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Breast Cancer (ML Telli, Section Editor)

Advancing Immunotherapy in Metastatic Breast Cancer

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Opinion statement

Despite many advances in the treatment of breast cancer, the development of metastatic disease remains an incurable and frequent cause of cancer death for women worldwide. An improved understanding of the role of host immunosurveillance in modulating breast cancer disease biology, as well as impressive survival benefits seen to checkpoint blockade in other malignancies have provided great hope for an expanding role of immunotherapies in breast cancer management. While these novel therapies are currently being investigated in clinical trials, signals of efficacy, and tolerability in early phase studies suggest these will eventually make their way into standard practice algorithms. Ongoing research has highlighted a high degree of intertumoural heterogeneity in tumour lymphocytic infiltrates, suggesting some tumours or subtypes are more immunogenic than others. Furthermore, tumour intrinsic mechanisms of immune evasion are beginning to be uncovered, potentially representing key therapeutic targets to use in combination with checkpoint blockade, exemplifying the emerging concept of personalised medicine approaches to immune therapies. Subsequently, different immunotherapeutic strategies may be required based on stratification by these factors—for the minority of tumours with a high level of pre-existing immunity, immune checkpoint blockade monotherapy may be sufficient. However, for the majority of tumours with lower levels of pre-existing immunity, combination approaches will likely be required to achieve maximal therapeutic effect. Results of ongoing clinical trials including combinations with chemotherapy, radiation therapy, and targeted therapies are eagerly awaited.

Introduction

Breast cancer (BC) is the most frequently diagnosed cancer, with one million new diagnoses each year [1], and is a leading cause of cancer-related death among females worldwide. It is a heterogeneous disease made up of subtypes with distinct molecular profiles, responses to treatments, and clinical outcomes [2]. While most BC patients will be diagnosed with localised tumours, roughly 6% of patients will present with de novo metastatic disease and around 10–40% of patients with localised primary tumours will relapse systemically [3]. The prognosis of patients with metastatic BC is heterogeneous and depends on pathological factors including hormone receptor status, human epidermal growth factor receptor 2 (HER2) status, as well as clinical parameters such as the sites of disease recurrence, disease burden, and the presence or absence of symptoms [3, 4].

Although metastatic BC is generally not considered curable, meaningful improvements in survival have been coincident with the introduction of newer systemic therapies [5]. Indeed, advances in clinical and translational research have dramatically improved our understanding of the molecular foundations of metastatic BC disease, including the discovery of various biomarkers, potential therapeutic targets, as well as hitherto mechanisms of resistance to treatment [6]. Consequently, the therapeutic arsenal available for metastatic BC patients has improved significantly and has impacted the treatment of metastatic disease.

By the mid-twentieth century, the development of chemotherapy was a significant advance for metastatic BC treatment leading to a median overall survival (OS) of 10 months [7, 8]. Later, newer improved chemotherapeutic agents were developed and extended the OS improvement to above 20 months when used as sequential monotherapy instead of combined use, becoming the standard care for metastatic BC, unless there is rapid disease progression [9]. Nevertheless, chemotherapy, as a systemic treatment, also affects non-tumour cells and contributes to generalised adverse secondary effects impairing the quality of patients' life. In search of more specific drugs, the introduction of monoclonal antibodies against the membrane receptor HER2 becomes especially relevant in BC treatment. In the late 1990s, trastuzumab was approved for clinical use. Clinical trials combining trastuzumab and chemotherapy regimens have consistently shown survival benefits for patients diagnosed with HER2-positive BC in both the early and advanced setting of disease [10, 11].

More recently, attention has turned to the role of immune infiltrates in BC. While not a new observation [12], its association with improved prognosis appears to vary by subtype. Loi et al. discovered that tumour-infiltrating lymphocytes (TILs) are an important prognostic factor in both HER2-positive BC and triple-negative BC (TNBC) and their presence is associated with improved survival as well as treatment response [13]. These findings highlight the potential importance of immunity as a determinant of BC outcomes and further suggest that some subtypes are more immunogenic and may be particularly amenable to immune modulating therapy. The discovery of immune checkpoints inhibitors and their success in the treatment of cancers such as melanoma and renal cell carcinoma has led to renewed interest in exploring the role of immune therapies in both metastatic and early BC disease. In the present article, we review the most promising and clinically relevant immunotherapeutic strategies, with a focus on immune checkpoint blockade, that are being evaluated for the treatment of BC.

The immune landscape of breast cancer

The immunoediting theory postulates the role of host immunosurveillance in recognising tumour-specific epitopes (termed neoantigens) with subsequent response in three phases—elimination, equilibrium, and escape [14]. Malignant tumours are most frequently diagnosed in the escape phase, highlighting one of the key hallmarks of cancer [15]. Host anti-tumour immune responses are thought to arise predominantly via adaptive T cell-mediated cytotoxic responses [16, 17], originating from the

recognition of neoantigens, the end product of expressed somatic cancer mutations [18, 19].

BC as a whole has not previously been considered a highly immunogenic tumour. However, numerous retrospective analyses of the tumour immune microenvironment in prospectively collected tumour samples from early BC reveal considerable diversity in the amplitude of lymphocytic infiltrate, suggesting that certain subsets may be more immunogenic than others [20, 21]. These analyses have utilised various methodologies to both quantify and characterise immune infiltrates; however, results have largely been consistent with each other. In keeping with the key role of adaptive T cell responses, immune infiltrates in BC are composed predominantly of T cells and variably lower levels of B cells, natural killer (NK) cells, dendritic cells, and macrophages [20, 22]. Importantly, studies evaluating the magnitude of lymphocytic infiltrate show a high degree of reproducibility [23, 24] and demonstrate notable associations with clinical outcomes [20].

Analysis by the clinically utilised subtypes demonstrates that HER2-positive BC and TNBC generally harbour higher TIL levels than ER-positive/HER2-negative BC [25–27], implying these subtypes are more immunogenic. Conversely, as luminal breast subtypes tend to harbour lower TIL levels, they are thought to be less immunogenic.

Significant associations of TILs with clinical endpoints suggest an important influence of host immunity in determining prognosis and response to therapy, but this also differs by BC subtype and is summarised in Table 1 [20]. TILs have most consistently been shown to have a positive prognostic association not only in TNBC in the context of adjuvant anthracycline chemotherapy [28] but also in the HER2-positive subset in patients undergoing adjuvant treatments both with and without HER2-targeted agents [29, 30]. Interestingly, the prognostic relationship in these subtypes is linear with increasing magnitude of lymphocytic infiltrate associated with improved survival. In direct contrast, increasing TILs have not been associated with an improvement in outcome in the ER-positive/HER2-negative subtype—most studies have reported no

Table 1. Summary of the prognostic and predictive association of TILs in breast cancer

	ER-positive, HER2-negative	HER2-positive	Triple-negative breast cancer
Predictive			
Pathological complete response to neoadjuvant therapy	+ [27]	+ [29, 31, 41]	+ [41–43]
Predictive of survival benefit	–	+ [32, 33]	–
Prognostic			
Various survival endpoints	–	+ [29, 30]	+ [25, 32, 44–47]
Overall survival	+ [27]	+ [48, 49]	+ [25, 44, 45, 47, 48]

A plus sign is shown if there has been a previous report of a statistically significant association. A minus sign is shown if there has not been a previous report of a statistically significant association. Various survival endpoints include all survival endpoints that are not overall survival. These differ by study and include breast cancer-free interval, breast cancer-specific survival, metastasis-free survival, distant disease-free survival, recurrence-free survival, and event-free survival. All studies are from analysis of TILs in the primary tumour in the early breast cancer setting with the exception of the analysis of TILs from the CLEOPATRA trial

significant association with prognosis; however, one report of patients who received neoadjuvant therapy demonstrated an association with worse survival in those with tumours that harboured higher levels of TILs [27]. However, analyses in the luminal subtypes may be hampered by a substantial degree of heterogeneity.

Immune infiltrates may also play important roles in maximising response and benefits to specific therapeutic agents, also summarised in Table 1. This has been evaluated in the setting of pathological complete response (pCR) rates to neoadjuvant therapy, predominantly not only in HER2-positive disease with HER2-targeted agents but also in TNBC, whereby higher TIL levels are associated with higher pCR rates [29, 31]. Additionally, statistical interaction terms have been used to evaluate whether the benefit of treatment on disease outcomes can significantly differ by TIL level. In the FinHER study, for example, patients with higher TIL levels demonstrated significantly improved distant disease-free survival benefit to trastuzumab compared with patients with lower TIL levels [32••]. Similarly, an improved benefit to trastuzumab in patients with higher TIL levels was also seen in a gene expression analysis of the NSABP B-31 clinical trial [33].

Given the findings of an important influence of host anti-tumour immunity in selected early BC, and encouraged by impressive survival benefits to immunotherapies observed in advanced melanoma [34, 35], non-small cell lung cancer [36, 37], and renal cell carcinoma [38], there is ample motivation to trial immunotherapies in BC. It should be noted, however, that the TIL landscape of advanced BC remains relatively unexplored—persistent immunoediting and the development of immune-evasive mechanisms are thought to lead to diminished immunogenicity over time as a tumour evolves to advanced disease. Consistent with this, small numbers of metastatic BC lesions have demonstrated lower TIL levels than in primary tumours [39, 40]. The relevance of this to the potential activity of checkpoint blockade in metastatic and treatment-experienced disease remains unclear.

Checkpoint blockade in breast cancer

Enhancing immunity with immune checkpoint blockade

Checkpoint inhibition with the use of immune-modulating antibodies has emerged as an exciting and promising form of immunotherapy in several cancer types [50]. Under normal physiologic conditions, various suppressive pathways in the immune system exist to promote self-tolerance and protect against autoimmunity. The latter is highly regulated by immune checkpoints. Tumours have been shown to exploit these immunosuppressive pathways in order to dampen anti-tumour responses and escape immune detection and elimination [51]. In cancer patients, effector T cells at the tumour site can remain in an 'exhausted' state as a result of chronic exposure to antigen and is characterised by the upregulation of checkpoint receptor inhibitors in order to prevent uncontrolled immune reactions. Blockade of one or several of these immune checkpoints with monoclonal antibodies (mAbs) has been shown to be able to rescue otherwise exhausted anti-tumour T cells and, most importantly, has been associated with objective clinical responses and survival benefits in cancer patients [52]. The use of checkpoint antibodies was initially tested for the treatment of melanoma and renal cell carcinoma, however given the immunogenic potential of BC subsets, checkpoint blockade represents a promising approach for the treatment of BC.

CTLA4 blockade

The first therapeutic immune checkpoint inhibitor introduced in clinical trials was ipilimumab (Yervoy^{R}, Bristol-Myers Squibb, NY, USA), which targets CTLA-4 [53]. This drug was approved for use by the US FDA based on an OS benefit for both untreated and treatment-refractory metastatic melanoma patients [34]. CTLA-4 is an inhibitory receptor expressed on T cells that competes with the co-stimulatory receptor CD28 for B7 ligands (B7.1 and B7.2). Binding of B7 ligands to CD28 receptor stimulates T cell amplification and a robust immune activation; however, binding to CTLA-4 produces T cell inhibitory signals that suppresses the immune response [51]. There are few reports on the use of anti-CTLA-4 immunotherapy in BC. The first was a phase I study done in metastatic hormone refractory BC patients testing tremelimumab, a fully human monoclonal antibody specific for CTLA-4, in combination with exemestane (aromatase inhibitor). The best overall response was stable disease for at least 12 weeks in 42% of patients. Most patients developed increased peripheral $CD4^{+}$ and $CD8^{+}$ T cells expressing inducible co-stimulatory receptors as well as an increase in the ratio of $CD4^{+}$ and $CD8^{+}$ T cells to regulatory T cells (Tregs) [54]. Another trial assessed the safety and tolerability of pre-operative cryoablation-mediated tumour antigen presentation and whether ipilimumab therapy induces immune modulation in women with operable BC (NCT01502592). In this study, ipilimumab was evaluated with or without cryoablation in 19 patients with early stage BC [55]. The combination approach with ipilimumab and cryoablation showed favourable immunological changes with T cell activation in the bloodstream and a modest increase in the ratio of tumour $CD8^{+}$/Tregs cells. Although the efficacy of anti-CTLA-4 monotherapy has been generally modest, combinations of anti-CTLA-4 with radiation therapy, cytotoxic chemotherapy, and other immunotherapies are thought to have therapeutic potential. As an example, another trial is currently evaluating the combination of a histone deacetylase inhibitor and immune checkpoint blockade with nivolumab and ipilimumab for metastatic solid tumours including locally advanced or metastatic HER2-negative BC (NCT02453620).

PD-1/PD-L1 blockade

Therapies targeting the interaction between programmed cell death-1 (PD-1) and programmed death ligand-1 (PD-L1) have arguably provided the most impressive improvements in survival of all checkpoint blockade agents, emphasising this checkpoint as a key regulator of tumour immune evasion in advanced disease. The PD-1 receptor is expressed on peripheral T cells, and its ligand, PD-L1, is expressed in peripheral tissues, such as tumour and stromal cells. Upon upregulation, PD-L1 delivers inhibitory signals that block T cell proliferation and induce immune tolerance [51, 56]. Likewise to anti-CTLA-4, anti-PD1 has also shown promising activity in treating melanoma [57]. Pembrolizumab (MK03475, Keytruda, Merck, White House Station, NJ) and nivolumab (ONO-4538/BMS-936558, Opdivo, Bristol-Myers-Squibb) were approved by the FDA in 2014 for treatment of patients with advanced melanoma, as supported by the positive results from several phase III studies of melanoma patients [58, 59]. Both antibodies inhibit the interaction between the PD-1 receptor and its immunosuppressive PD-L1 ligand preventing the neutralisation of T cells by the tumours and thereby enhancing the anti-tumour immunity.

Several reported early phase studies have investigated the use of PD-1/PD-L1 checkpoint inhibitors either in patients with all BC subtypes [60], or in specific BC subtypes including TNBC [28, 61, 62], and ER-positive/HER2-negative BC [63]. The therapeutic agents utilised and relevant checkpoint targets are summarised in Table 2. Despite significant heterogeneity among trial participants in these trials, these agents given as monotherapy have been shown to be relatively safe and have demonstrated efficacy with an overall response rate (ORR) between 5 and 20%. Furthermore, some responses have displayed durability, suggesting the possibility of prolonged tumour responses to immune therapy. Similar to the TNBC subtype, HER2-positive BC is also associated with increased number of TILs, higher PD-L1 tumour expression levels, and a high mutation rate that can produce neoantigens inducing immune responses as compared with other BC subtypes [25, 49•]. Accordingly, the HER2-positive BC subtype may also be amenable to anti-PD-1/PDL-1 antibodies, although this is currently being investigated and is not yet reported. A phase I/II trial (PANACEA trial; NCT02129556) to determine whether the addition of pembrolizumab can reverse trastuzumab resistance in patients with HER2-positive disease that have previously progressed on trastuzumab treatment is ongoing.

Given the promising safety and efficacy demonstrated in early phase trials, randomised phase III trials of several different approaches are also currently underway, with results expected in the near future. One such study (NCT02555657/KEYNOTE-119) is investigating the superiority of single agent pembrolizumab (MK-3475) versus physician's choice chemotherapy in patients with metastatic TNBC who have previously received one or two prior lines of systemic therapy.

An alternative approach shown in other phase III trials has been to use rational combinations with other therapies that could either induce immunogenicity or relieve mechanisms of immune suppression, discussed in more detail in later sections. For example, one trial (NCT02819518/KEYNOTE-355) is assessing the safety and efficacy of the first-line

Table 2. Summary of early phase anti-PD-1/PD-L1 trials in breast cancer

Drug compound	Checkpoint target	Trial	Phase	Study population	Overall response rate
Avelumab	PD-L1	JAVELIN [60]	Phase Ib	All subtypes[a]	4.8%
Pembrolizumab	PD-1	KEYNOTE-012 [61]	Phase I	TNBC	18.5%
Atezolizumab	PD-L1	NCT01375842 [62]	Phase I	TNBC[b]	19%
Nivolumab	PD-1	NCT02499367	Phase II	TNBC	Not reported
Pembrolizumab	PD-1	KEYNOTE-028 [63]	Phase Ib	ER-positive/HER2-negative	12%

Each study had different eligibility criteria with respect to PD-L1 status (JAVELIN and NCT01375842 was PD-L1 unselected; KEYNOTE-012 and KEYNOTE-028 required PD-L1 ≥1% of tumour cells or any staining in the stroma)

PD-1 programmed cell death protein 1, *PD-L1* programmed death ligand 1, *BC* breast cancer, *TNBC* triple-negative breast cancer

[a]Overall response rate is shown for all subtypes regardless of PD-L1 status for the JAVELIN trial. Higher response rates were observed with higher PD-L1 expression (33.3% for tumours with ≥10% PD-L1 expression in immune cell 'hotspots')

[b]The study population was not selected for by PD-L1 status; however, only participants with PD-L1 expression ≥5% have been reported

pembrolizumab combined with chemotherapy versus chemotherapy alone for the treatment of locally recurrent inoperable or metastatic TNBC. A similar trial is investigating the use of atezolizumab with nab-paclitaxel versus chemotherapy alone as the first-line therapy for advanced TNBC (NCT02425891/Impassion130).

Enhancing patient selection for immunotherapy

While several trials in other solid tumour types have demonstrated impressive survival benefits and safety profiles to immunotherapies, most notably checkpoint blockade, it is evident that not all patients will derive a benefit. Moreover, the financial costs of these novel therapies remain very high. Subsequently, it is crucial that ongoing efforts are made to develop accurate predictive biomarkers.

PD-L1 expression and the tumour immune microenvironment

PD-L1 expression from immunohistochemical staining, in particular, has emerged as having a potential role as a predictive biomarker, although reports across tumour types have been inconsistent [36, 64]. This may be due in part to the heterogeneity in methodologies of PD-L1 evaluation, the use of different cut-off values for positivity, the use of relevant tissue (archival tissue versus fresh metastatic biopsies), as well as the inducible nature of PD-L1 [65]. Efforts are currently underway to determine the magnitude of these differences and the origin of discordant results [66]. Alternatively, differential predictive significance may also reflect differences in immune-evasive mechanisms between different cancer types.

Phase III trials of anti-PD1/PD-L1 checkpoint blockade have often utilised PD-L1 cut-off levels obtained from early phase trials that predicted improved response rates. For example, the KEYNOTE-024 study of pembrolizumab as the first-line therapy for non-small cell lung cancer demonstrated superior OS compared with chemotherapy using an intratumoural PD-L1 cut-off of 50%—a value obtained from a response rate from earlier trials [67•]. In contrast, the Checkmate-026 study of nivolumab as the first-line therapy for non-small cell lung cancer did not demonstrate superiority to chemotherapy using a PD-L1 cut-off of 1% or greater [68]. While this suggests that predictive correlations from early phase trials could have a role in improving patient selection for larger phase III clinical trials, it is not entirely clear that predictors of tumour response necessarily correlate with OS benefit. Indeed, observations of immunotherapy responses have shown occasional unconventional response patterns, delayed survival benefits [64], and improvements in OS without associated improvements in progression-free survival [36, 38]. Notably, higher response rates have generally been observed in patients with higher PD-L1 expression from early phase BC trials of checkpoint blockade. For example, in the JAVELIN clinical trial [60], the ORR to single agent avelumab (anti-PD-L1) across all BC subtypes unselected for PD-L1 was 4.8%, whereas the ORR for those with PD-L1 levels 10% or greater was 33.3%. Larger studies will be

required to determine the potential predictive role of PD-L1 expression in response to checkpoint blockade in BC.

Breast cancer subtype

As previously described, TIL levels are generally higher in TNBC and HER2-positive BC compared with the more common ER-positive/HER2-negative subtype. PD-L1 expression levels exhibit similar patterns between the BC subtypes [60]. Despite these correlations, PD-1 checkpoint blockade has demonstrated activity even in the ER-positive/HER2-negative subtype, achieving an overall response rate of 12% in a phase I expansion cohort using single agent pembrolizumab in patients with metastatic disease [63]. Importantly, patients were only eligible for this trial if they were PD-L1 positive, using a definition of ≥1% expression on tumour cells or any expression in the stroma. In total, 248 patients were screened for the trial, with only 19.4% meeting the PD-L1 eligibility criteria. Intriguingly, this suggests that PD-L1 expression may be a more relevant predictor of response than BC subtype; however, further studies would be required to confirm this.

Disease burden

The optimal timing of immune-based treatments also warrants further consideration. Immunogenicity appears to decline with advancing disease in the context of immunoediting and the establishment of immune-evasive mechanisms [39, 40]. The impact of this decline in immunogenicity on the efficacy of checkpoint blockade remains unknown. Furthermore, the extent of disease may be important—a retrospective report demonstrated improved response to checkpoint blockade in patients with advanced melanoma and metastatic disease burden limited to the lymph nodes and lungs [69]. Subsequently, patients who are heavily pretreated and with extensive disease burden may be less likely to respond to a single agent checkpoint blockade than patients with earlier disease and lesser metastatic burden.

Therapeutic modulation of the immune microenvironment

Optimising immunotherapy requires treatments that affect multiple aspects of the immune response. There is accumulating evidence that standard BC therapies can affect tumour immunity. This raises the possibility that rational combinations of different treatment modalities can be used to remodel and enhance host immunity against cancer.

Chemotherapy and radiation therapy

The immunomodulatory effects of chemotherapy and radiation therapy have been well documented. More recent data indicate that the therapeutic efficacy of some of these cytotoxic treatments is in part facilitated by an induced anti-tumour immune response. As previously noted, the level of TILs is observed to increase after neoadjuvant chemotherapy with taxanes or anthracyclines in breast cancer patients [22, 70]. Additionally, greater density of intra-tumoural and stromal TILs in BC lesions that persist after neoadjuvant and adjuvant chemotherapy is associated with improved metastasis-free and OS [25, 71]. This

observed increase in TILs could be a result of the induction of a prognostic type I interferon gene signature with anthracycline treatment in BC patients [72]. Additionally, ionising radiation of BC cells induces expression and release of chemokine CXCL16 which promotes recruitment of effector $CD8^+$ T and $CD4^+$ T helper1 cells [73].

These treatments, when given at a standard dose and schedule, can induce a form of tumour cell death that elicits an adaptive immune response specific for the tumour: immunogenic cell death [74, 75]. Immunogenic cell death is characterised by the release of damage-associated molecular pattern molecules such as ATP and high mobility group box-1 (HMGB1) as well as increased exposure of calreticulin on the tumour cell surface [76]. These signals initiate a cascade of immune activation. Genetic or pharmacological interventions that limit the availability of extracellular ATP have been shown to markedly reduce the immunogenic potential of cells succumbing to immunogenic cell death. The enzymatic activities of ectonucleosidase triphosphate diphosphohydrolase 1 (best known as CD39) and 5′-nucleotidase (best known as CD73) convert ATP to AMP and AMP to adenosine, respectively [77]. This drives a shift from an ATP-driven proinflammatory environment to an anti-inflammatory microenvironment induced by adenosine, which exerts potent immunosuppressive effects. Interestingly, both CD39 and CD73 are often overexpressed by cancer cells, reflecting the exploitation of a mechanism to evade immunosurveillance and promote treatment resistance [78].

The binding of HMGB1 released from cells undergoing immunogenic cell death to toll-like receptor 4 (TLR4) has been found to be vital for the eradication of tumours by chemotherapy and radiation therapy [79]. This is in accordance with clinical analyses of BC patients treated with anthracyclines: patient carriers of a *TLR4* mutation that prevents binding of HMGB1 and TLR4 correlated with early relapse post treatment [79, 80]. Neoadjuvant chemotherapy with epirubicin/docetaxel increased concentration of circulating HMGB1 only in BC patients who responded to treatment [81]. Upon disease progression, BC cells have been shown to downregulate HMGB1 [82].

Calreticulin acts as a phagocytic signal for dendritic cells to engulf apoptotic cells and process associated tumour antigens for presentation [83]. However, tumour cells often suppress the activity of phagocytes by expressing cell surface protein CD47 [84]. In luminal BC, circulating $CD47^+$ tumour cell frequency as well as CD47 expression levels are correlated with lower survival and increased metastasis [85, 86].

These findings suggest that the impairment of immunogenic cell death is a mechanism of resistance against chemotherapy or radiation therapy, which contribute to therapeutic failure in some BC patients. Agents that antagonise these mechanisms such as anti-CD73 and anti-CD47 are recent clinical developments and have been demonstrated in preclinical settings to be promising therapeutic targets to overcome treatment resistance in patients [87, 88].

Targeted therapy

The molecular understanding of cancer biology has advanced substantially, revealing crucial pathways that drive tumour growth. Therapies that can inhibit activity of these specific pathways or mutant proteins have been rationally designed and developed as a result. Several of the targeted pathways are additionally shown to influence diverse aspects of the immune response including

enhancing tumour antigen presentation, T cell effector function and activation, as well as antagonising tumour-mediated immunosuppression. This suggests the possibility that targeted therapies may help to optimise anti-tumour immune responses from immunotherapies.

Inhibitors of tumour-associated receptor kinases such as HER2 and vascular endothelial growth factor-A (VEGF) have been evaluated for clinical efficacy against BC [89, 90]. Trastuzumab (anti-HER2-antibody) treatment increased tumour antigen presentation by inducing more rapid ubiquitination of HER2 is associated with increased level of infiltrating immune cells and enhanced cytotoxic T lymphocyte-mediated lysis [29]. In patients receiving trastuzumab treatment, the presence of TILs is associated with improved outcomes [29, 32]. Interestingly, the increase in immune checkpoint PD-L1 was found to limit the effect of HER2 antibody [91]. Accordingly, further preclinical studies have shown that simultaneous inhibition of PD-L1 and HER2 activity is synergistic against HER2-positive BC [92].

VEGF is a primary stimulant for tumour angiogenesis [93]. To date, clinical outcomes of anti-VEGF drugs have been unconvincing in BC where results of clinical trials with bevacizumab failed to demonstrate OS advantage [94, 95]. Nonetheless, VEGF also has an important and complex immunologic role. Anti-VEGF therapy has been shown to reduce tumour-associated macrophage infiltration, modulate cytokine production to increase TILs as well as enhance dendritic cell maturation and frequency in peripheral blood of BC patients [96]. Study shows an inverse association between PD-L1 and the expression levels of genes in the VEGF pathway [97, 98]. Further preclinical work substantiated that VEGF enhances expression of PD-1, involved in $CD8^+$ T cell exhaustion [99]. The immune suppression associated with VEGF was reverted by agents targeting VEGF. These results indicate potential synergy between anti-angiogenic molecules with inhibitors of immune checkpoints in VEGF-producing tumours.

The PI3K/AKT/mTOR pathway has long been recognised as crucial regulators of metabolism, growth, and survival. More recent data indicate that PI3K, AKT, and mTOR molecules are also integral coordinators of innate and adaptive immune responses [100]. Inhibitors of the PI3K-AKT pathway are targeted agents that sensitise tumour cells to immune-mediated destruction, partly by promoting apoptosis [101, 102]. mTOR is well-known to promote immune activity. Blockade of mTOR elicits pervasive immunosuppressive effects in both innate and adaptive immune responses: broadly by impairment of dendritic cell maturation and function as well as inhibition of T cell proliferation [103, 104]. mTOR inhibitors have been used as an immunosuppressant in the clinics for preventing transplant rejections [105]. However, recent studies indicate that low doses of mTOR inhibitors can increase memory precursor effector cell frequency and the subsequent pool of long-lived $CD8^+$ T cells [106]. It has been further demonstrated that combined blockade of immune checkpoint CTLA-4 and mTOR further improves immune memory responses to tumour challenges [107].

PARP (poly-ADP-ribose polymerase) is a protein that has several roles in cellular processes, most notably in DNA repair and programmed cell death [108]. Recently, PARP inhibitors demonstrated multiple durable anti-tumour responses in patients with advanced germline *BRAC1/2* mutated ovarian and BC [109]. Accordingly, various phase I/II trial are currently ongoing investigating the safety and efficacy of using niraparib (PARP inhibitor) combined with pembrolizumab (NCT02657889) as well as the combination of olaparib

(PARP inhibitor) with the anti-PD-L1 antibody durvalumab (MEDI4736) (NCT02734004) to treat advanced TNBC.

Conclusion

The practice of cancer immunotherapy has seen dramatic advancement during the last 20 years. In particular, the recent success seen with the use of novel immunotherapy in the treatment of cancers such as melanoma has renewed interest in harnessing the power of immune system in the fight against cancer. Accordingly, immune checkpoint blockade has emerged as a promising clinical approach for the treatment of advanced BC. The characterisation of the BC tumour microenvironment and the immune landscape of all BC subtypes have provided the rationale to explore the use of immune-modulating strategies in the treatment of BC. Several preclinical and clinical studies have suggested so far that immunotherapy has the potential to improve clinical outcomes for BC patients, in particular for TNBC patients. However, the use of checkpoint blockade as a monotherapy may be insufficient in patients with advanced BC indicating that there is still much to be done to implement immunotherapy in this disease. Therefore, research efforts have moved toward a focus on combining immunotherapy with new or standard treatment modalities including chemotherapy, radiotherapy, specific targeted therapy, and other immunotherapies, as well as on identifying reliable biomarkers to identify patients that would most benefit from immunotherapy, and/or early diagnosis to prevent cancer progression as a vital part of that therapy.

Compliance with Ethical Standards

Conflict of Interest
The authors declare that they have no conflict of interest.

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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