Immunothe[16]rapy in breast cancer: the subtype story

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Abstract
Great hopes have been accorded to the potential of immunotherapy to exploit host anti-tumour immunity and deliver improved survival outcomes. Impressive results in cancers known to be immunogenic have led to a plethora of immunotherapy trials in several cancer types, including breast cancer. Descriptions of tumour-infiltrating lymphocytes in early breast cancer have unravelled the landscape of immunogenicity across the breast cancer subtypes, and provide rationale for investigation into immunotherapeutic approaches. Subsequently, numerous clinical trials have been launched, predominantly with checkpoint blockade. While triple negative and HER2-positive breast cancers appear to be more immunogenic than ER-positive/HER-2 negative breast cancers, responses to checkpoint blockade are still seen in this subtype, suggesting that subtype alone may not be a sufficient predictor of response to immunotherapies. Moreover, tumour-intrinsic contributors towards immunogenicity and immune-evasion are increasingly being explored, as is the ability of conventional therapies to modulate the immune microenvironment. Reports from early phase trials in breast cancer show that while immunotherapeutic approaches may not be suitable for all breast cancer patients, there are promising signs for a potential role of immunotherapy in the treatment of selected breast cancers.

The presence of lymphocytic infiltrates in breast cancer were noted as early as 1922,1 demonstrating that host immune-surveillance has the potential to recognise cells that have undergone neoplastic transformation. More recently, retrospective analyses of prospectively collected tissue samples from clinical trials has served as a means to not only quantify the landscape of tumour-infiltrating lymphocytes in breast cancer, but also to examine its influence on response to treatment and disease outcomes. Together these results suggest anti-tumour immunity can be a key determinant of disease outcomes in breast cancer and provides justification for evaluation of immunotherapeutic approaches.

Compared with several other solid tumours, clinical trials of immunotherapy agents in breast cancer are in the developmental stage. Immune checkpoint blockade, in particular, has demonstrated clinical activity and reasonable safety profiles in early phase trials. Given the modest response rates observed in advanced disease, much consideration has been given to enhanced patient selection with the use of predictive biomarkers and an improved understanding of the relevance of pre-existing anti-tumour immunity. Furthermore, combinations of standard or targeted therapies with checkpoint blockade may have the potential to further improve therapeutic benefits through immune modulation.

This review aims to describe the immune landscape across breast cancer subtypes and summarise results from early phase immunotherapy trials in breast cancer. Finally, we will discuss some of the future directions and the emerging prospects of immunotherapy in breast cancer. While this review focuses predominantly on checkpoint blockade, it should be noted that other promising immune approaches are increasingly being tested in breast cancer, including vaccination.

Immune infiltrates and patterns of pre-existing immunity

Immune infiltrates in breast cancer subtypes
Adaptive T-cell mediated cytotoxic responses have been recognised as a dominant mechanism of host anti-tumour immune responses,2,3 and are thought to arise from the recognition of tumour-specific epitopes (neoantigens), the by-product of expressed somatic cancer mutations.4-6
Investigation into the interaction between host immunity and breast tumours has predominantly been carried out through evaluation of tumour-infiltrating lymphocytes (TILs). Consistent with a key role of adaptive T-cell immunity, lymphocytic infiltrates are most commonly dominated by T cells, with variably lower levels of B cells, NK cells, macrophages, and dendritic cells. Various methodologies have been employed to quantify and characterise TILs in breast cancer, most commonly using light microscopy of haematoxylin and eosin stained sections of tumour samples, but also by immunohistochemistry and gene expression. Each of these methodologies has its own strengths and weaknesses, however the results have been mostly consistent with each other.

While not generally considered a strongly immunogenic tumour, numerous retrospective studies evaluating TILs in prospectively collected tumour samples from early breast cancer have now revealed remarkable diversity in the degree of lymphocytic infiltrates, implying that certain subsets of breast cancer are more immunogenic than others. Among the clinically utilised subtypes, HER2-positive and triple negative breast cancers generally harbour higher TIL levels than ER-positive/HER2-negative breast cancers, suggesting that luminal breast tumours are generally less immunogenic. It should be noted, however, that substantial heterogeneity exists even among luminal tumours, suggesting a subgroup of luminal tumours may be more immunogenic than others.

**Clinical implications of host immunity**

Beyond the mere presence of tumour-infiltrating lymphocytes, the clinical implications of host immunity have been demonstrated by significant associations with prognosis and response to therapies, although this differs by breast cancer subtype. The prognostic significance of TILs has been most consistently shown in the TNBC subtype in the context of anthracycline chemotherapy, but also independently in the HER2-positive subtype in patients undergoing a variety of treatments both with and without HER2-targeted agents. This relationship is linear with increasing percentage of lymphocytic infiltrates associated with improved survival. By contrast, no prognostic significance of TILs has been found in the ER-positive/HER2-negative subtype, although this may be hindered by substantial heterogeneity.

Increasing TIL levels have also been demonstrated to be predictive of pathological complete response (pCR) to neoadjuvant therapy, predominantly in HER2-positive disease with HER2-targeted agents, but also in TNBC. Using statistical interaction terms, some studies have also shown improved disease outcomes to treatment in those with higher levels of TILs. For example, in the FinHER study, those with higher TIL levels had a significantly improved distant disease-free survival benefit to trastuzumab. Taken together, these studies suggest that immune infiltrates can play important roles in maximising the efficacy of specific therapeutic agents.

**Rationale for immunotherapeutic approaches**

Evaluation of TILs in early breast cancer has established that host anti-tumour immunity can exert an influence in disease and treatment outcomes in selected breast tumours, most notably in TNBC and HER2-positive subtypes. Interestingly, the quantity of immune infiltrate appears to be most strongly associated with disease outcomes, implying the amplitude of pre-existing immunity is of key importance. Armed with this knowledge, and spurred on by impressive survival benefits to immunotherapy seen in melanoma, non-small cell lung cancer, and renal cell carcinoma, multiple clinical trials of checkpoint blockade as monotherapy, as well as in combination with standard and targeted therapies, have been launched in specific breast cancer subtypes.

**Checkpoint blockade**

While lymphocytic infiltrates appear to play an important biological role, the diagnosis of breast cancer ultimately represents escape from immune control. Subsequent research into the full spectrum of immune-evasive mechanisms has intensified. In particular, immune checkpoints have emerged as key regulators of self-tolerance and regulation of established immune responses. Checkpoint blockade efficacy is thought to be mediated by re-engagement pre-existing immune responses. There have been relatively few studies of checkpoint blockade with reported outcomes in breast cancer compared with other cancer types - of these, most have focused on TNBC. At the time of this report, there had been no published phase III trials of checkpoint blockade in breast cancer.
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**Anti-CTLA4 checkpoint blockade**

Anti-CTLA4 immunotherapy has been utilised only in small exploratory studies in breast cancer, primarily to investigate changes in peripheral immune cell profiles. The first was a phase I trial of 26 patients with ER-positive advanced breast cancer with the use of tremelimumab in dose-escalation in combination with exemestane.\(^{31}\) No objective responses were reported. However an increase in CD4+ and CD8+ T cells expressing inducible costimulatory was noted, as well as an increase in the ratio of CD4+ and CD8+ T cells to regulatory T cells. In a second study, 18 patients with early breast cancer were treated with either Ipilimumab alone, cryoaulation plus ipilimumab, or cryoaulation alone prior to mastectomy.\(^{32}\) The combination approach with cryoaulation plus ipilimumab was reported to lead to potentially favourable immunological changes in peripheral blood as well as in tumour. While these studies suggest a potential role for anti-CTLA4 checkpoint blockade, the lack of evidence of efficacy thus far has been disappointing. Moreover, toxicity observed with these agents is common, similar to toxicities profiles observed in other studies of anti-CTLA4 treatment. If it is to play a role in the treatment of breast cancer, it appears unlikely to be as a monotherapy.

**Anti-PD1/PD-L1 checkpoint blockade**

Several early phase trials of anti-PD1 and anti-PD-L1 checkpoint blockade as monotherapy have been reported with response rates and toxicity profiles suggestive that anti-PD1/PD-L1 immunotherapies are worthy of further investigation (table 1).\(^{33-36}\) There is significant heterogeneity among these trials with regard to investigational agent used, included breast cancer subtypes, PD-L1 evaluation methodology and eligibility, and prior treatment experience. Overall response rates in these trials varied from 5%-19%, with some responses exhibiting durability, even in patients who have experienced heavy treatment.

In order to improve patient selection, PD-L1 expression has been marked as having a potential role as a predictive biomarker and has been evaluated in all the described early phase trials. In other cancer types, PD-L1 expression has shown some promise as a predictive biomarker of PD-1/PD-L1 checkpoint blockade response, however this has been inconsistent.\(^{26,27,37,38}\) Furthermore, PD-L1 evaluation has been hampered by heterogeneity in methodologies, the use of different cut-off values for positivity, and the use of different tissue types given the inducible nature of PD-L1 (archival tissue versus fresh metastatic biopsies).\(^{39,40}\) Despite this, higher response rates have generally been observed in those with higher PD-L1 expression in early phase checkpoint blockade trials in breast cancer. For example, in the JAVELIN clinical trial,\(^{31}\) using a PD-L1 cut-off value of ≥ 10%, the overall response rate was 33.3% in the whole cohort, and 44.4% in the TNBC cohort, implying that PD-L1 expression could improve patient selection, although this requires validation in larger studies. The ability of TILs to add to PD-L1 in helping to identify responders to PD-1/PD-L1 inhibition as immunotherapy is unknown and under active investigation.

Questions still remain as to whether there are significant differences in response rates and efficacy to checkpoint blockade between the breast cancer subtypes. As previously described, TIL levels are reported to be higher in TNBC and HER2-positive breast cancer compared with the ER-positive/HER-2 negative subtype. Interestingly, PD-L1 expression has shown a similar pattern among breast cancer subtypes.\(^{33,41}\) Despite this, a response rate of 14% was seen in a phase I expansion cohort of single agent pembrolizumab in ER-positive/HER-2 negative metastatic breast cancer,\(^{36}\) demonstrating that PD1/PD-L1 checkpoint blockade can have activity in this breast cancer subtype. It should be noted however, that patients were only eligible if they were PD-L1 positive (using a definition of ≥ 1% expression on tumour cells or any expression in the stroma) - 248 patients were screened with only 19.4% being PD-L1 positive. Taken together, PD1/PD-L1 checkpoint blockade has demonstrated efficacy in selected patients across all breast cancer subtypes. While PD-L1 expression is incompletely predictive of treatment response, it may better predict response than breast cancer subtype. Further studies will be required to confirm these findings.

**Combination therapy with checkpoint blockade**

Given the modest response rates observed in early phase trials, strong consideration has been given to combining conventional and targeted therapies with immune checkpoint blockade to improve outcomes. The rationale behind this approach stems from observations across several studies that mechanisms of response to conventional agents including chemotherapy, radiotherapy and targeted therapies may be partially mediated by immune effects – via modulation of the tumour immune microenvironment, or by stimulation of immunogenicity.\(^{42,43}\) This may occur via a process of enhanced apoptosis of immunogenic cell death, whereby release of tumour neoantigens on tumour cell death.
results in enhanced uptake by antigen presenting cells and subsequent recruitment of effector T cells.\textsuperscript{44,45} Several studies are currently underway investigating the approach of combination chemotherapy or radiotherapy with checkpoint blockade in breast cancer.\textsuperscript{46,47} One early phase trial investigated the combination of taxane chemotherapy with atezolizumab (anti PD-L1 checkpoint blockade) in advanced TNBC,\textsuperscript{48} demonstrating reasonable safety profiles, as well as encouraging response rates, with the highest response rates observed in patients receiving therapy in the first line metastatic setting. Subsequently, a phase III randomised, placebo-controlled trial is currently underway (clinicaltrials.gov identifier: NCT02425891).\textsuperscript{46}

Specific genomic and transcriptomic alterations are increasingly recognised as contributors towards immune escape.\textsuperscript{49} Some of these alterations may be targetable, suggesting a possible personalised approach to immunotherapy with the combination of checkpoint blockade with targeted therapies. In HER2-positive disease for example, trastuzumab and trastuzumab emtansine conjugate (T-DM1) treatment has been observed to be able to induce tumour lymphocytic infiltration.\textsuperscript{50,51} Additionally, combination T-DM1 and checkpoint blockade has demonstrated enhanced efficacy in animal models with primary resistance to immunotherapy.\textsuperscript{51} Subsequently, several studies of PD-1/PD-L1 inhibition in combination with HER2-targeted agents are underway.\textsuperscript{52-54} Similarly, in TNBC, MEK inhibition in mouse models with Ras-MAPK genomic alterations has demonstrated the potential to relieve immune suppression and upregulate interferon gamma-mediated antigen presentation and PD-L1 expression.\textsuperscript{55} Therefore, a rational approach may be the combination of MEK inhibition with PD1/PD-L1 checkpoint blockade in patients with TNBC harbouring genomic alterations in the Ras/MAPK pathway. The role of MEK inhibition in TNBC is currently being explored in a clinical trial in combination with paclitaxel (clinicaltrials.gov identifier: NCT02322814).

Future prospects of immunotherapy in breast cancer

Further research into the intersection between the unique genomic and transcriptomic profiles of breast cancer and anti-tumour immunity will provide a clearer picture of the tumour-intrinsic determinants of immunogenicity and immune-evasion.\textsuperscript{56} This will provide further considerations for optimising combination immunotherapy approaches. Moreover, phenotyping of the constituents of immune responses will allow us to better understand the balance between an effective immune response and a suppressed immune response, including the establishment of multiple immune checkpoints. Several other checkpoint targeted therapies are in drug development and may also be rational combination partners with PD-1/PD-L1 checkpoint blockade.

The timing of immune based treatment warrants important consideration. Metastatic tumour biopsies have generally harbourd lower TIL levels than primary disease,\textsuperscript{57} perhaps through extensive immunoediting, or the establishment of immune-evasive mechanisms. Therefore, highly treatment experienced patients with extensive disease burden are expected to be less likely to respond to checkpoint blockade as a single agent than earlier disease with a lesser extent of disease. Finally, it appears unlikely that all unselected patients will benefit from an immunotherapeutic approach, exemplifying the need to stop biomarkers that may indicate when a patient can be prescribed treatment that will be effective.

Conclusion

Recent breakthroughs in immunotherapy, particularly checkpoint blockade, have led to a great deal of excitement regarding potential therapeutic benefits in all cancer types. Furthermore, detailed quantification and characterisation of the immune microenvironment of breast cancer and its subtypes have provided ample justification for evaluation of immunotherapy in breast cancer. Reports from several early phase trials show response rates that have maintained our enthusiasm. However it has become clear that checkpoint blockade as monotherapy will be insufficient in many patients with advanced breast cancer. To combat this, research efforts are now focusing on the key determinants of immunogenicity, and the factors that contribute towards immune-escape. If these are fully understood, a personalised approach to combination immunotherapy may have great potential to enhance breast cancer outcomes in the right patients.
The study cohort was not selected for by PD-L1 status, however only participants with PD-L1 expression ≥ 5% have been reported. Confirmed overall response rate shown - the highest overall response rate was observed in those undergoing treatment in the first line advanced setting. Abbreviations: TNBC, triple negative breast cancer; PD-L1, programmed cell-death ligand 1; ORR, overall response rate.

### Table 1 - Summary of early phase anti-PD1/PD-L1 trials in advanced breast cancer

<table>
<thead>
<tr>
<th>Trial</th>
<th>Phase</th>
<th>Study population</th>
<th>Number of participants</th>
<th>Study compound</th>
<th>PD-L1 status</th>
<th>ORR</th>
<th>Median duration of response</th>
<th>Median time to response</th>
</tr>
</thead>
<tbody>
<tr>
<td>JAVELIN33</td>
<td>Phase Ib</td>
<td>All subtypes</td>
<td>168</td>
<td>Avelumab</td>
<td>Unselected</td>
<td>4.8%</td>
<td>28.7 weeks</td>
<td>11.4 weeks</td>
</tr>
<tr>
<td>KEYNOTE-012</td>
<td>Phase I</td>
<td>TNBC</td>
<td>32</td>
<td>Pembrolizumab</td>
<td>PD-L1 ≥ 1% of tumour cells or any staining in the stroma</td>
<td>18.5%</td>
<td>Not reached</td>
<td>17.9 weeks</td>
</tr>
<tr>
<td>NCT01375842</td>
<td>Phase I</td>
<td>TNBC</td>
<td>21</td>
<td>Atezolizumab</td>
<td>PD-L1 ≥ 5% of infiltrating immune cells†</td>
<td>19.0%</td>
<td>Not reached</td>
<td>Not reported</td>
</tr>
<tr>
<td>KEYNOTE-028</td>
<td>Phase Ib</td>
<td>ER-positive/HER2-negative</td>
<td>25</td>
<td>Pembrolizumab</td>
<td>PD-L1 ≥ 1% of tumour cells or any staining in the stroma</td>
<td>14.0%</td>
<td>Not reached</td>
<td>8.0 weeks</td>
</tr>
<tr>
<td>NCT01633970</td>
<td>Phase Ib</td>
<td>TNBC</td>
<td>32</td>
<td>Atezolizumab + nab-paclitaxel</td>
<td>Unselected</td>
<td>42.0%</td>
<td>Not reported</td>
<td>Not reported</td>
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References


