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REVIEW

Novel classes of immunotherapy for breast cancer

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Abstract

Immune-checkpoint inhibitors have profoundly changed the treatment landscape for many tumor types. Despite marked improvements in disease control for highly immunogenic cancers, the clinical impact of checkpoint inhibitors in breast cancers to date is limited. Breast cancer is a heterogeneous disease with different levels of PD-L1 expression and variable tumor microenvironment (TME) composition according to molecular subtype. With emerging evidence of the role of different factors involved in immune evasion, there are promising new immunotherapy targets that will reshape early drug development for metastatic breast cancer. This review examines the available evidence for existing and emerging immuno-oncology (IO) approaches including small molecules targeting different regulators of the cancer-immunity cycle.

Keywords Breast cancer · Immunotherapy · Drug development

Introduction

Immune-checkpoint inhibitors (ICIs) that target programmed cell death protein-1 (PD-1) or its ligand (PD-L1), as well as cytotoxic T-lymphocyte antigen 4 (CTLA-4) are now approved to treat multiple tumor types [1–5]. However, the overall response rate (ORR) as monotherapy in pre-treated metastatic breast cancer with PD-1 or PD-L1 ICI monotherapy is generally less than 20% [6, 7]. Breast cancer has historically been considered a non-immunogenic tumor [8]. Accordingly, the prevalence of predictive biomarkers of IO sensitivity, such as PD-L1 expression in tumor and/or immune cells and tumor mutation burden (TMB) is low, with differences according to molecular subtype, testing based on primary versus metastatic tumor tissue and assay characteristics [9–11].

Many factors are linked to the immunological quiescence observed in breast cancer, suggesting that more extensive biomarker profiling beyond TMB, tumor infiltrating lymphocytes (TILs) density and PD-L1 expression may be required to individualize immunotherapy treatments. Immunosuppressive cytokines including IL-6, IL-8 and TGF-β and tumor epithelial-to-mesenchymal transition (EMT) may have a role in tumor progression, metastatic spread, and immune resistance. Furthermore, different cells such as tumor-associated M2 macrophages, regulatory T (Treg) and B cells, myeloid derived suppressor cells (MDSC), or tumor-associated fibroblasts may also play a role in immune evasion [8]. Altogether, this may explain the limited response rates observed with single agent ICI in metastatic triple negative breast cancer (mTNBC). Immunotherapy combinations targeting different immunosuppressive elements within the TME may expand clinical responses to ICIs in metastatic breast cancer [12].

Thus far, PD-1 or PD-L1 ICIs in combination with chemotherapy are approved for the first-line treatment of PD-L1 expressing mTNBC. IMpassion130 showed a progression free survival (PFS) improvement in the intention-to-treat population for nab-paclitaxel and atezolizumab as compared to chemotherapy alone. Although a numerically higher median overall survival (OS) was observed among patients with PD-L1 positive tumors by immunohistochemistry, no significant differences were observed in OS in the intention-to-treat population [13, 14]. Notably, the recently reported negative results of the combination of atezolizumab and paclitaxel in the IMpassion131 raises the question of the best chemotherapy backbone to use with ICIs [15]. KEYNOTE-355 showed a PFS improvement for the combination of pembrolizumab plus either paclitaxel, nab-paclitaxel, or gemcitabine plus carboplatin

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as compared to placebo-chemotherapy in mTNBC patients with a PD-L1 immunohistochemistry combined positive score (CPS) ≥ 10% (CPS ≥ 10%) [16].

While these results are an important advance for patients with PD-L1 positive mTNBC, there are many mTNBC patients who do not benefit from ICI monotherapy or ICI+ chemotherapy. This situation, together with the fact that most patients with mTNBC receive poly-agent chemotherapy in the (neo)-adjuvant setting, has motivated the development of clinical trials testing ICIs in combination with molecularly targeted therapies to improve efficacy [13, 16]. There is current enthusiasm in the field for PARP inhibitor and ICIs combinations based upon promising early phase data for patients with germline BRCA1/2 mutations [17]. Larger studies are ongoing to examine PARP inhibitor and ICIs combinations as maintenance treatment in mTNBC [18, 19]. Androgen blockade has additionally been a focus of attention due to the negative role of the androgen pathway in T-cell activation [20–22]. Examples of combination treatments with androgen blockade and immunotherapy include (NCT03650894) and (NCT02971761). The PI3K/AKT/mTOR pathway plays a central role in the biology of specific molecular subtypes of TNBC [23, 24]. Moreover, the activation of this axis leads to recruitment of MDSCs, Tregs, and PD-L1 upregulation [25, 26]. IPI-549 is an inhibitor of the phosphoinositide-3-kinase (PI3K)-gamma that is currently being evaluated in addition to atezolizumab and nab-paclitaxel for patients with mTNBC [27]. Investigation of the AKT inhibitor ipatasertib in combination with chemotherapy, with or without atezolizumab was also pursued in mTNBC. Despite promising results in the Phase 1b (NCT03800836) with an ORR of 73% for the combination of ipatasertib, atezolizumab, and chemotherapy, the Phase III Ipatunity 170 (NCT04177108) did not reach its primary endpoint and has been terminated [28]. Similarly, the BEGONIA trial is currently studying the role of capivasertib, a small molecule inhibiting AKT, in combination with durvalumab and paclitaxel [29]. Another potential partner for ICI combinations are MEK inhibitors. In preclinical models, MEK inhibition has been shown to increase the cytotoxic effects of paclitaxel [30]. Furthermore, Ras/MAP pathway activation has been associated with immune evasion in mTNBC supporting combination strategies with ICIs [31]. Despite the preclinical rationale for combination testing, the COLET phase 2 trial did not show additive effect for the addition of cobimetinib to paclitaxel as compared to paclitaxel single agent or an increase in ORR for the combination of atezolizumab and cobimetinib with either paclitaxel or nab-paclitaxel [32, 33]. Lastly, different trials are studying the potential additive effects of antibody drug conjugates (ADCs) and ICIs combinations. In this respect, the initial results from the arm 6 of the BEGONIA trial testing durvalumab in combination with trastuzumab deruxtecan, were recently presented. Confirmed responses were observed in 8/12 evaluable patients [34]. Sacituzumab govitecan, an ADC against the Trop-2 antigen and ladiratuzumab vedotin in combination with pembrolizumab are currently being tested for mTNBC in two clinical trials (NCT04468061) and (NCT03310957).

The estrogen receptor (ER) positive (ER+)/HER2-negative (HER2−) and HER2-positive (HER2+) subtypes account for up to 90% of BC and lack effective FDA-approved immunotherapies [35]. An increased understanding of the specific genomic and molecular pathways associated with these subtypes has enabled the development of different immunotherapy combinations. To date, the efficacy of HER2-targeted therapies in combination with ICIs for metastatic HER2+ BC has been limited [36–38]. For ER+/HER2− breast tumors, lower responsiveness to IO agents have been observed as compared to mTNBC which could be secondary to lower TIL infiltration, lower values of TMB and PD-L1 expression [39–42]. While ICI+ chemotherapy is being investigated in ER+, disease specific treatment approaches in this molecular subtype include combinations of ICIs with either endocrine therapy or cyclin dependent kinase 4/6 (CDK-4/6) inhibitors [43–46].

All these examples suggest that more in-depth understanding of the molecular underpinnings leading to immune evasion in breast cancer may lead to the development of co-inhibitory or co-stimulatory agents that in addition to ICIs may help to overcome intrinsic immune resistance [8]. Selected results of clinical trials involving ICIs in combination with other targeted agents in later phases of development are summarized in Table 1 and are not the focus of this review. Herein, we describe emerging IO approaches in breast cancer, including novel therapeutic strategies based on the PD-1/PD-L1 axis and small molecules targeting different points of the cancer-immunity cycle in early stages of drug development.

Novel immunotherapies as single agents or in combination with ICIs

Drugs targeting co-inhibitory or co-stimulatory pathways

Co-inhibitory immune pathways

Anti-tumor responses are regulated by both stimulatory and inhibitory pathways that dictate antigen recognition by T-cells. Theoretically, the blockade of existent co-inhibitory pathways in the tumor and TME may enable responses in non-immunoreactive tumors [47]. In addition to PD-1/PD-L1 and cytotoxic T-lymphocyte-associated antigen 4 (CTLA4), other inhibitory molecules are being explored as potential

Table 1 Selected results of clinical trials involving ICIs in combination with targeted agents

Therapeutic class	NCT ID	Description	Phase	Primary endpoint	Status	Results
ICI combinations						
Endocrine therapy + IO						
	NCT02395627	Vorinostat, tamoxifen and pembrolizumab in ER+ breast cancer	II	ORR and safety	Terminated	ORR = 4%; TEAES G3/4 = 11
	NCT03650894	Nivolumab, ipilimumab and bicalutamide for HER2− breast cancer patients	II	Clinical benefit rate	Active	n/a
	NCT02971761	Pembrolizumab and enobosarm for androgen receptor positive mTNBC	II	ORR and safety	Active not recruiting	n/a
	NCT03280563	Fulvestrant and atezolizumab for HER2−/HR+ breast cancer (MORPHEUS Experimental arm 2)	I, II	ORR	Active	n/a
	NCT03393845	Pembrolizumab plus fulvestrant in HER2−/HR+ breast cancer	II	ORR	Active	n/a
CDK inh. + IO						
	NCT02779751	Study of abemaciclib combined with pembrolizumab in patients with NSCLC or HR+/HER2− breast cancer	I	Safety	Active not recruiting	n/a
	NCT02778685	Pembrolizumab, letrozole, and palbociclib in postmenopausal patients with metastatic ER+ breast cancer	II	ORR	Active	n/a
Anti-HER2+ IO						
	NCT02129556	Pembrolizumab and trastuzumab in trastuzumab resistant advanced HER2+ breast cancer (PANACEA)	Ib,II	Safety (Ib). ORR (Phase II)	Completed	ORR = 15% (PD-L1 +) and 0% (PD-L1 -); AES (≥ G3) = 50%
	NCT02649686	Durvalumab and trastuzumab in HER2+ metastatic breast cancer (CCTG IND.229)	Ib	Safety	Completed	ORR = 0%; No dose limiting toxicities
	NCT02924883	Trastuzumab emtansine plus atezolizumab vs trastuzumab placebo in HER2+ (KATE2)	II	PFS	Completed	PFS HR = 0.82 (0.55–1.23)

Table 1 (continued)

Therapeutic class	NCT ID	Description	Phase	Primary endpoint	Status	Results
	NCT02605915	Atezolizumab with trastuzumab emtansine or trastuzumab+ pertuzumab in HER2+ breast cancer (GO29831)	Ib	Safety	Completed	n/a
	NCT04042701	DS8201 and pembrolizumab in participants with metastatic breast cancer	I	Safety and ORR	Active	n/a
PARP inhibitor + IO						
	NCT02734004	Olaparib and durvalumab for germline BRCA-mutated breast cancer (MEDIOLA)	I, II	Safety and ORR	Active not recruiting	ORR = 63.3%; AES (≥ G3) = 12%
	NCT02657889	Niraparib and pembrolizumab in patients with mTNBC (TOPACIO/KEYNOTE-162)	I, II	Safety and ORR	Active not recruiting	ORR = 29%; AES (≥ G3) = 50%
	NCT02849496	Olaparib with or without atezolizumab in patients with HER2- metastatic breast cancer	II	PFS	Active	n/a
PI3K/AKT pathway + IO						
	NCT03961698	Eganelisib in combination with atezolizumab and nab-paclitaxel (MARIO-3)	II	Complete response rate	Active	n/a
	NCT03800836	Ipatasertib in combination with atezolizumab and either paclitaxel or nab-paclitaxel in mTNBC	I	ORR	Active not recruiting	n/a
	NCT04177108	Ipatasertib in combination with atezolizumab and paclitaxel in untreated mTNBC (Ipatunity 170)	III	PFS and OS	Terminated	n/a
	NCT03742102	Novel anti-cancer agents in patients with mTNBC (BEGONIA). Arm 2: durvalumab + paclitaxel + capivasertib	I,II	Safety and ORR	Active	n/a

Table 1 (continued)

Therapeutic class	NCT ID	Description	Phase	Primary endpoint	Status	Results
MAPK/ERK pathway + IO						
	NCT02322814	Cobimetinib plus paclitaxel or atezolizumab + paclitaxel or atezolizumab + nab-paclitaxel in mTNBC	II	PFS (Cohort 1) and ORR (Cohort 2–3)	Active not recruiting	n/a
ADC + IO						
	NCT03742102	Novel anti-cancer agents in patients with mTNBC (BEGONIA). Arm 6: durvalumab + trastuzumab deruxtecan	Ib,II	Safety and ORR	Active	ORR = 66.7%; AES (≥ G3) = 38.1%
	NCT04468061	Sacituzumab govitecan ± pembrolizumab in mTNBC	II	PFS	Active	n/a
	NCT03310957	SGN-LIV1A and pembrolizumab for patients with mTNBC	I	ORR and safety	Active	n/a

TEAES treatment emergent adverse events; *AES* adverse events

targets in breast cancer. Indoleamine 2,3 dioxygenase 1 (IDO) is a tryptophan (Trp)-catabolizing enzyme released by both MDSC and tumor cells negatively impacting in T-cell function [48]. Pembrolizumab in combination with the IDO1 inhibitor epacadostat was tested in mTNBC where a 10% ORR was observed, not substantially different from the expected pembrolizumab monotherapy response rate [49].

CD73 is a cell surface enzyme that is expressed in TNBC and is associated with chemotherapy resistance, metastatic potential, and T-cell impairment. CD73 releases adenosine acting as a potent immunosuppressor in the TME [50–52]. There are ongoing studies evaluating CD73 inhibitors for mTNBC (NCT03454451, NCT04148937, and NCT03549000). Adenosine can increase in the TME due to the action of CD73. Subsequently, activation of the adenosine A2A receptor (A2AR) can eventually lead to inhibition of the effector function of NK and T-cells [53–57]. CPI-444 is an adenosine A2A receptor inhibitor currently under investigation as single agent or with atezolizumab in selected solid tumors including mTNBC [58]. Also, the phase I/II SYNERGY trial is evaluating the safety and efficacy of the combination of chemotherapy (paclitaxel + carboplatin) with durvalumab ± the anti-CD73 antibody oleclumab for patients with mTNBC [59]. Additionally, for early-stage luminal B breast cancer, oleclumab in addition to chemotherapy and radiotherapy is being investigated in the neoadjuvant setting prior to surgery [60]. Other A2AR inhibitors under investigation in TNBC include NIR178 and IPI-549 (NCT03742349 and NCT03719326, respectively).

Lymphocyte-Activation Gene 3 (LAG-3) is a marker of exhausted $CD8^+$ T-cells [61, 62], which has generated substantial interest. LAG-3 binds to MHC Class II with more affinity than $CD4^+$, acting as a negative regulator for T-cell expansion and promoting Treg function [63, 64]. The co-expression of both PD-L1 and LAG-3 in TILs for metastatic breast cancer supports the rational for treatment combinations [62]. Ongoing phase I clinical trials are evaluating anti-PD-1/L1 ICIs combined with LAG-3 inhibitors (NCT03250832). LAG525 (anti-LAG-3) is being explored in combination with the anti-PD-1 spartalizumab and carboplatin or with carboplatin alone for mTNBC (NCT03499899). A soluble dimeric recombinant form of LAG-3 (IMP321) is being explored in addition to standard chemotherapy in subjects with hormone receptor positive (HR+) metastatic breast cancer [65]. Additionally, the I-SPY trial is investigating the anti-LAG-3 REGN3767 with the anti-PD-1 cemiplimab in the neoadjuvant setting for operable breast cancer (NCT01042379).

TIGIT is a receptor of the Ig superfamily that when activated downregulates both adaptive and innate immune responses [66]. The phase I trial (NCT03628677) is investigating AB154 (domvanalimab) a monoclonal antibody targeting TIGIT alone or in combination with zimberelimab (anti-PD-1). B7-H4 is a PD-L1 family member that is also

being tested as a rational partner for checkpoint inhibition. FPA150 is an anti-B7H4 antibody currently under investigation [67]. TIM-3 is an inhibitory receptor that binds to galectin-9 negatively impacting in IFN-gamma production by CD4+ T-cells. Preclinical data have shown synergistic effects by combining anti-PD-1 and TIM-3 blockade [68]. The phase I clinical trial (NCT02608268) studied the combination of MBG453 and spartalizumab in patients with solid tumors. The combination was well tolerated, but with an ORR of only 5% [69].

Co-stimulatory immune pathways

Multiple co-stimulatory molecules regulate T-cell responses against cancer cells [70]. Immunotherapy combinations including ICIs and agents providing agonistic signals through co-stimulatory molecules could re-establish immune surveillance [47]. OX40 and 4-1BB are members of the TNF receptor superfamily which contributes to T-cell activation and survival [71]. OX40 agonists in combination with anti-PD-1/PD-L1 agents are under investigation for mTNBC (NCT03971409). 4-1BB (also known as CD137) is a co-stimulatory immune checkpoint expressed in T-cells. The interaction with its ligand protects $CD8^+$ T-cells from apoptosis, enhances effector functionalities and promotes memory cell differentiation ultimately leading to enhanced anti-tumor responses. Urelumab is a monoclonal antibody currently being tested in early phase clinical trials as single agent (NCT00309023) or in combination with nivolumab (NCT02253992 and NCT02534506) [72]. PRS-343 is a HER2/4-1BB bispecific construct that was investigated in a phase I clinical trial (NCT03330561) involving refractory HER2 + breast cancer. Treatment with PRS-343 was well tolerated with a 11% ORR [73].

STING (stimulator of interferon genes) has been identified as an important mediator of immune response, and STING agonists have been developed for the treatment of solid tumors. In breast cancer, STING agonists enhance innate and adaptive immune responses by driving the production of cytokines such as Type I interferons [74, 75]. Until now, mainly intratumoral administration of STING agonists has been explored given ubiquitous expression of cGAS/STING, narrow therapeutic index, and poor metabolic stability [76]. Intratumoral vaccination with 2′3′-cGAMP led to accumulation of macrophages and repolarization toward a M1 phenotype in preclinical models [77]. The phase I clinical trial CA046-006 (NCT03956680) is investigating the combination of an intratumoral STING agonist with nivolumab and ipilimumab in patients with solid tumors [78], including breast cancer. Additionally, ongoing Phase 1 trials have begun to explore intravenous STING agonists in different solid tumors including breast cancer (NCT04420884).

Dectin-1 is a pattern recognition receptor expressed in dendritic cells. Dectin-1 activation is fundamental for NK-mediated killing of tumor cells and polarization of $CD4^+$ T-cells into Th9 cells [79]. Mechanistically, Th9 cells secrete interleukin (IL)-9 improving $CD8^+$ T-cells responses [80]. Imprime PGG is an agonist of the dectin receptor. The IMPRIME 1 trial studied pembrolizumab and Imprime PGG in patients with mTNBC following progression on at least two prior lines of treatment. Mechanistically, Imprime PGG requires anti-beta glucan antibodies (ABA) to enhance anti-tumor immunity. Therefore, eligibility was restricted to patients with ABA IgG ≥ 20 mcg/ml. The ORR was 15.9% and the median OS was 16.4 months. Intriguingly, there appeared to be greater benefit for patients who were initially HR+ with 50% ORR, though given the small sample size and clinical heterogeneity of the cohort, the implications of this finding remain to be further characterized [81].

Oncolytic viruses

Oncolytic viruses are a promising approach to enhance T-cell infiltration. Oncolytic viruses are specifically engineered to replicate and lyse tumor cells minimizing their effects on normal tissues. Thus, expected effects include increasing T-cell priming, homing, and antagonism of immunosuppressive signals in the TME. Various non-pathogenic viruses have been employed for this purpose [82, 83]. Talimogene Laherparepvec (T-VEC) is a human herpes virus that induces the expression of colony-stimulating factor (GM-CSF) through viral replication in cancer cells producing anti-tumor immune responses [84, 85]. The combination of pembrolizumab and T-VEC was feasible in metastatic melanoma patients with a promising 62% ORR, exceeding the responses observed with either T-VEC or pembrolizumab as single agents [86, 87]. In localized TNBC, the addition of T-VEC to neoadjuvant chemotherapy based on weekly paclitaxel followed by doxorubicin/cyclophosphamide was safe, with a promising pathological complete response observed in five out of nine patients enrolled [88]. The ongoing SOLTI-1503 PROMETEO evaluates the combination of T-VEC with atezolizumab in patients with operable TNBC or Luminal B-like/HER2- breast cancer with residual disease after neoadjuvant chemotherapy. The primary end point of this study is to evaluate whether this strategy increases the expression of a T-cell gene signature [89].

Adoptive cell therapies

Adoptive cell therapy involves the transfer of therapeutic effector cells into a patient. One approach involves isolating TILs from the host, expansion and eventually infusion after lymphocyte-depleting chemotherapy. Proof-of-concept for this approach was demonstrated for a patient with refractory HR+ breast cancer who received TILs in combination with pembrolizumab [90]. The phase 1 clinical trial

(NCT00027807) explored anti-CD3-activated T-cells in combination with IL-2 and anti-CD3/anti-HER2 bispecific antibody achieving a limited 5% ORR and manageable toxicity profile in HER2+ breast cancer [91]. Lastly, the (NCT00228358) study explored T-cell infusion primed with a HER2/neu vaccine. A promising ORR of 43% was observed [92]. The second approach within adoptive cell strategies, involves the use of chimeric antigen receptor (CAR)-T therapies. CAR-T cells are genetically modified T-cells to express either a tumor specific T-cell receptor (TCR) or a synthetic chimeric antigen receptor (CAR) that are able to identify specific antigens [93]**.** Several cancer antigens are currently being evaluated as potential targets [94]. For example, mesothelin is overexpressed in 36% of TNBC [95]. Clinical trials involving mesothelin-CAR-T cells enrolling breast cancer patients are ongoing (NCT02792114) and (NCT02414269) [96].

Interleukins in breast cancer

Cytokines are small peptides or glycoproteins with a short half-life released in response to different stimuli resulting in intracellular signaling and transcription modifications. Cytokines can have either tumor promoting or anti-cancer effects [97]. Recent optimization of the pharmacodynamic and pharmacokinetic profiles of cytokine-based agents has led to an increased interest in the development of clinical trials of cytokines in combination with ICIs or other immunotherapy agents [98]. There are a variety of novel therapeutic cytokines that are being evaluated in breast cancer clinical trials. The phase Ib/II (NCT01131364) explored the safety and tolerability of the F16-IL2 an antibody-cytokine fusion protein in combination with doxorubicin in metastatic breast cancer with an observed clinical benefit rate (CBR) defined as disease stability at 8 months of 57%. Targeting IL-7 has also been studied [99]. In the phase Ib/II KEYNOTE-899 trial, GX-I7, a long-acting IL-7, was evaluated in combination with pembrolizumab mTNBC. The toxicity profile was favorable, however, ORR was only 5.9%. IRX-, a biologic of physiologically derived T-helper type 1 cytokines, was studied as intratumoral injection in 16 patients with early-stage breast cancer before surgical resection. This approach was feasible and upregulation of RNA inflammatory signatures and TILs were observed [100].

Bispecific antibodies

Bispecific antibodies are proteins with at least two epitope recognizing sequences [101]. Molecular characterization of breast cancer subtypes has facilitated the development of bispecific antibodies engineered to bind to tumor specific antigens and receptors on T-cell surface [102]. TGF- β is a pleiotropic cytokine with a role in immune evasion in breast cancer [103]. M7824 targeting PD-L1 and TGF-β, is currently under investigation for localized HER2+ breast cancer (NCT03620201). KN046 is a bispecific antibody that blocks PD-L1 and CTLA-4 by interaction with PD-1 and CD80/86. The preliminary results of the (NCT03733951) were presented in ASCO 2020 for patients with nasopharynx cancer or non-small cell lung cancer. Objective responses were observed in 3 patients out of 26 (12%) [104]. Currently, NCT03872791 is evaluating KN046 alone or in combination with nab-paclitaxel in patients with mTNBC. MGD013 is a dual-affinity retargeting protein (DART) targeting both PD-1 and LAG-3 [105]. Out of 23 patients with mTNBC, 2 partial responses were observed [106].

Probody

Probody therapeutics are proteolytically activated antibody prodrugs designed to be activated and to target specific receptors in the TME rather than in peripheral tissues [107]. In this regard, the results of the PROCLAIM-CX-072 phase I clinical trial described the safety and efficacy of the CX-072 a PD-L1 probody therapeutic as single agent or in combination with Ipilimumab in patients with solid tumors. 3 out of 12 patients with mTNBC responded when used as monotherapy, confirming single agent activity, and with response rate similar to established PD-1/L1 inhibitors. CX-072 both in monotherapy and in combination with ipilimumab was well tolerated with few immune related adverse events reported [108].

Cancer vaccines

T-cell priming, an initial step in the cancer-immunity cycle, can be enhanced by the use of cancer vaccines. Breast cancer vaccines have been designed by using different platforms with the ultimate goal of activating T-cells to recognize cancer cells in a variety of settings including disease prevention, interception of minimal residual disease in the adjuvant setting, and treatment of metastatic disease [109, 110]. MAG-Tn3 glycopeptide vaccine was engineered to activate $CD4^+$ T-cell responses by binding to a wide range of HLA-DRB molecules. MAG-Tn3 was well tolerated and induced humoral responses [111]. The P10s-PADRE vaccine was designed to induce functional antibodies against carbohydrate antigens (TACAs) for patients with metastatic breast cancer. In the (NCT01390064) phase 1 clinical trial, IgG and IgM anti-P10 were long lasting and persisted high one year after vaccination [112]. Additional trials have explored using HER2 as a target for vaccine design including strategies based in the HER2 protein [113, 114] or relying in viral vectors to HER2-specific cell-mediated or humoral immunity [115, 116]. Recently, synthetic mRNA has emerged as novel vaccine format [117]. In this respect, TNBC-MERIT

(NCT02316457) is a phase 1 clinical trial assessing the safety and tolerability of a liposome-formulated intravenous RNA vaccine encoding different tumor antigens in patients with early-stage TNBC [118].

Epigenetic drugs

Some described mechanisms of resistance to ICIs may be reverted with the use of epigenetic modulation [119]. Lysine-specific histone demethylase 1A (LSD1) inhibitors, DNA methyl-transferase (DNMT) inhibitors, and histone deacetylase (HDAC) inhibitors can impact on adaptive immunity by inducing dsRNA production from endogenous retrovirus genes leading to a type I interferon response [120–122]. Moreover, DNMT inhibitors are also known to influence chemokine production increasing the expression of CXCL12, a key regulator of T-cell homing [123]. Other examples include increased production of CXCL9 and CXCL10 chemokines by enhancer of zeste homologue 2 (EZH2) modulation [124].

Different approaches combining epigenetic modifiers and immunotherapy have been tested in breast cancer [125]. In ER+ endocrine-resistant metastatic breast cancer, a Phase II trial (NCT02395627) tested the combination of tamoxifen, vorinostat (HDAC inhibitor), and pembrolizumab. CBR defined as the sum of partial response and stable disease for more than 6 months was observed in 5 out of 28 patients (18%) [126]. The ENCORE 602 (TRIO025) Phase II trial tested atezolizumab with or without entinostat, an oral class I-selective HDAC. This trial did not show a benefit for entinostat and atezolizumab compared to atezolizumab alone (HR: 0.87, 95% CI 0.52–1.46; $p = 0.59$) [127]. Lastly, the investigator initiated METADUR trial studied the combination of the PD-L1 ICI durvalumab and the hypomethylating agent oral azacitidine (CC-486) in ER+/HER2- breast cancer. This combination was shown to be safe, however, no clinical activity and limited pharmacodynamic changes were observed. Consequently, the underlying basis for the combination strategy could not be evaluated [128].

Immune targeted kinase inhibitors

Small molecules targeting pathways related to T-cell inhibitory signals or immunosuppressive cells in the TME are a feasible approach and a very attractive partner for immunotherapy combinations. As compared to ICIs, small molecules can penetrate into the TME and be directed to different intracellular proteins. Several tyrosine kinase inhibitors (TKIs) in combination with ICIs are currently under investigation [129]. Among them, HPK1 inhibitors have the potential to become a successful approach by acting in multiple steps of the cancer-immunity cycle [130–133]. HPK1 activation is linked to the MAPK/NfKB pathways and plays a negative regulatory role in T-cell activation and cell adhesion regulation. Notably, HPK1 inhibition resulted in increased IL-2 production in mouse T-cells [131]. CFI-402411 is an oral immunomodulatory kinase inhibitor currently being tested in a Phase 1 trial (NCT04521413) alone or in combination with pembrolizumab in patients with advanced solid tumors. BGB-15025 is another example of HPK1 inhibitor currently under study as single agent and in combination with anti-PD-1 (NCT04649385).

Conclusions

While PD-1/L1 inhibitors added to chemotherapy have established the first clinical role for immunotherapy in PD-L1 + mTNBC and high risk early TNBC, this strategy is currently relevant only to a minority of breast cancer patients [13, 16, 134, 135]. Substantial unmet need remains for advanced disease with lower level or absent PD-L1 expression, and other subtypes of breast cancer where checkpoint inhibitor therapy, alone or in combination with standard chemotherapy, has not been shown to improve treatment outcomes. As novel IO compounds enter into the clinical arena of breast cancer drug development, there are important questions about whether personalized interventions with doublets or triplets may be capable of overcoming immune resistance. There are many steps in the cancer-immunity cycle that might be susceptible to modification by novel IO compounds seeking to restore immune surveillance Fig. 1. Considering the complexity and variability of tumor-host immune interactions, as well as the observed differences in anti-tumor activity with ICIs in advanced and early-stage disease breast cancer according to PD-L1 expression, "one-size-fits-all" treatment approaches are unlikely to be successful. The development of robust predictive biomarkers will be required to individualize IO interventions in breast cancer.

The recent success of immunotherapy and chemotherapy combinations and the relatively limited efficacy of ICIs as monotherapy in breast cancer provides many important lessons to guide future drug development. First, better outcomes have been observed in advanced TNBC with both ICIs as monotherapy or in combination with chemotherapy in patients selected by PD-L1 expression and this marker may be considered a surrogate of ongoing immunologic activation [13, 136, 137]. Second, the additive benefit of immunotherapy appears to be greater in early-stage disease and may not differ based upon PD-L1 expression [134]. Third, there are clinical factors, such as absence of liver metastases, low lactate dehydrogenase (LDH) levels, and lymph node predominant metastatic disease, associated with an increased responsiveness to immunotherapy in the metastatic setting [7]. However, several unanswered questions remain from the available clinical data, such as whether

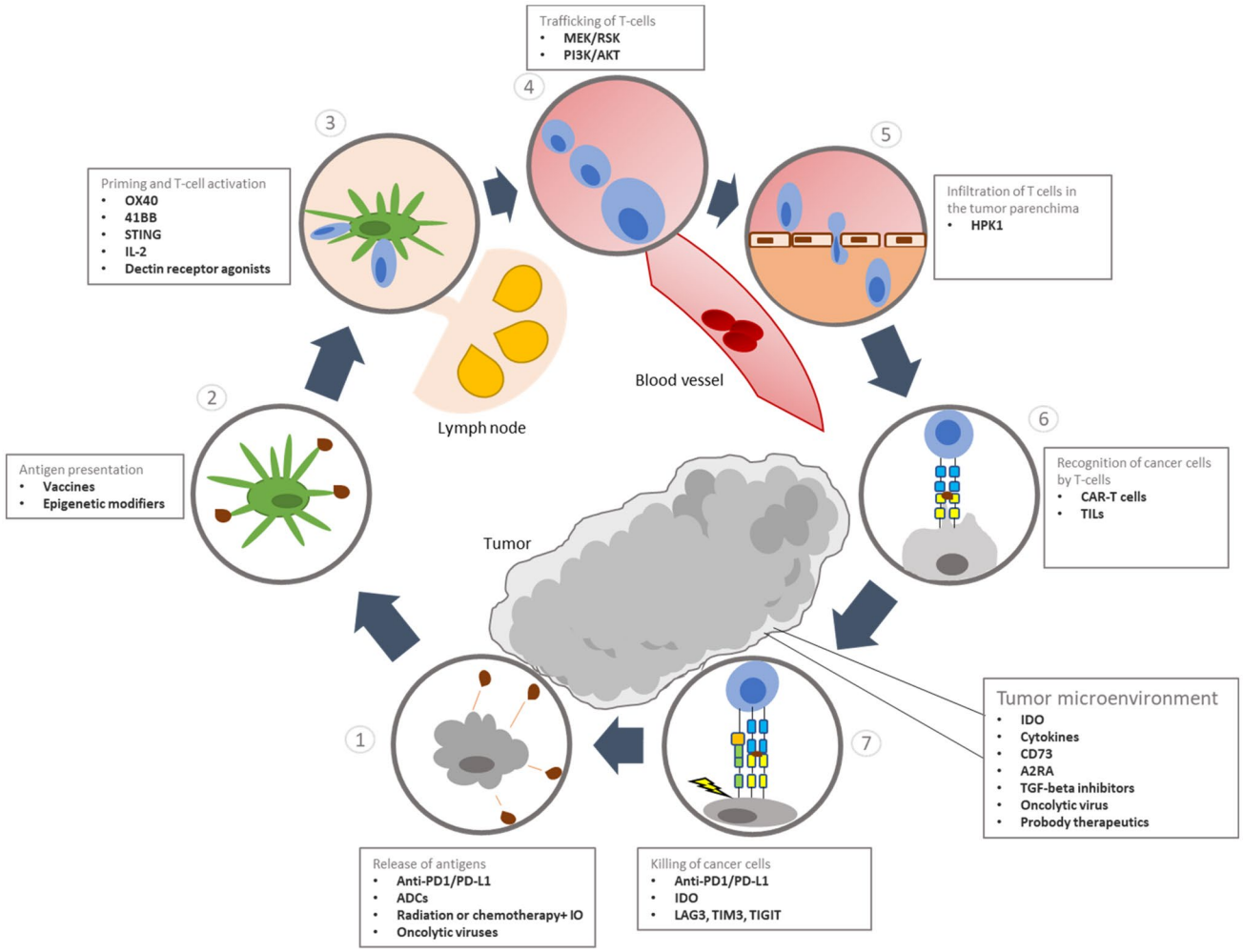

Fig. 1 Selected novel therapeutics currently being tested in the immuno-oncology field and its interaction with elements of the immune-cell cycle ([138]. Adapted from Chen and Mellman. Immunity 2013

loss of hormone receptor expression during evolution from localized to metastatic disease converts a non-immunogenic microenvironment to an immunogenic microenvironment and whether single-agent PD-1/L1 inhibitors without chemotherapy might be effective in a subset of patients with immunogenic TNBC to minimize chemotherapy-associated side effects. Moreover, mechanisms of both intrinsic resistance and acquired resistance to ICIs in breast cancer are largely unknown. Particularly with the adoption of immunotherapy for early disease, it will be important to understand which approaches may be relevant in second line settings for patients who initially respond to the standard first-line treatments. Biomarkers of acquired resistance are critical to develop rational combinations for the second line setting and beyond.

All of the above suggest that future immunotherapy combinations should take into account the highly complex TME in breast cancer and incorporate biomarkers for patient selection. Establishing clear signals of activity for novel combinations with PD-1 or PD-L1 ICIs is challenging. To date, most studies include small, single-arm cohorts of highly selected patients with refractory breast cancer. With few responses observed, it is difficult to determine whether there is synergistic or even additive activity when the expected response rate to PD-1 or PD-L1 ICI monotherapy is 10–20% and is highly dependent on patient selection. Establishing proof of mechanism a priori benchmarks of pharmacodynamic activity will be critical to determine which combinations should be accelerated to randomized clinical trials.

We envision a future immunotherapy landscape in breast cancer with greater individualization of treatment based upon characterization of targetable tumor alterations, understanding of host-related factors and TME interactions, ongoing response monitoring and identification of adaptive resistance mechanisms.

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Declarations

Conflict of interest Alberto Hernando-Calvo reports travel grants from Kyowa Kirin, Merck Serono, MSD and Bristol Myers. Dave Cescon reports Consulting/Advisory: Agendia, AstraZeneca, Dynamo Therapeutics, Exact Sciences, GlaxoSmithKline, Merck, Novartis, Pfizer, Puma Biotechnology, and Roche. Research funding to institution: GlaxoSmithKline, Pfizer, and Roche. Patent (US62/675,228) for methods of treating cancers characterized by a high expression level of spindle and kinetochore associated complex subunit 3 (ska3) gene. Philippe Bedard reports consulting or advisory role with Seattle Genetics, Lilly, Amgen, Merck, BMS (institution) and Pfizer (institution). Research funding from Bristol-Myers Squibb (institution), Sanofi (institution), Astrazeneca (institution), Genentech/Roche (institution) Servier (institution), GlaxoSmithKline (institution), Novartis(institution), PTC Therapeutics (institution), Nektar (institution), Merck (institution), Merck (institution), Seattle Genetics (institution) Mersana (institution), Immunomedics (institution), Lilly (institution), Amgen (institution) and Bicara (institution).

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Research involving human and animal rights Additional declarations for articles in life science journals that report the results of studies involving humans and/or animals.

Consent to participate All the authors have consented to participate in this review.

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