7 (expressing oestrogen, progesterone, and glucocorticoid receptors), the combination of C10 and tamoxifen resulted in an enhanced anticancer response than either treatment administered alone (33). As TLR3 activation can enhance tumour cell growth and proliferation, TLR3 inhibitors can potentially allow mediation of the immune responses within the tumour microenvironment. C10 can block the production of 1L-6, a cytokine that has a known role in tumour growth and helps to drive STAT3, an oncogene which also helps promote cancer development and metastasis (33). The combination of C10 with tamoxifen enhanced tamoxifen's cytotoxic potential by over 50% compared to tamoxifen alone and decreased cellular migration (33). A more potent derivative of C10 has also been developed, COB-141 and both compounds were able to further inhibit IL-6 secretion in a TNBC cell line, MDA-MB-231 (61). COB-141 furthermore reduced IL-6 secretion in two additional TNBC cell lines, MDA-MB-468 and Hs578T, and in all three TNBC cell lines there was a decrease in NF-kB DNA binding, but interestingly was not found to limit metabolic activity, as was seen in MCF7 with C10 (61). This highlights differences in cells from different cancer types, and the need to consider different mechanisms and efficacy across cancer subtypes (61).

4. Conclusion

Immunotherapies are a promising, novel method for the treatment of breast cancers. TLRs, specifically TLR3, are a type of receptor that is expressed on a variety of different cells, including tumour cells. The activation of TLR3 has been shown to have both carcinogenic and anticancer properties and therefore should be further researched as potential cancer treatment options. The subtype of cancer needs to be considered, as many TLR3-based therapies have not been broadly tested against subtypes, and likelihood of success is tied to the expression of TLR3 in the patient-specific tumour. In most breast cancer studies, TLR3-agonists have reported anticancer effects, however, as there is evidence of TLR-antagonists limiting breast cancer cell metabolism, there is a need for more research (61). In particular, the use of 2D monoculture breast cancer cell lines fail to explore the role of the tumour microenvironment. Creation of more relevant models, such as co-culture of tumour cells and immune cells, could provide more relevant information into the development of TLR-based therapies, and could help further clarify the paradoxical pro- and anti-cancer responses of TLR3 stimulation. Immunostimulatory nucleic acids, such as the majority of TLR agonists, require an effective delivery system to improve stability and efficacy, current research leads to nanocarriers to serve this purpose. Up to this point, research conducted on TLR3 agonist therapies in cancer treatment has made it evident that the use of combination therapies compared to monotherapies will most likely be more effective in treating breast cancers.

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Competing interests

The authors declare that they have no competing interests.

References

1. Sancho-Garnier H and Colonna M: Breast cancer epidemiology. Presse Med 48: 1076-1084, 2019 (In French).
2. Waldman AD, Fritz JM and Lenardo MJ: A guide to cancer immunotherapy: From T cell basic science to clinical practice. Nat Rev Immunol 20: 651-668, 2020.
3. Oiseth SJ and Aziz MS: Cancer immunotherapy: A brief review of the history, possibilities, and challenges ahead. J Cancer Metastitis Treat 3: 250-261, 2017.
4. Le Naour J, Galluzzi L, Zitvogel L, Kroemer G and Vacchelli E: Trial watch: TLR3 agonists in cancer therapy. Oncoimmunology 9: 1771143, 2020.
5. Kawasaki T and Kawai T: Toll-like receptor signaling pathways. Frontiers Immunol 5: 461, 2014.
6. Iwasaki A and Medzhitov R: Toll-like receptor control of the adaptive immune responses. Nat Immunol 5: 987-995, 2004.
7. Vacchelli E, Eggermont A, Sautès-Fridman C, Galon J, Zitvogel L, Kroemer G and Galluzzi L: Trial Watch: Toll-like receptor agonists for cancer therapy. Oncoimmunology 2: e25238, 2013.
8. Chen X, Zhang Y and Fu Y: The critical role of Toll-like receptor-mediated signaling in cancer immunotherapy. Med Drug Disc 14: 100122, 2022.
9. Bianchi F, Pretto S, Tagliabue E, Balsari A and Sfondrini L: Exploiting poly(I:C) to induce cancer cell apoptosis. Cancer Biol Ther 18: 747-756, 2017.
10. Muresan XM, Bouchal J, Culig Z and Souček K: Toll-like receptor 3 in solid cancer and therapy resistance. Cancers (Basel) 12: 3227, 2020.
11. Matsumoto M, Takeda Y, Tatematsu M and Seya T: Toll-Like receptor 3 signal in dendritic cells benefits cancer immunotherapy. Front Immunol 8: 1897, 2017.
12. Yu M and Levine SJ: Toll-like receptor 3, RIG-I-like receptors and the NLRP3 inflammasome: Key modulators of innate immune responses to double-stranded RNA viruses. Cytokine Growth Factor Rev 22: 63-72, 2011.
13. Holm CK, Petersen CC, Hvid M, Petersen L, Paludan SR, Deleuran B and Hokland M: TLR3 ligand polyinosinic: Polycytidylic acid induces IL-17A and IL-21 synthesis in human Th cells. J Immunol 183: 4422-4431, 2009.
14. Weber A, Kirejczyk Z, Besch R, Potthoff S, Leverkus M and Hacker G: Proapoptotic signalling through Toll-like receptor-3 involves TRIF-dependent activation of caspase-8 and is under the control of inhibitor of apoptosis proteins in melanoma cells. Cell Death Differ 17: 942-951, 2010.
15. Shime H, Matsumoto M and Seya T: Double-stranded RNA promotes CTL-independent tumor cytolysis mediated by CD11b+Ly6G+ intratumor myeloid cells through the TICAM-1 signaling pathway. Cell Death Differ 24: 385-396, 2017.
16. Bondhopadhyay B, Moirangthem A and Basu A: Innate adjuvant receptor Toll-like receptor 3 can promote breast cancer through cell surface. Tumor Biol 36: 1261-1271, 2015.
17. Paone A, Galli R, Gabellini C, Lukashev D, Starace D, Gorlach A, De Cesaris P, Ziparo E, Del Bufalo D, Sitkovsky MV, *et al*: Toll-like receptor 3 regulates angiogenesis and apoptosis in prostate cancer cell lines through hypoxia-inducible factor 1 alpha. Neoplasia 12: 539-549, 2010.
18. Zhan Z, Xie X, Cao H, Zhou X, Zhang XD, Fan H and Liu Z: Autophagy facilitates TLR4-and TLR3-triggered migration and invasion of lung cancer cells through the promotion of TRAF6 ubiquitination. Autophagy 10: 257-268, 2014.
19. Zhao J, Zhang Z, Xue Y, Wang G, Cheng Y, Pan Y, Zhao S and Hou Y: Anti-tumor macrophages activated by ferumoxytol combined or surface-functionalized with the TLR3 agonist poly (I: C) promote melanoma regression. Theranostics 8: 6307-6321, 2018.
20. Fan L, Zhou P, Hong Q, Chen AX, Liu GY, Yu KD and Shao ZM: Toll-like receptor 3 acts as a suppressor gene in breast cancer initiation and progression: A two-stage association study and functional investigation. Oncoimmunology 8: e1593801, 2019.
21. Salaun B, Coste I, Rissoan MC, Lebecque SJ and Renno T: TLR3 can directly trigger apoptosis in human cancer cells. J Immunol 176: 4894-4901, 2006.
22. Wulff S, Pries R and Wollenberg B: Cytokine release of human NK cells solely triggered with Poly I: C. Cell Immunol 263: 135-137, 2010.
23. Harashima N, Minami T, Uemura H and Harada M: Transfection of poly (I: C) can induce reactive oxygen species-triggered apoptosis and interferon-β-mediated growth arrest in human renal cell carcinoma cells via innate adjuvant receptors and the 2-5A system. Mol Cancer 13: 217, 2014.
24. Chew V, Tow C, Huang C, Bard-Chapeau E, Copeland NG, Jenkins NA, Weber A, Lim KH, Toh HC, Heikenwalder M, *et al*: Toll-like receptor 3 expressing tumor parenchyma and infiltrating natural killer cells in hepatocellular carcinoma patients. J Natl Cancer Inst 104: 1796-1807, 2012.
25. Wischke C, Zimmermann J, Wessinger B, Schendler A, Borchert HH, Peters JH, Peters JH, Nesselhut T and Lorenzen DR: Poly (I:C) coated PLGA microparticles induce dendritic cell maturation. Int J Pharm 365: 61-68, 2009.
26. Zhu X, Fallert-Junecko BA, Fujita M, Ueda R, Kohanbash G, Kastenhuber ER, McDonald HA, Liu Y, Kalinski P, Reinhart TA, *et al*: Poly-ICLC promotes the infiltration of effector T cells into intracranial gliomas via induction of CXCL10 in IFN-alpha and IFN-gamma dependent manners. Cancer Immunol Immunother 59: 1401-1409, 2010.
27. Kyi C, Roudko V, Sabado R, Saenger Y, Loging W, Mandeli J, Thin TH, Lehrer D, Donovan M, Posner M, *et al*: Therapeutic immune modulation against solid cancers with intratumoral poly-ICLC: A pilot trial. Clin Cancer Res 24: 4937-4948, 2018.
28. Navabi H, Jasani B, Reece A, Clayton A, Tabi Z, Donninger C, Mason M and Adams M: A clinical grade poly I: C-analogue (Ampligen) promotes optimal DC maturation and Th1-type T cell responses of healthy donors and cancer patients in vitro. Vaccine 27: 107-115, 2009.
29. Brodsky I, Strayer DR, Krueger LJ and Carter WA: Clinical studies with ampligen (mismatched double-stranded RNA). J Biol Response Mod 4: 669-675, 1985.
30. Martins KA, Bavari S and Salazar AM: Vaccine adjuvant uses of poly-IC and derivatives. Expert Rev Vaccines 14: 447-459, 2015.
31. Hubbell HR: Synergistic antiproliferative effect of human interferons in combination with mismatched double-stranded RNA on human tumor cells. Int J Cancer 37: 359-365, 1986.
32. Gatti G. Nunez NG, Nocera DA, Dejager L, Libert C, Giraudo C and Maccioni M: Direct effect of ds RNA mimetics on cancer cells induces endogenous IFN-β production capable of improving dendritic cell function. Eur J Immunol 43: 1849-1861, 2013.
33. Schwartz AL, Dickerson E, Dagia N, Malgor R and McCall KD: TLR signaling inhibitor, phenylmethimazole, in combination with tamoxifen inhibits human breast cancer cell viability and migration. Oncotarget 8: 113295-113302, 2016.
34. Sharma S, Zhu L, Davoodi M, Harris-White M, Lee JM, St John M, Salgia R and Dubinett S: TLR3 agonists and proinflammatory antitumor activities. Expert Opin Ther Targets 17: 481-483, 2013.

35. Gosu V, Basith S, Kwon OP and Choi S: Therapeutic applications of nucleic acids and their analogues in Toll-like receptor signaling. Molecules 17: 13503-13529, 2012.
36. Owen AM, Fults JB, Patil NK, Hernandez A and Bohannon JK: TLR agonists as mediators of trained immunity: Mechanistic insight and immunotherapeutic potential to combat infection. Front Immunol 11: 622614, 2021.
37. Pahlavanneshan S, Sayadmanesh A, Ebrahimiyan H and Basiri M: Toll-Like receptor-based strategies for cancer immunotherapy. J Immunol Res 2021: 9912188, 2021.
38. Kaczanowska S, Joseph AM and Davila E: TLR agonists: Our best frenemy in cancer immunotherapy. J Leukoc Biol 93: 847-863, 2013.
39. Ding L, Ren J, Zhang D, Li Y, Huang X, Ji J, Hu Q, Wang H, Ni Y and Hou Y: The TLR3 agonist inhibit drug efflux and sequentially consolidates low-dose cisplatin-based chemoimmunotherapy while reducing side effects. Mol Cancer Ther 16: 1068-1079, 2017.
40. Matsuo A, Oshiumi H, Tsujita T, Mitani H, Kasai H, Yoshimizu M, Matsumoto M and Seya T: Teleost TLR22 recognizes RNA duplex to induce IFN and protect cells from birnaviruses. J Immunol 181: 3474-3485, 2008.
41. Bernardo AR, Cosgaya JM, Aranda A and Jiménez-Lara AM: Synergy between RA and TLR3 promotes type I IFN-dependent apoptosis through upregulation of TRAIL pathway in breast cancer cells. Cell Death Dis 4: e479, 2013.
42. Aznar MA, Planelles L, Perez-Olivares M, Molina C, Garasa S, Etxeberría I, Perez G, Rodriguez I, Bolaños E, Lopez-Casas P, *et al*: Immunotherapeutic effects of intratumoral nanoplexed poly I:C. J Immunother Cancer 7: 116, 2019.
43. Ultimo A, Giménez C, Bartovsky P, Aznar E, Sancenón F, Marcos MD, Amorós P, Bernardo AR, Martínez-Máñez R, Jiménez-Lara AM and Murguía JR: Targeting innate immunity with dsRNA-Conjugated mesoporous silica nanoparticles promotes antitumor effects on breast cancer cells. Chemistry 22: 1582-1586, 2016.
44. Khodadust R, Alpsoy A, Ünsoy G and GÜndÜz U: Poly (I: C)-and doxorubicin-loaded magnetic dendrimeric nanoparticles affect the apoptosis-related gene expressions in MCF-7 cells. Turk J Biol 44: 133-144, 2020.
45. Sheikhzadeh S, Delirezh N and Hobbenaghi R: Mannosylated polylactic-co-glycolic acid (MN-PLGA) nanoparticles induce potent anti-tumor immunity in murine model of breast cancer. Biomed Pharmacother 142: 111962, 2021.
46. Colapicchioni V, Palchetti S, Pozzi D, Marini ES, Riccioli A, Ziparo E, Papi M, Amenitsch H and Caracciolo G: Killing cancer cells using nanotechnology: Novel poly (I: C) loaded liposome-silica hybrid nanoparticles. J Mater Chem B 3: 7408-7416, 2015.
47. Sultan H, Salazar AM and Celis E: Poly-ICLC, a multi-functional immune modulator for treating cancer. Semin Immunol 49: 101414, 2020.
48. Jasani B, Navabi H and Adams M: Ampligen: A potential toll-like 3 receptor adjuvant for immunotherapy of cancer. Vaccine 27: 3401-3404, 2009.
49. Keshavarz A, Pourbagheri-Sigaroodi A, Zafari P, Bagheri N, Ghaffari SH and Bashash D: Toll-like receptors (TLRs) in cancer; with an extensive focus on TLR agonists and antagonists. IUBMB Life 73: 10-25, 2021.
50. Mu QG, Lin G, Jeon M, Wang H, Chang FC, Revia RA, Yu J and Zhang M: Iron oxide nanoparticle targeted chemo-immunotherapy for triple negative breast cancer. Mat Today (Kidlington) 50: 149-169, 2021.
51. Le UM, Yanasarn N, Lohr CV, Fischer KA and Cui Z: Tumor chemo-immunotherapy using gemcitabine and a synthetic dsRNA. Cancer Biol Ther 7: 440-447, 2008.
52. Slingluff C, Mauldin I, Gaughan E, Dillon P, Opyrchal M, Puzanov I, Kruse M, Gastman B, Friedlander P, Marron T, *et al*: 337 Intratumoral immune therapy for recurrent breast cancer with polyiclc, and tremelimumab combined with systemic durvalumab. J Immunother Cancer 9: A1–A1054, 2021.
53. Isakoff SJ, Adams S, Soliman HH, Tung N, Barry WT, Hu J, Trippa L, Deering R, Parker J, Park H, *et al*: Abstract P3-09-15: A phase 1b study of PVX-410 (PVX) vaccine plus durvalumab (DUR) as adjuvant therapy in HLA-A2+ early stage triple negative breast cancer (eTNBC) to assess safety and immune response. Cancer Res 80: (suppl 4): P3-06-15, 2020.
54. Dillon PM, Petroni GR, Smolkin ME, Brenin DR, Chianese-Bullock KA, Smith KT, Olson WC, Fanous IS, Nail CJ, Brenin CM, *et al*: A pilot study of the immunogenicity of a 9-peptide breast cancer vaccine plus poly-ICLC in early stage breast cancer. J Immunother Cancer 5: 92, 2017.
55. Brockwell NK, Owen KL, Zanker D, Spurling A, Rautela J, Duivenvoorden HM, Baschuk N, Caramia F, Loi S, Darcy PK, *et al*: Neoadjuvant interferons: Critical for effective PD-1-based immunotherapy in TNBC. Cancer Immunol Res 5: 871-884, 2017.
56. Glaffig M, Stergiou N, Schmitt E and Kunz H: Immunogenicity of a fully synthetic MUC1 glycopeptide antitumor vaccine enhanced by poly(I:C) as a TLR3-Activating adjuvant. ChemMedChem 12: 722-727, 2017.
57. Salaun B, Zitvogel L, Asselin-Paturel C, Morel Y, Chemin K, Dubois C, Massacrier C, Conforti R, Chenard MP, Sabourin JC, *et al*: TLR3 as a biomarker for the therapeutic efficacy of double-stranded RNA in breast cancer. Cancer Res 71: 1607-1614, 2011.
58. Conforti R, Ma Y, Morel Y, Paturel C, Terme M, Viaud S, Ryffel B, Ferrantini M, Uppaluri R, Schreiber R, *et al:* Opposing effects of toll-like receptor (TLR3) signaling in tumors can be therapeutically uncoupled to optimize the anticancer efficacy of TLR3 ligands optimizing poly (A: U) anticancer therapy. Cancer Res 70: 490-500, 2010.
59. Lacour J, Lacour F, Spira A, Michelson M, Petit JY, Delage G, Sarrazin D, Contesso G and Viguier J: Adjuvant treatment with polyadenylic-polyuridylic acid in operable breast cancer: Updated results of a randomised trial. Br Med J (Clin Res Ed) 288: 589-592, 1984.
60. Venkatesh A, Nandigam H, Muccioli M, Singh M, Loftus T, Lewis D, Pate M and Benencia F: Regulation of inflammatory factors by double-stranded RNA receptors in breast cancer cells. Immunobiology 223: 466-476, 2018.
61. Noori MS, O'Brien JD, Champa ZJ, Deosarkar SP, Lanier OL, Qi C, Burdick MM, Schwartz FL, Bergmeier SC, McCall KD and Goetz DJ: Phenylmethimazole and a thiazole derivative of phenylmethimazole inhibit IL-6 expression by triple negative breast cancer cells. Eur J Pharmacol 803: 130-137, 2017.