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Combination Immunotherapy Strategies in Breast Cancer

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Abstract

Purpose of Review We summarize combination immunotherapy strategies for the treatment of breast cancer, with a focus on metastatic disease. First, a general overview of combination approaches is presented according to breast cancer subtype. Second, additional review of promising combination approaches is presented.

Recent Findings Combination strategies utilizing chemotherapy or radiotherapy with immune checkpoint inhibition are being evaluated across multiple phase III trials. Dual immunotherapy strategies, such as dual immune checkpoint inhibition or combined co-stimulation/co-inhibition, have supportive preclinical evidence and are under early clinical investigation. Modulation of the immune microenvironment via cytokines and vaccination strategies, as well as locally focused treatments to enhance antigenic responses, are active areas of research.

Summary Pre-clinical and translational research sheds new light on numerous ways the immune system may be modulated to fight against cancer. We describe current and emerging combination approaches which may improve patient outcomes in metastatic breast cancer.

Keywords Metastatic breast cancer · Combination immunotherapy · Treatment

Introduction

Immunotherapy attracts interest as a therapeutic strategy in breast cancer due to recognition of immune system involvement in the tumor microenvironment, observation that a robust immune response may confer a favorable prognosis, and achievement of meaningful clinical outcomes with immune-based therapies [1, 2••]. Enhanced survival utilizing immunotherapy as monotherapy has been limited, and it is increasingly accepted that in breast cancer, a combination of immunotherapy with other systemic or locally focused treatments may be helpful to induce an immunogenic cell death which promotes and sustains an immune-mediated response [3–5]. The purpose of this review is to describe combination strategies for immunotherapy in the treatment of breast cancer, with a focus on metastatic disease. First, a general overview of combination approaches across breast cancer subtypes is presented, and second, a more comprehensive review of various combination approaches is reviewed.

This article is part of the Topical Collection on *Immuno-oncology*

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Summary of Combination Approaches According to Tumor Subtype

Hormone-Receptor Positive Metastatic Breast Cancer

There is growing recognition of the effects of androgens and estrogens on immune function, which opens new possibilities for combination treatment strategies utilizing hormone blockade [6]. The androgen receptor (AR) is expressed in thymic tissue, and under experimental conditions, blockade of AR leads to increases in thymic volume and maturation of naïve T cell clones, which have the potential to become tumor reactive [7, 8]. AR inhibition may also directly inhibit tumor growth, as the AR is expressed in 60–80% of breast cancers and is also involved in the PI3K/Akt/mTOR and MAPK pathways [9]. In prostate cancer, AR antagonist therapy (with

enzalutamide) was associated with increases in dendritic cell expression of programed death ligands 1 (PD-L1) and 2 (PD-L2), relative to untreated patients. [10]. A number of trials are ongoing evaluating the role of AR inhibition plus anti-PD-1/L1 in hormone-receptor positive breast cancer. (Table 1)

CDK 4/6 inhibitors such as palbociclib, ribociclib, and abemaciclib are now standard-of-care options for hormone-receptor positive breast cancer, and function primarily by blocking the transition from G1 to S phase of the cell cycle [11]. It has been demonstrated preclinically that CDK4/6 inhibitors may incite anti-tumor immune responses due to interferon production, reduced proliferation of Tregs, and stimulation of effector T lymphocytes which augments the effect of immune checkpoint inhibitors (ICI) [12, 13]. Mouse models have also shown synergistic effects of CDK4/6 and PI3K inhibition with PD-1 and CTLA-4 inhibition [14]. Recently, the combination of abemaciclib plus pembrolizumab (anti-PD-1) was found to be safe in metastatic hormone receptor positive breast cancer, with an ORR of 28.6% at 24 weeks [15]. In comparison, the phase II MONARCH 1 study of abemaciclib monotherapy in HR+/HER2- mBC achieved an ORR of 10.6% with abemaciclib monotherapy at the same 24-week time point and at final 12-month analysis had an ORR of 19.7% [16]. A study assessing the combination of aromatase inhibition with abemaciclib and pembrolizumab has just completed accrual (NCT02779751). Additional trials are in progress evaluating combinations of ICI with small molecule targeted therapies (Table 1).

Because CDK4/6 inhibition may upregulate PD-L1 within the tumor microenvironment, another avenue of investigation in hormone-sensitive breast cancer is to evaluate combination immunotherapy approaches at the time of progression following receipt of CDK4/6 inhibitor. The MORPHEUS trial is a multi-

Table 1 Selected combination ICI trials in metastatic breast cancer

ICI agent	Combination agent	Tumor type	Phase of study	NCT identifier
Chemotherapy plus ICI (TNBC)				
Atezolizumab	Paclitaxel	TNBC#	Phase III	NCT03125902
Atezolizumab	Gemcitabine/carboplatin or capecitabine	TNBC##	Phase III	NCT03371017
Pembrolizumab	Nab-paclitaxel or paclitaxel or gemcitabine/carboplatin	TNBC###	Phase III	NCT02819518
Targeted therapies + ICI				
Atezolizumab	Entinostat or ipatasertib or fulvestrant	HR+ HER2-	Phase I/II	NCT03280563
Lodapolimab	Abemaciclib	HR + HER2-	Phase I	NCT02791334
Atezolizumab	Pertuzumab + trastuzumab	HER2+	Phase II	NCT03417544
Pembrolizumab	HER2 bi-armed activated T cells	HER2+	Phase I/II	NCT03272334
Atezolizumab	Paclitaxel + trastuzumab + pertuzumab	HER2+	Phase III	NCT03199885
Durvalumab	Olaparib	TNBC	Phase II	NCT03167619
Durvalumab	Olaparib	TNBC	Phase II	NCT03801369
MEDI4736 (anti-PD-L1)	Olaparib +/- cediranib	TNBC	Phase I/II	NCT02484404
Avelumab	Talazoparib	TNBC/ HR+	Phase II	NCT03330405
Dual ICI				
Ipi/nivo	RT or capecitabine	TNBC	Phase II	NCT03818685
Ipi/nivo		HER2-	Phase II	NCT03789110
Ipi/nivo	Bicalutamide	HER2-	Phase II	NCT03650894
Ipi/nivo	Cyclophosphamide + doxorubicin	HER2+	Phase I/II	NCT03409198
Ipi/nivo	INCAGN01876 (GITR agonist)	mBC	Phase I/II	NCT03126110
Nivo (phase I), Ipi/nivo (phase II)	NKTR-214	TNBC	Phase I/II	NCT02983045
Nivo	NKTR-214, NKTR-262 (TLR agonist)	TNBC	Phase I/II	NCT03435640
Ipi/nivo	Entinostat (HDAC inhibitor)	mBC	Phase I	NCT02453620
Combination ICI/co-stimulatory				
Nivo +/- ipi	BMS-986178 (OX40 agonist)	mBC	Phase I/II	NCT02737475

NCT, national clinical trials; *ICI*, immune checkpoint inhibition; *TNBC* triple negative breast cancer; *HR+*, hormone receptor positive; *HER2+*, HER-2-Neu positive; *HER2-*, HER-2-Neu negative; *Ipi*, ipilimumab; *nivo*, nivolumab; *TLR.* toll like receptor; *mBC*, metastatic breast cancer

[#] ≥ 12 months since prior chemotherapy

[##] ≤ 12 months since prior chemotherapy

[###] ≥ 6 months since prior chemotherapy

[^] following clinical benefit from platinum-based chemo

arm study that aims to evaluate second-line hormone–directed therapy (fulvestrant) in combination with immunotherapy (atezolizumab, anti-PD-l/L1) with or without various targeted therapies, including tyrosine kinase inhibitors (e.g., the AKT inhibitor, ipatasertib), angiogenesis inhibitors (e.g., bevacizumab), and epigenetic modifiers (e.g., the histone deacetylase inhibitor, entinostat) (NCT03280563). These therapies are associated with unique immunomodulatory effects. For example, AKT signaling is implicated in macrophage M1/M2 polarization [17], whereas blockade of vascular endothelial growth factor with bevacizumab may be associated with influx and activation of immune cells into tumors [18], and entinostat is associated with neutralization of myeloid-derived suppressor cells and enhanced efficacy of anti-PD-1/L1 [19].

Another potential immunotherapy target in hormone sensitive breast cancer is transforming growth factor beta (TGFβ), a multipotent cytokine which is present at high levels in the tumor microenvironment and is immunosuppressive. TGFβ can directly suppress the effector function of CD4+ and CD8+ T cells by transcriptional regulation of cytotoxic mediators granzyme, perforin, and interferon [20]. In addition, TGFβ limits T cell proliferation and differentiation [21, 22] but may also exclude T cells from the tumor microenvironment by promoting fibrosis and extracellular matrix deposition [23–25]. TGFβ gene signatures have been found to be enriched among less proliferative luminal-type tumors, raising interest in TGFβ as a potential target for hormone sensitive breast cancer [26]. Furthermore, TGFβ is one of the most abundant factors secreted within bone and is known to stimulate breast cancer bone metastases, which is a common site of metastasis in hormone-sensitive breast cancer [27]. In a mammary carcinoma model, TGFβ blockade at the time of radiation improved radiosensitivity in vitro and in vivo by attenuating DNA damage responses [28], as well as mediating interferon gamma (IFNγ) production [29]. Addition of anti-PD1 to radiation and TGFβ blockade further improved survival in murine mammary carcinoma models [29]. In light of these data, a number of TGFβ antagonists are being developed in combination with anti-PD-1/L1 in breast cancer. M7824, a bispecific antibody that targets both PD-L1 and TGFβ has demonstrated in murine models an ability to increase CD8+ T cell and NK cell activity, and increase MHC and PD-L1 expression within the tumor [30]. In a small trial, anti-TGFβ (fresolimumab) was evaluated in combination with palliative radiotherapy in metastatic breast cancer. Three of 23 subjects experienced a best response of stable disease; however, in a post hoc analysis, subjects randomized to the higher dose of fresolimumab had a significantly higher median OS (HR 2.73, 95% CI 1.02–7.30, p = .039) [31].

Human Epidermal Growth Factor Receptor 2–Positive Metastatic Breast Cancer

HER2 overexpression is found in 15–20% of invasive breast cancers and is associated with aggressive behavior and poor survival related to metastatic recurrence [32]. Targeted anti-HER2 treatments function by inhibiting intracellular signaling, but also by facilitating antibody-dependent cellular cytotoxicity (ADCC), which relies on both the innate and adaptive immune system [33–35]. Furthermore, anti-HER2 antibodies have been shown to synergize with anti-PD-1/L1 in mammary carcinoma models [36]. A number of trials are evaluating anti-PD-1/L1 with anti-HER2 antibodies +/- chemotherapy. The phase Ib/II PANACEA trial assessed pembrolizumab plus trastuzumab in trastuzumab-resistant advanced HER2+ BC, and showed an overall response rate (ORR) of 15% (90% CI 7–29%) among patients with PD-L1 positive tumors and ORR of 0% in patients with PD-L1 negative tumors [37]. There is an ongoing phase III study evaluating first-line paclitaxel plus trastuzumab plus pertuzumab with or without atezolizumab for metastatic HER2-positive breast cancer (NCT03199885).

Ado-trastuzumab emtansine (T-DM1, Kadcyla) is an antibody-drug conjugate that combines trastuzumab with a potent cytotoxic moiety, emtansine. In preclinical evaluations, T-DM1 potently synergized with ICIs including anti-CTLA-4 and anti-PD-1, resulting in massive T cell infiltration, tumor rejection, Th1 helper T cell polarization, and T regulatory depletion [38]. However, the phase II KATE2 study of trastuzumab-emtansine plus placebo versus atezolizumab in HER-2+ advanced BC failed to demonstrate a clinically significant improvement in PFS (HR 0.82, 95% CI 0.55–1.23, p = .33)[39]. Similar to the PANACEA trial, subjects with PD-L1-positive tumors had numerically higher PFS and ORR [39], indicating that biomarker-driven patient selection may be important for further clinical development of anti-PD-1/anti-HER2 combination approaches in HER2-positive breast cancer.

Additional immunotherapies are being developed to capitalize upon ADCC as a mechanism of tumor cell death. Margetuximab is an anti-HER2 antibody with a genetically enhanced fragment crystallizable (Fc) region that allows for increased FcγRIIIA receptor affinity, which may optimize ADCC-dependent tumor killing by natural killer (NK) cells, particularly in patients with a CD16A low-affinity binding genotype. In a recent phase III trial, margetuximab was associated with modest increases in PFS compared with trastuzumab in HER2+ BC 5.8 mos vs 4.9 mos (HR 0.76, 95% CI 0.59–0.98, p = 0.033), and of comparably greater benefit in patients with a low-affinity CD16A-F allele PFS 6.9 mos vs 5.1 (HR 0.68 95% CI 0.52–0.90, p = 0.005) [40, 41•]. Margetuximab may have unique promise if evaluated in combination with other modulators of ADCC and adaptive immune response. For example, a phase I study of margetuximab plus pembrolizumab is currently in progress (NCT02689284). There are tri-specific antibodies in development that also exhibit enhanced Fc receptor binding in addition to targeting of two surface antigens [42].

Another unique aspect of HER2-positive metastatic breast cancer is the potential for the HER2 protein to serve as a tumor-associated antigen. Nelipepimut-S, also known as E75,

is a 9-amino acid peptide from the extracellular domain of HER2/neu and is capable of eliciting an anti-HER2 immune response. Preclinical data suggested the addition of granulocyte-macrophage colony–stimulating factor (GM-CSF) to nelipepimut (NeuVax) may induce proliferation, maturation, and migration of dendritic cells [43]. Recently, the phase III PRESENT study failed to show benefit of nelipepimut plus GM-CSF for prevention of cancer recurrence in patients with early-stage low-intermediate HER2 positivity [44]. Similarly, a recent phase IIb trial evaluating nelipepimut plus trastuzumab failed to improve disease free survival (DFS) among the intention-to treat (ITT) population (HR 0.62, 95% CI 0.31–1.25, $p = 0.18$); however, there was a significant benefit in the triple negative breast cancer (TNBC) subgroup (HR = 0.26, $p = .013$) [45]. Setbacks in clinical development of short peptide–based vaccines has fueled ongoing investigation into different modes of vaccination, including autologous cells, DNA, and dendritic cell (DC)–based vaccines, as well as ongoing evaluation of combinations with various adjuvant therapies.

Triple Negative Metastatic Breast Cancer

Lack of targets and limitations of standard cytotoxic chemotherapy have prompted exploration of combination immunotherapy strategies in TNBC. In a phase III trial (Impassion130), the addition of atezolizumab to chemotherapy (nab-paclitaxel) was demonstrated to improve PFS and lead to a clinically significant improvement in OS among PD-L1-positive unresectable/metastatic TNBCs (PFS 7.5 v 5.0 mo, HR = 0.62; 95% CI, 0.49 to 0.78; $p < 0.001$), leading to Food and Drug Administration (FDA) approval for this indication [2••]. Interim analysis of the ongoing phase III KEYNOTE-522 trial which evaluates pembrolizumab plus chemotherapy in the neo-adjuvant and the adjuvant settings in TNBC demonstrated improvement in the pathological complete response rate regardless of PD-L1 status [46•]. Additionally, there are several ongoing phase III trials evaluating various chemotherapy backbones combined with anti-PD1/L1 (Table 1). Other emerging targets for combination therapy in TNBC include poly(ADP-Ribose) polymerase 1 (PARP) inhibitors, tyrosine kinase inhibitors, immune co-stimulatory/co-inhibitory antibodies, androgen receptor antagonists, and epigenetic modulators [47]. For more in-depth reading, see article by Kim (this issue).

Deficiencies in homologous recombination correlate with improved response to platinum-based chemotherapy and PARP inhibitors [48–51], particularly in breast cancers with germline/somatic mutations in the BRCA1/2 gene [47]. There is emerging interest in combination ICI with PARP inhibitors [52]. Preclinical data suggests that PARP inhibition upregulates PD-L1 expression in breast cancer cell lines and animal models. Furthermore, blockade of cytotoxic T lymphocyte antigen 4 (CTLA-4)—a T cell co-inhibitory molecule—was shown to be effective in combination with PARP inhibitors in an ovarian cancer BRCA-deficient model [53, 54]. A phase I/II trial of niraparib with pembrolizumab in advanced TNBC has achieved an ORR of 28% and disease control rate (CR/PR or SD ≥ 16 weeks) in evaluable patients of 80% [55]. Several ongoing trials are evaluating PARP inhibition plus ICI (NCT03167619, NCT 03801369, NCT02484404, NCT03330405) in breast cancer.

Emerging Combination Immunotherapy Approaches

A recent systematic review identified 107 molecules targeting 16 immune checkpoint pathways in clinical development in published literature [56•] (Table 2). A comprehensive review of all possible combination approaches is beyond the scope of this paper, but selected combination approaches and mechanisms will be reviewed (Fig. 1).

Combination with Initiators of Immunogenic Cell Death

Immunogenic cell death is a form of cell death sufficient to induce an adaptive immune response through molecular signaling [57]. Damage-associated molecular pattern (DAMP) signaling activates DCs via toll-like receptors (TLRs) and results in initiation of tumor-specific B cell and T cells adaptive responses [58]. Standard approaches for the treatment of breast cancer, including radiotherapy (RT) and chemotherapy, have the ability to induce immunogenic cell death. In a melanoma model, anti-CTLA4 with

Table 2 Next-generation immune modulator pathways classified by cell type and action

	Stimulatory	Inhibitory
Lymphoid	OX-40 GITR 4-1BB (CD137) ICOS	LAG-3 TIM-3 TIGIT Adenosine signaling pathway
Non-lymphoid	PAMP/DAMP receptors CD-40	IDO1 CSF-1/CSF-1-receptor TGF-β CD47/SIRPα Chemokines
Natural killer	KIR-2 IL-15	NKG2A

GITR, glucocorticoid-induced tumor necrosis factor-receptor (TNFR)-related; *ICOS*, Inducible co-stimulator; *LAG-3*, lymphocyte activation gene-3; *TIM-3*, Transmembrane immunoglobulin and mucin domain 3; *TIGIT*, T cell immunoglobulin and ITIM domain; *PAMP*, Pathogen-associated molecular pattern; *DAMP*, Damage-associated molecular pattern; *IDO-1*, Indoleamine-2,3-dioxygenase 1; *CSF-1*, Colony-stimulating factor-1; *TGF-β*, Transforming growth factor-beta; *SIRPα*, Signal-regulatory protein alpha; *KIR2R*, Killer-cell immunoglobulin-like receptor; *IL-15*, Interleukin-15; *NKG2A*, natural killer gene 2A

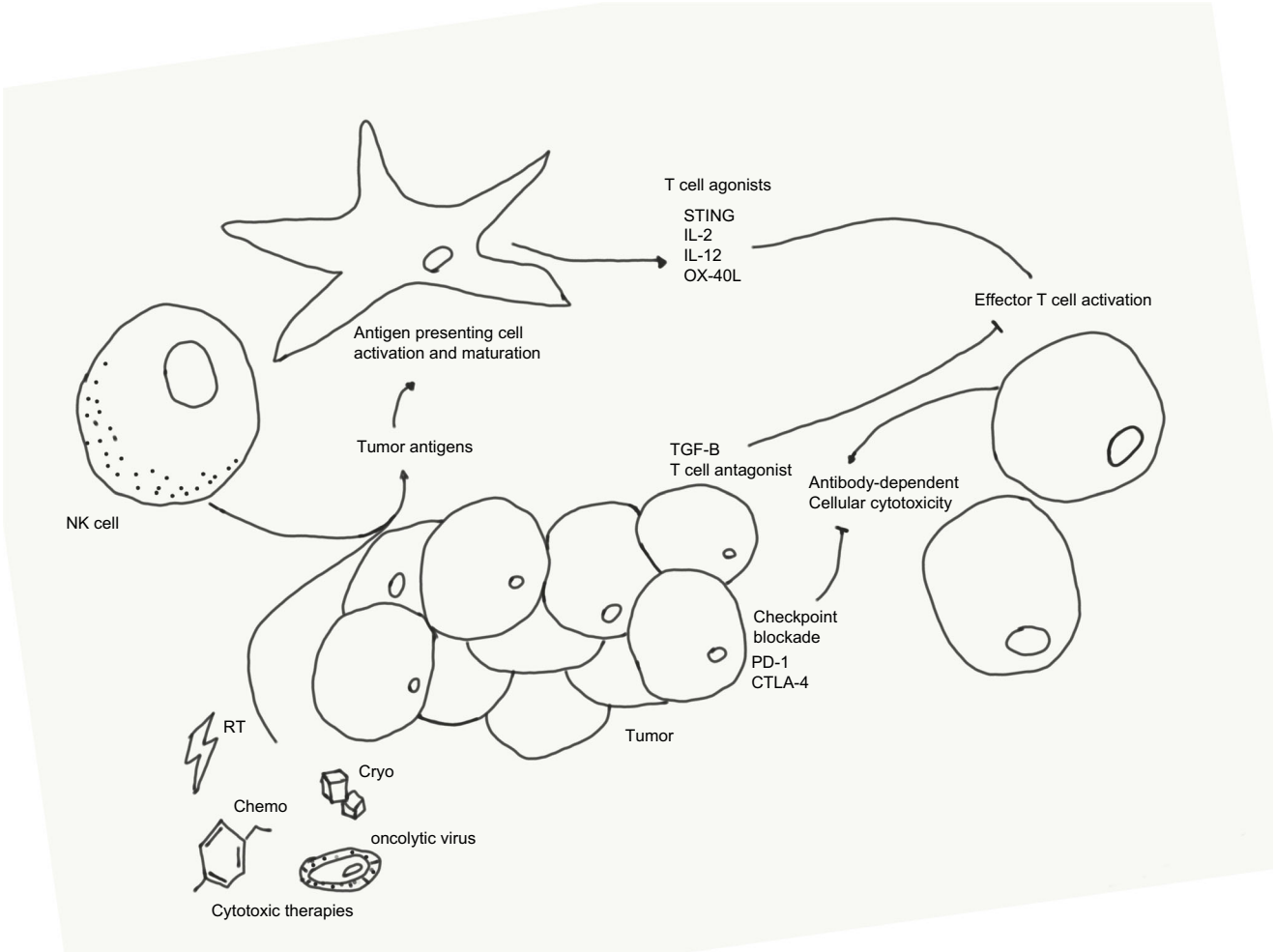

Fig. 1 Illustration of selected mechanisms modulating immunogenic cell death. Cytotoxic therapies such as radiotherapy, cryoablation, chemotherapy, and oncolytic viruses in addition to natural killer (NK) cells induce tumor cell antigen release. Tumor antigens are associated with damage-associated molecular pattern (DAMP), which activates antigen-presenting cells. Subsequent T cell activating signaling by STING pathway, IL-2 and IL-12, and OX-40L induces effector T cell maturation and activation. Effector T cells are capable of antibody-dependent cellular cytotoxicity (ADCC) against tumor cells. Tumors are capable of immune-evasion strategies such as PD-1 and CTLA-4 expression to counter ADCC and TGF-β signaling to suppress effector T cell activation

RT was associated with PD-L1 upregulation, and the addition of anti-PD-L1 reversed T cell exhaustion, promoted clonal T cell expansion within the tumor, and enhanced response [59]. Numerous current trials are ongoing to determine optimal dosing and schedule of RT for immunogenic purposes. Cytotoxic chemotherapy has immune-modulatory effects such as expanding or activating NK cells, DC cells, and T cells; depleting tumor-associated macrophages, myeloid derived suppressor cells, T-regs, and IFNγ and PD-L1 upregulation [60–63]. Since the FDA approval of atezolizumab with nab-paclitaxel, this has become a robust area of research, with several phase III trials assessing utility of dual chemotherapy with ICI (Table 1).

Dual Co-inhibition

Dual ICI co-inhibition with anti-CTLA-4 plus anti-PD-1/L1 is associated with improvements in PFS and OS in melanoma, and there is preclinical data to support its use in breast cancer [64]. A pilot trial of durvalumab (anti-PD-L1) plus tremelimumab (anti-CTLA-4) in metastatic breast cancer resulted in an ORR of 17% with 0% ORR among ER-positive patients, but an ORR of 43% among TNBC patients, suggesting patients with TNBC, may be more likely to benefit [65]. There are several phases I and II studies in breast cancer utilizing dual ICI (see Table 1). One legitimate concern is toxicity, with a recent analysis of melanoma/renal cell carcinoma trials demonstrating increased efficacy but a near doubling of grade 3–4 toxicity compared with single-agent ICI [66, 67••, 68, 69]. Retrospective analyses have demonstrated that responses to dual ICI may persist well beyond treatment discontinuation related to toxicity [70•]. A number of guidelines have been published to guide clinicians on how to effectively manage immune-related toxicities. Clinical trials are ongoing to evaluate whether toxicities could be mitigated by reducing

Table 3 Selected vaccine/oncolytic combination trials in metastatic breast cancer

Vaccine agent	Combination agent	Phase of study	NCT identifier
Oncolytic virus–based vaccine			
T-VEC	Atezolizumab	Phase I	NCT03256344
T-VEC	Paclitaxel	Phase I/II	NCT02779855
T-VEC		Phase II	NCT02658812
T-VEC	Paclitaxel or endocrine therapy	Phase I	NCT03554044
Dendritic cell–based vaccine			
Tumor blood vessel antigen	Gemcitabine	Phase I	NCT02479230
Tumor cell–based vaccine			
SV-BR-1-GM (GM-CSF secreting line)	Pembrolizumab	Phase I/II	NCT03328026
Peptide-based vaccines			
PVX-410 (XBP1, CD138, CS1)	Pembrolizumab	Phase I	NCT03362060
HER2 intracellular domain	Polysaccharide-K + pertuzumab or trastuzumab	Phase I/II	NCT01922921
Personalized synthetic long peptide	Nab-paclitaxel + durvalumab	Phase II	NCT03606967
LTX-315 (oncolytic peptide)	Ipilimumab or pembrolizumab	Phase I	NCT01986426
Carbohydrate-based vaccines			
Globo H carbohydrate antigen	Cyclophosphamide	Phase II/III	NCT01516307

T-VEC, Talimogene laherparepvec; *GM-CSF*, granulocyte-macrophage colony-stimulating factor; *XBP1*, X-box binding protein 1; *CS1*, Cyclin D3 (CCND3) subset 1

dosing and/or frequency of anti-CTLA-4 [71]. Additionally, there exist strategies to block tumor cell evasion by targeting alternative immune checkpoints such as TIM-3, LAG-3, and BTLA-4. (Table 2)

To date, the majority of clinically investigated ICIs target T cells, yet there are additional cell types that may facilitate anti-tumor immunity, each with targetable co-inhibitory molecules. For example, CD47 is expressed on tumor cells and interacts with signal regulatory protein alpha on macrophages to trigger a "don't eat me" signal [72]. CD47 is an innate immune checkpoint whose overexpression correlates with poor prognosis [73]. Targeting the CD47 protein may be relevant in combination with anti-HER2 antibody therapy or other antibody-based therapies, as blockade of CD47 may enhance antibody-mediated phagocytosis of tumor cells via ADCC. In a phase I study, an anti-CD47 molecule (ALX148) was safely combined with trastuzumab, and was associated with an ORR of 22% among trastuzumab-resistant gastric cancers [74]. Although CD47 signaling involves innate immune cells, murine models also suggest that CD47 blockade improves CD8+ T cell response [75, 76]. ALX148 was also safely combined with pembrolizumab (anti PD-1), with encouraging activity in non-small cell lung cancer and head/neck squamous cell cancer cohorts [77].

A number of antibody-drug conjugates are being developed in breast cancer, such as trastuzumab-deruxtecan (DS-8201a) which is a novel drug-antibody combination which pairs anti-HER targeting with a topoisomerase-inhibitor. There are two ongoing phase Ib trials assessing DS-8201a in combination with ICI in mBC: one with pembrolizumab (NCT04042701) and one with nivolumab (NCT03523572). Sacituzumab govitecan combines Trop-2 targeting with a topoisomerase-inhibitor and was shown to be active in pretreated TNBC, with an ORR of 33.3% (95% CI 24.6–43.1) and median duration of response 7.7 months (95% CI 4.9–10.8) [78]. There are no reported preclinical or clinical trials evaluating the combination of sacituzumab with ICIs or other immunotherapies in breast cancer; however, this is an active area of interest.

Bispecific dual immunomodulators combining two inhibitory functions are being explored [79]. Ongoing is a phase 1 trial of XmAb20717, a combined PD-1 x CTLA-4 antibody in selected advanced solid tumors (NCT03517488). Its safety data will be reviewed with interest as combinations of anti-PD-1/L1 plus anti-CTLA-4 are known to be more toxic than monotherapy. LAG-3 is a surface molecule which binds to major histocompatibility complex II (MHCII) on antigen presenting cells, and may serve to block T cells from binding MHCII and becoming activated. [80] A number of antibodies against LAG-3 are in development, as well as bispecific antibodies that engage both LAG-3 and PD-1/L1. (NCT03219268, NCT03440437).

Co-stimulation and Co-inhibition Combination Approaches

There exist numerous co-stimulatory targets including the tumor necrosis factor receptor (TNFR) family members OX40, 4-1BB, and GITR [81–84]. OX40 is expressed on CD4+ and CD8+ T cells and when ligated, has the ability expand T cells, improve T cell effector function, improve T cell memory, and facilitate

tumor clearance [85]. In mammary carcinoma models, anti-OX40 plus anti-PD-L1 was more effective than monotherapy in inducing regression [86, 87], and was associated with increases in tumor-specific T cells. [86] In an independent study, anti-OX40 plus anti-CTLA-4 plus HER2 vaccine seemed to reverse T cell anergy, enhance CD8+ T cell effector function, and increase longevity of memory T cell response [88]. A bispecific antibody targeting CTLA-4 and OX40 (ATOR-1015) has demonstrated efficacy in tumor models and is being tested in a phase 1 trial (NCT03782467) [89]. Timing of PD1/L1 blockade may be crucial for the efficacy of combination therapy. For example, in mammary carcinoma models, sequential administration of OX-40 followed by anti-PD1 was more effective than monotherapy, whereas concurrent blockade was not effective [90, 91], and was associated with high levels of peripheral cytokine production. Anti-OX40 has also been combined with radiation in a phase I trial (NCT01862900) which provided stereotactic body RT to metastatic lesions in the liver or lung with aOX40-mAb in metastatic breast cancer [92]. These targets may be more effective when combined with modulators of innate immunity, such as with ligands of the DNA-sensing stimulator of interferon genes (STING) pathway [93]. STING protein is expressed in multiple cell types including macrophages, T cells, DCs, and can trigger an anti-cancer immune response [94]. In a mouse model, STING signaling improved tumor clearance in combination with anti PD-1 and anti-OX40 [93].

Tumor Microenvironment Modulation

Cytokines may exhibit inhibitory or stimulating effects and, therefore, can be therapeutically targeted [95, 96]. One example TGFβ, a multipotent cytokine which is described above in the context of hormone-sensitive breast cancer. Other cytokines being evaluated with anti-PD-l/L1 in breast cancer include intratumoral IL-12 and pegylated IL-2. IL-12 is a potent inflammatory cytokine that induces IFNγ production and Th1 T cell response, but is too toxic for systemic administration. In a mouse model, intratumoral IL-12 was associated with improved antitumor responses when delivered in combination with anti-PD-1 [97]. A pilot study has been conducted in metastatic TNBC whereby intratumoral IL-12 plasmid was safely administered by electroporation, and was associated with increases in TIL count by immunohistochemistry (from mean 3 to 11% by day 28 of treatment, vs 5% in untreated tumors by day 28) [98]. A phase II study combining intratumoral plasmid IL-12 with pembrolizumab is in progress [99].

IL-2 is a central factor for orchestrating an anti-tumor immune response, and is associated with activation and proliferation of both CD8+ and CD4+ T cells. Systemic administration is FDA-approved for the treatment of metastatic melanoma and renal cell carcinoma (RCC); however, the therapy is toxic with a narrow therapeutic window and requires inpatient administration. The pegylated IL-2 prodrug, NKTR-214, is associated with prolonged half-life and favorable pharmacokinetics, enabling outpatient administration and reduced toxicity. NKTR-214 was effective in combination with ICI in both melanoma and mammary carcinoma models [100]. In a phase I study, NKTR-214 was shown to be well-tolerated relative to systemic IL-2 in a phase I study (grade III adverse event rate of 18%), with preliminary signals of clinical activity in melanoma/RCC [101]. The combination of NKTR-214 and nivolumab is currently being assessed in phase I and II studies in breast cancer (NCT02983045, NCT03435640).

Antigenic Cell Death: Radiation Therapy and Cryoablation

The abscopal effect, whereby the immune system creates a robust response against distant metastases following local treatment, powerfully illustrates the concept of immune-mediated cell death [102]. Radiotherapy and cryotherapy are well-established locally focused anti-cancer treatments which are being combined with immunotherapy in attempt to enhance known immunogenic effects against cancer [103]. The combinations of RT with immunotherapy and cryoablation with immunotherapy face hurdles to development such as establishing effective dosing, the optimal number of treatments, timing of intervention, and optimal immunotherapeutic combination agent.

There are numerous clinical trials investigating RT with ICI for treatment of breast cancer. In the mTNBC setting, a single-arm phase II study assessing the combination of RT with pembrolizumab demonstrated a partial response of 33% in 9 of 17 patients eligible at 13 weeks, 11% with stable disease in patients unselected for PD-L1 expression [104]. In contrast, a single-arm phase II study in HR+/HER2- mBC evaluated RT with pembrolizumab in the palliative setting and did not demonstrate a clinical benefit, though reported no unexpected adverse events [105]. Ongoing are several other studies including a pilot trial investigating the combination of preoperative pembrolizumab and RT boost prior to standard of care surgery and adjuvant RT (NCT03366844). In metastatic breast cancer, a combination of brain radiation with tremelimumab and durvalumab is being assessed in a pilot phase for patients with mBC with intracranial involvement (NCT02563925), and a phase II study is currently evaluating the efficacy of pembrolizumab with RT in mTNBC and high-risk hormone-positive disease (NCT02730130).

The possible permutations of RT with existing immunotherapies are vast, and combinations of RT with PARP inhibition, OX-40 signaling, TGF-β inhibition, and vaccines among others which will be discussed in a separate review in more detail (this issue).

Several existing trials of cryotherapy in combination with immunotherapy in local non-metastatic cancers offer implications for future treatment of metastatic disease. A pilot study utilizing cryoablation with ipilimumab demonstrated safety in women with operable breast cancer [106]. A phase II randomized study of perioperative ipilimumab, nivolumab, and cryoablation versus standard care in resectable TNBC following standard of care neoadjuvant therapy is ongoing [107]. The number of cryoablative treatments and timing for optimal outcome needs to be established; in one retrospective observational study of patients with metastatic breast cancer, multiple cryoablations were associated with greater median OS compared with single cryoablations (76 months vs 48 months, $p = 0.0005$) [108].

Antigen Delivery and Antigenic Cell Death: Vaccines and Oncolytic Viruses

Vaccines may be in the form of peptides, carbohydrates, organelles, and cells. They have the potential to enact powerful effector functions, or alter the tumor microenvironment to support an immune response. Critical to the success of vaccines is the proper selection of antigen, vector, adjuvant, route, dose, and schedule [58]. Combinations of vaccines with radiotherapy and chemotherapy are ongoing areas of research and covered in more detail in accompanying reviews (this issue). In addition to HER2-directed vaccines, another promising target is the mannose receptor, as shown in a phase II study of oxidized mannan-MUC1 which demonstrated encouraging reductions in recurrence rate (12.5% versus 60%) in a small study [109]. The multivalent poxviral–based cancer vaccine, PANVAC, which targets CEA and MUC1 and also contains genes for costimulatory molecules B7.1, ICAM-1, and LFA-3, showed a numerically increased PFS of 7.9 months vs 3.9 months when combined with docetaxel (HR 0.65, 95% CI 0.34–1.14, $p = 0.09$) [110].

Cell-based vaccines can induce broad activation of the immune system, and decreased resistance of tumor cells. SV-BR-1-GM (GVAX) is composed of tumor cells transfected with the GM-CSF gene. These cells over-express genes encoding tumor-associated antigens, and express MHC II and other immunostimulatory proteins which facilitate a coordinated anti-tumor response [111]. In a phase I study, GVAX was associated with regression of distant metastases [111, 112]. GVAX is currently in phase II trials (NCT03328026) in combination with pembrolizumab.

Proteasome inhibition thus poses an attractive target by which to enhance the accumulation of misfolded protein, which triggers an unfolded protein response and leads to cell cycle arrest and apoptosis, an approach that is promising in breast cancer models [113–115]. A phase II trial of 12 patients did not show any benefit against mTNBC as monotherapy [116]. However, a small study demonstrated a trend of improved PFS in hormone-sensitive breast cancer when combined with fulvestrant (PFS 5.4 v. 9.0 months, HR 0.73, 95% CI 0.49–1.09 $p = 0.06$) [117]. Inhibition of the proteasome can also been explored as a method of generating vaccines against intracellular proteins that are otherwise sequestered from antigen presentation via the autophagy process [118]. An ongoing study is evaluating an autophagy-based vaccine in combination with anti-PD-1 and anti-OX40 in metastatic TNBC (NCT02737475).

Oncolytic viruses have been approved for use in melanoma. Talimogene laherparepvec (T-VEC) is an HSV-1 virus modified to replicate in tumor cells and express GM-CSF to increase tumor-antigen presentation by dendritic cells. T-VEC is currently being evaluated in breast cancer in combination with anti-CTLA4, chemotherapy, or endocrine therapy (Table 3). CVA21 (CAVATAK) is a coxsackie-based oncolytic virus which adheres to ICAM-1 in order to enter a cell, then eventually lyses the cell releasing more viruses which can perpetuate cell lysis [119]. In mammary carcinoma models, CAVATAK in combination with doxorubicin resulted in synergistic cell death [120]. A non-viral oncolytic strategy utilizing the peptide LTX-315 is studied in a phase I trial in combination with ipilimumab or pembrolizumab (NCT01986426).

Conclusion

Combination immunotherapy strategies are intriguing as they may generate a more complete and durable response against tumors. However, at this point, the field is dominated by preclinical data and phase I evaluations. The sheer number of possible combination approaches is daunting and presents a unique challenge for the future of drug development. Novel adaptive clinical trial designs will hopefully enable more efficient screening of these combination approaches, as well as the development of next-generation biomarkers that will allow us to personalize combination immunotherapy according to the biological characteristics of a patient's tumor and pre-existing immune response.

Compliance with Ethical Standards

Conflict of Interest David Page reports personal fees from Genentech; grants and personal fees from Merck; personal fees from Novartis; personal fees from Puma; personal fees from Nanostring; personal fees from Nektar; personal fees from Syndax; grants and personal fees from Brooklyn Immunotherapeutics; and grants and personal fees from Bristol Myers-Squibb outside the submitted work. Heather McArthur has consulted for Amgen, Bristol-Myers Squibb, Celgene, Eli Lilly, Genentech/Roche, Immunomedics, Merck, OBI Pharma, Pfizer, Puma, Spectrum Pharmaceuticals, Syndax Pharmaceuticals, Peregrine, Calithera, and TapImmune. Dr. McArthur also has research supported by Bristol-Myers Squibb, MedImmune, LLC/AstraZenica, and Merck. Brie Chun declares no conflicts of interest relevant to this manuscript.

Human and Animal Rights and Informed Consent This article does not contain any studies with human or animal subjects performed by any of the authors.

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Papers of particular interest, published recently, have been highlighted as:
- • Of importance
- •• Of major importance

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