Editorial on Immunotherapy and Tumor Microenvironment

Innovative approaches to immunotherapy in breast cancer

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Introduction

Breast cancer impacts on the health of many women each year, with approximately 17,000 new diagnoses and 3,000 deaths per year in Australia alone in 2016 (1). Significant progress has been made with screening and early treatment of breast cancers, leading to a 5-year survival rate of 98.8% for individuals diagnosed with localised disease (2). The next wave of progress will include immunotherapies, which use the immune system or immune mechanisms to control or eliminate cancerous cells. Breast cancer represents an exciting target for these new therapies.

Breast cancers are heterogeneous but they can be subdivided into 4 main molecular subtypes based on expression of estrogen receptor (ER), progesterone receptor (PR), the human epidermal growth factor receptor 2 (Her2) and Ki67, which is a marker of active cell division. Luminal A breast cancer expresses ER and/or PR but not Her2 and Ki67. Luminal B breast cancer expresses ER and/or PR, it may be Her2+ or Her2- and it has high levels of Ki67. Her2-enriched (Her2+) breast cancer does not express ER or PR but it is Her2+. Triple negative breast cancer (TNBC) lacks expression of ER, PR and Her2 but it is more common in women that have *BRCA1* gene mutations.

Given the distinct receptor usage, these 4 subtypes can be clinically managed in different ways, depending on how advanced the tumour is. Common courses of treatment include surgery along with radiotherapy, chemotherapy, hormonal therapies as appropriate based on the expression of hormonal receptors and Her2-targeted therapy with Trastuzumab (described below), administered as neoadjuvant and/or adjuvant therapies. While these therapies can be very effective in early breast cancer, metastatic breast cancer still has a very poor prognosis, with a 5-year survival rate of 27.4% (2). New therapies are therefore urgently needed to prevent and/or manage metastatic disease.

Intrinsic immunogenicity of breast cancers

In the past, breast cancer had been thought of as poorly immunogenic, with low levels of inflammation in the tumour microenvironment (TME). However, it is now clear that distinct molecular subtypes are more likely to exhibit indicators of immunogenicity-specifically tumour mutational burden (TMB), tumour infiltrating lymphocytes (TILs) and expression of immuno-inhibitory molecules.

With regard to TMB, a higher level of mutation leads to increased generation of neoantigens, which makes the tumour more immunogenic. Breast cancer was thought to have modest rate of mutation [1 per megabase (MB)] relative to other forms of cancer, such as melanoma (10 per MB) (3). However, TNBC and Her2+ tumours can have markedly higher rates of mutation (4). With regard to TILs, these cells are enriched for specificity against tumour-associated antigens (TAAs) and their presence indicates that the tumour is immunogenic. Most breast cancers contain some TILs (5) but a subset of patients, particularly those with TNBC and Her2+ tumours, can exhibit high levels of TILs (5,6). For example, lymphocyte-predominant breast cancer (LPBC) is characterised by >50% lymphocyte infiltration into the tumour tissue, with the frequency of LPBCs at 20% for TNBC, 16% for Her2+ and 6% for ER+ luminal breast cancers (5). A number of studies have demonstrated that increased TIL frequency correlates with improved prognosis in both neoadjuvant and adjuvant therapy settings with breast cancer (5,7). With regard to expression of inhibitory molecules such as PD-L1/2 by the tumour (or TILs), this can indicate that the tumour has been under immune pressure, which has resulted in the

upregulation of these molecules. Increased expression of PD-L1 in breast cancers correlates with increased TILs and it is, again, more prevalent in TNBC and Her2+ molecular subtypes (8).

The increased immunogenicity of TNBC and Her2+ breast cancer subtypes makes them a primary target for novel immunotherapies. However, all breast cancers may be relevant targets for some form of immunotherapy. A recent study of over 10,000 different tumours defined 6 major tumour subtypes: wound-healing, IFNγ enriched, inflammatory, lymphodepleted, TGFβ dominant and immunologically quiet (9). When 944 breast cancer samples were similarly profiled, there were clear immunological features in all breast cancers profiled and none were classified as immunologically quiet (10). This suggests that heterogeneous immunological mechanisms are at play in all breast cancers and more innovative approaches to target these mechanisms are required.

Targeted therapies for breast cancer

One of the first monoclonal antibody (mAb)-based therapeutics was Trastuzumab (Herceptin®), which was developed for Her2+ breast cancer. The principal mode of action was thought to be that the mAb bound to the Her2 receptor and blocked signalling from epidermal growth factor, which would otherwise drive malignant growth of Her2+ tumours. However, it is now apparent that Trastuzumab also augments immune-mediated control of Her2+ tumours in a number of ways, such as mediating antibody-dependent cell-mediated cytotoxicity from natural killer (NK) cells (11) and promoting antibody-dependent cellular phagocytosis from tumour-associated macrophage (12). Trastuzumab also appears to alter the TME to facilitate entry of cytotoxic T cells, with increased frequencies of TILs correlating the frequency of responses in treated patients and increasing survival after treatment (7). Trastuzumab is, therefore, a one example of how recruiting and activating immune cells, such as NK cells and tumour-associated macrophages, may disrupt the TME and be sufficient to increase the immunogenicity of breast cancers. More recently, Trastuzumab has been conjugated to a chemotherapeutic agent, called DM1, to generate a conjugate drug, ado-trastuzumab emtansine. This conjugate uses the specificity of the mAb for Her2 to deliver DM1 directly to the target tumour cell, where undergoes receptor-mediated internalisation to inhibit microtubule assembly and block cell proliferation.

Monotherapy or combined therapy with immune checkpoint blockade (ICB)

ICB has clearly changed the landscape of therapy for tumours with high TMB, such as lung cancers and melanomas. The two current clinical therapeutic modalities are to target and block CTLA-4, which would otherwise block *de novo* CD4 and CD8 T cell activation, and to target and block PD-1/PD-L1/2 binding, which would otherwise inhibit re-activation of exhausted CD8 T cells. While mAbs directed against CTLA-4 or PD-1/PD-L1/2 can be effective as monotherapies for tumours with high TMB, combining each of these modalities together clearly augments responses, likely due to the synergistic effect of combining distinct mechanisms (13).

There have been clinical trials of ICB as a monotherapy for breast cancer, which have demonstrated modest efficacy (14). For example, the anti-PD-1 mAb, pembrolizumab (Keytruda®), has been trialled as a monotherapy in heavily pretreated patients with PD-L1-positive TNBC (15). It generated an overall response rate (ORR) of 18.5% with similar levels of toxicity as was seen in other ICB trials. The anti-PD-L1 mAb, atezolizumab (Tecentriq®), has since been trialled as a monotherapy in patients with metastatic TNBC as a first-line and second-line or greater agent (16). It generated an ORR of 24% as a first line agent, 6% as a second-line or greater agent and increased expression of PD-L1 on immune cells in the tumour correlated with higher ORRs and other outcomes. While such results are promising, the heterogeneity of breast cancers may necessitate targeting multiple inhibitory pathways simultaneously.

With the aim of targeting multiple pathways, there are a number of clinical trials that are actively recruiting to test multiple mAbs targeting the PD-1/PD-L1/2 signalling axis in combination with other inhibitory molecules, such as lymphocyte-activation gene 3 (LAG-3), TIGIT and B7-H4 and cohorts are targeting solid tumours including breast cancer (10). Another way to engage multiple mechanisms is to combine ICB with chemo-therapeutic agents. This may seem paradoxical, as certain chemotherapy regimens can be cytotoxic and deplete immune cells, but the dose and mode of delivery can be optimised to facilitate both cytotoxic and immune effects of a combined ICB/chemotherapy regimen. One recently licenced approach is to combine atezolizumab with a nanoparticle albumin-bound form of paclitaxel, nab-paclitaxel (Abraxane®). The combination of atezolizumab and nab-paclitaxel was compared to placebo and nab-paclitaxel in patients with

untreated metastatic TNBC in the IMpassion130 trial (14). While the addition of atezolizumab lead to modestly more adverse events, it extended overall survival from 17.6 to 21.3 months in all patients and from 15.5 to 25 months in patients with PD-L1+ tumours. Other molecular therapy agents are also in trials as combined approaches with ICB, including poly (ADP-ribose) polymerase inhibitors such as Olaparib, which prevents single-stranded DNA break repair, and cyclin dependent kinase inhibitors such as Abemaciclib (Verzemio®), which inhibits cyclin dependent kinase 4/6 to block cell division (17). Accordingly, the combination of ICB mAbs and other chemo- and molecular therapeutics is one potential pathway towards better control of advanced breast cancer.

Vaccines for breast cancer

Cancer vaccines are in development for a number of tumour targets, but the key challenge for cancer vaccines is antigen selection. There are a small group of TAA that are commonly ectopically or over-expressed in breast cancer, which include Her2, Mucin 1, carcinoembryonic antigen and Wilms tumour 1 (18). However, these are self-antigens, which makes it difficult to break tolerance with endogenous T cell populations and immune responses generated could lead to autoimmunity.

To avoid this challenge, a cancer vaccine could be targeted towards neoantigens, in an approach known as a personalised mutanome vaccine (19,20). This requires DNA sequencing of a patient's tumour, identification of mutation-derived neoantigens that are predicted to generate immunogenic epitopes, based on a patient's HLA haplotype, and formulation of a vaccine based on these epitopes. While cancer vaccine strategies have not yet been licenced for breast cancer, these more personalised vaccines hold significant potential, as demonstrated by robust responses and reduced metastases in patients with melanoma (19,20).

Adoptive cell therapies (ACT) for breast cancer

ACT rely on the activation and expansion of TAA-specific T cells that are then transferred back into the patient. One approach for ACT is to take the TILs and expand them *in vitro* before reinfusing them into a patient that has undergone lymphodepletion. In a recent case study, transfer of expanded TILs was able to induce a complete response in a patient with advanced breast cancer (21). The TIL protocol was modified by first DNA sequencing the patient's tumour, identifying mutations and the resulting putative neoantigens, screening TILs that expanded in response to these neoantigens and then transferring these TILs back into the patient. In addition, this patient also received pembrolizumab to mitigate exhaustion in the transferred TILs. This highly personalised therapy resulted in a remarkable remission but it raises the question of how feasible it will be in the future to define the antigenic landscape of individual tumours. If the aim of personalisation is to tailor the therapeutic to a heterogeneous cancer, such as breast cancer, then it may be an attractive avenue to pursue.

Another approach for ACT is to take peripheral blood mononuclear cells and genetically modify them with a TAA-specific chimeric antigen receptor (CAR) to generate CAR T cells. These are again activated and expanded *in vitro* before reinfusion into the patient. Critically, CAR T cell therapy requires identification and targeting of a TAA and these can be challenging to identify for breast cancer, as described above. In addition, CAR T cells can generate incredibly potent immune responses to low levels of TAA, which can lead to "on target, off tumour" side effects. This was observed with one version of a Her2-specific CAR, which generated a robust immune response directed against low levels of Her2 expression in the lung that was fatal to the patient (22). Approaches to control and tune the sensitivity of Her2-specific CARs to avoid reactivity to low levels of antigen are in development (23), as are CARs directed to other TAAs for breast cancer, such as cMET and mesothelin.

Key barriers and novel solutions for immune-based therapies in breast cancer

While a number of innovative, immune-based approaches are being developed, there are a several universal challenges to implementing immunotherapies in breast cancer; specifically, selecting the appropriate immunotherapy for the tumour, managing the potential toxicity of immunotherapy and ensuring access of immunotherapy to the solid TME.

First, a good example of the importance of selecting appropriate therapies is the enhanced efficacy of anti-PD-1 and -PD-L1 mAb-based treatments in patients with tumours that have high expression of PD-L1 (14-16). PD-L1 is just one molecular marker and, given the heterogeneity of breast cancer and the diversity of immune mechanisms engaged within these tumours, typing of a number of biomarkers will be critical to select the optimal immunotherapy for each patient. Second, managing

toxicity with immune-based therapies is critical. ICBs can drive autoimmunity and ACT can lead to tumour lysis syndrome, cytokine release syndrome and CAR T cell-related encephalopathy syndrome, alongside the desired immune responses (23). Currently, these side effects are managed with corticosteroids and mAbs directed against inflammatory cytokines but the immune cells that drive these toxicities can be difficult to control or eliminate once activated. Finally, the TME in solid tumours is complex and often immuno-suppressive. For some immunotherapies to work effectively, cells may need to migrate into this suppressive environment at high enough frequencies to elicit a biological response.

To address some of these issues, a more recent approach is to use bi-specific antibodies, particularly a form known as bi-specific T cell engagers (BiTEs). Bispecific antibodies and BiTEs can be engineered to bind to a TAA on one end and a T cell target on the other end, to force the association of cytotoxic cells with their tumour cell targets and drive T cell activation *in vivo* (24). For example, blinatumomab (Blincyto®) is a BiTE directed against CD19 as a TAA and CD3 on T cells, and it is currently in clinical use for acute lymphoblastic leukaemia. BiTE therapy can be discontinued if serious complications occur and the small molecular weight of BiTEs allows them potentially greater access into the TME, to activate TILs *in situ*.

More recent work has highlighted that bispecific antibodies may be a powerful adjunct for CAR T cell therapy for solid tumours (25). Effective CAR T cell therapy relies on expansion of CAR T cells *in vivo* after reinfusion: with haematological cancers, this expansion is robust but, with solid tumours, the expansion is minimal, likely due to sequestration of TAA within the immunosuppressive TME. To augment *in vivo* expansion, a bispecific antibody has been engineered to bind to the CAR on one end and to an antigen presenting cell antigen on the other end (25). When co-administered with CAR T cells in a solid tumour model, these antibodies direct interactions between CAR T cells and APCs, to expand the reinfused CAR T cells and eliminate solid tumours. This approach combines targeted mAbs and ACT to generate remarkably robust immunity but the dose of bispecific antibody can be controlled, to control the T cell expansion *in vivo* and manage toxicity.

Summary

A wide array of immunotherapies are currently in development for breast cancer. Key challenges for these therapies include the heterogeneity of breast cancers, which may require more tailored or even personalised formulations. However, the fact the breast cancers are clearly immunologically active, TNBC and Her2+ tumours in particular, highlights their potential as targets of immune-based therapies. Into the future, these approaches will be refined and rationally combined in an effort to improve outcomes for more advanced and metastatic forms of breast cancer.

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