Immunotherapy in Breast Cancer

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Introduction

Significance of the Tumor Immune Microenvironment
An important role of the tumor microenvironment and host immune response in breast cancer has been recognized for several decades [1, 2].

The presence of tumor-infiltrating lymphocytes (TILs) has long been linked to a favorable prognosis. However, only our current understanding of breast cancer subtypes has led to the realization that the prevalence of TILs as well as their prognostic and predictive meaning vary between these subtypes.

The highest prevalence of TILs can be observed in triple-negative breast cancers (TNBC) and HER2-positive disease whereas TILs are less abundant in luminal type breast cancers, with the lowest amount of immune infiltration being observed in luminal A-like disease [3].

In TNBC, the presence of TILs is a strong and independent prognostic factor, and several immune gene signatures capturing this immune infiltrate have been shown to correlate with a favorable prognosis in patients without adjuvant chemotherapy [4–6]. In addition, TILs also predict response to neoadjuvant chemotherapy as determined by pathologic complete response (pCR) [7–15].

An international working group has recently published recommendations for the evaluation of TILs, allowing a reliable quantification in local laboratories. Quantification relies on stromal as opposed to intratumoral TILs, although both contain prognostic and predictive information. However, stromal TILs can be quantified more reliably and are more prevalent [16].

In the adjuvant setting, each 10% increase in TILs has been associated with a 19% relative risk reduction for distant recurrence in TNBC. About 10% of TNBC can be categorized as lymphocyte predominant breast cancer [9] whereas 15–20% show no relevant lymphocyte infiltrate. In TNBC patients who do not achieve a pCR, the presence of abundant TILs in the residual tumor, which can be observed in about 10% of cases, predicts a good prognosis even in the case of residual nodal involvement [17].
In HER2-positive breast cancer, TILs and immune gene signatures provide similar prognostic and predictive information; however, the data are more complex, possibly because of the use of HER2-directed antibodies like trastuzumab and pertuzumab which at least in part rely on immunologic effects such as antibody-dependent cellular cytotoxicity to achieve response and might attract new TILs in tumors which were negative before therapy [18–22].

In estrogen receptor (ER)-positive breast cancer, the prevalence of TILs is significantly lower and their impact seems less pronounced at least in low-grade luminal A-like disease [23].

T-lymphocytes, followed by B-cells and macrophages, are the predominant cell types within immune cell infiltrates. However, their composition is a complex interplay and equilibrium of tumor-suppressing as well as tumor-promoting immune cells such as regulatory T-cells (Tregs) and myeloid-derived suppressor cells [16, 24]. Efforts to decipher the composition of the tumor immune microenvironment of breast cancer have revealed a strong co-expression of immunosuppressive (e.g. PD-1, PD-L1, CTLA4, IDO1) as well as immune-activating (e.g. CXCL9, CCL5, CD8A, CD80, CXCL13, IGKC, CD21) markers [25]. All of these markers, including the immunosuppressive markers, are positively correlated with each other and seemingly paradoxically associated with better survival and higher pCR rates.

These observations are in concordance with the immune-editing hypothesis which ascribes a dual role in cancer to the local immune response. It can suppress tumor growth by immune-mediated cell death and slow down tumor growth or cause stagnation. Likewise, the local immune response can create an inflammatory environment which promotes growth of those tumor cells escaping immune surveillance. This hypothesis could explain why even in clinically apparent cancers which escape immune surveillance some degree of control via the immune system is maintained, as well as the favorable prognosis observed in tumors harboring strong immune infiltrates [26].

The Role of Tumor Antigens

Genetic and epigenetic alterations form the basis for the development and progression of tumors and allow the immune system to distinguish tumor cells from their normal counterparts within the body and recognize tumor cells as foreign. This recognition is based on antigens which can either be overexpressed in cancer cells in contrast to a limited or absent expression in normal cells (so-called tumor-associated antigens, TAAs) or be specific to tumor cells, like viral proteins in virus-related cancers (e.g. cervical cancer) or so-called neoantigens caused by non-synonymous mutations. In contrast to TAAs, tumor-specific antigens and neoantigens are not subject to central tolerance in the thymus. In fact, a high mutational load is positively associated with TILs and improved survival [27, 28]. Likewise, tumors caused by long exposure to mutagens (e.g. cigarette smoke and ultraviolet light), such as non-small cell lung cancer (NSCLC) and melanoma, also exhibit the highest non-synonymous mutation burden [29] and are among those with the best responses to novel immune checkpoint inhibitors (see below). Likewise, tumors caused by defects in mismatch repair genes (like Lynch syndrome-associated cancers) or mutations in proofreading DNA polymerases (e.g. POLE mutations in endometrial cancer), which both are associated with a very high mutation burden, also demonstrate superior efficacy of these therapies [30, 31]. However, most non-synonymous mutations do not lead to the generation of immunogenic neoantigens. Such neoantigens have to fulfill several requirements to be effectively recognized by T-cells: the mutated protein has to be expressed and adequately processed into small peptides which finally have to have a sufficiently high affinity to form stable complexes with major histocompatibility complex (MHC) molecules for antigen presentation. In fact, the neoantigens appear to confer more prognostic (and predictive?) information than the overall mutation burden [27, 32–34]. Tumor-specific CD8+ T-cells reacting specifically to the mutated form of the antigen but not the wild type form, which has been observed in patients responding to pembrolizumab and ipilimumab, provide evidence of an important role of these neoantigens in the anti-tumor immune response [30, 35].

Breast Cancer Vaccines

The potential of cancer vaccines is appealing and has been a strong focus of research for decades. Considering this long effort, breast cancer vaccines, except for some encouraging preliminary results, have demonstrated very limited efficacy. A rapidly increasing and improved understanding of tumor immune escape mechanisms and promising strategies to encounter these have raised new hope and led to new strategies.

One of the prerequisites of a tumor vaccine is that it has to be distinguished from self-antigens by the host’s immune system and identified as foreign. It has been recognized that the immune system is capable of recognizing several types of antigens on tumor cells referred to as TAAs.

TAAs are characterized by a strong overexpression in tumor cells compared to normal cells, as described for HER2/neu, MUC-1, CEA, or hTERT, as well as for the so-called cancer/testis antigens which constitute protein antigens with normal expression restricted to adult testicular germ cells and aberrant overexpression in various human cancers [36], and antigens which are mainly expressed during embryonic development.

As these antigens are in principal self-antigens, the major challenge for vaccination strategies based on TAAs is to overcome both central tolerance (eliminating autoreactive T-cells in the thymus during development) as well as peripheral tolerance (whereby mature T-cells are suppressed by regulatory mechanisms such as Tregs, myeloid-derived suppressor cells, as well as immune checkpoints like CTLA-4 and PD1/PD-L1) [33].

Antigens used for vaccination can be administered via several possible platforms, such as peptides, proteins, naked DNA, viral vectors, whole cell-based vaccines (autologous, heterologous), and dendritic cell (DC)-based vaccines. Antigen delivery is mostly combined with the co-administration of an adjuvant to enhance
the response [37]. All of these current vaccine platforms have their advantages and limitations.

Early cancer vaccine trials have focused on short MHC-I-restricted peptides, but it has been realized that the CD8+ T-cell responses elicited by this strategy are mostly weak and short-lived. Therefore, longer peptides and mixtures of epitopes have subsequently been evaluated to trigger both CD4+ and CD8+ responses to optimize CD8+ T-cell response and in addition a humoral immune response [38].

Peptide vaccines have several potential advantages including that they are easy to manufacture, their immune response can be easily evaluated, and they have a favorable toxicity profile [38]. However, most peptide vaccines are restricted to HLA-A2, therefore limiting the number of patients who could potentially benefit.

**Anti-HER2 Vaccines**

HER2/neu constitutes one of the most thoroughly studied TAAs for breast cancer vaccines in clinical trials. In fact, some patients develop spontaneous anti-HER2-specific immunity, either during or prior to conventional adjuvant treatment. The development of a HER2-specific immunity has been linked to improved survival [39].

Several HER2-directed vaccines are currently undergoing clinical development. The HER2 peptide E75 vaccine (nelipepimut-S; NeuVaxTM, Galena Biopharma, San Ramon, CA, USA) (aa 369–377), derived from the extracellular domain, is the most advanced in terms of clinical development. It is limited to HLA-A2+ patients. Recently, the final results from a larger phase I/II trial including 195 women with early breast cancer and a HER2 expression score on immunohistochemistry (IHC) of ≥1 were reported. Patients screened negative for HLA-A2/3 within the trial served as a control group. 5-year disease-free survival (DFS) in vaccinated patients was 89.7% compared to 80.2% among controls (p = 0.08). In patients receiving a booster inoculation (n = 53), the 5-year DFS rate was even higher (95.2%; p = 0.11 vs. controls). These results appear encouraging, and results from the large randomized phase III trial is expected. The vaccine demonstrated some clinical activity, including a complete response [48, 49].

**Anti MUC-1 Vaccines**

MUC-1, a membrane glycoprotein, is frequently found to be overexpressed and aberrantly glycosylated on cancer cells. A soluble form, more widely known as CA15-3, is also used to monitor disease activity in patient sera. The abnormally glycosylated form has been found to be immunogenic and can trigger cytotoxic T-cell responses. The presence of specific antibodies in patients with early breast cancer has been associated with improved survival [43].

A synthetic antigen mimicking the sialyl-Tn carbohydrate epitope found on MUC-1 as well a variety of glycoproteins on tumor cells has been used as a vaccine conjugated to keyhole limpet hemocyanin (KLH) as a carrier. A large randomized phase III trial in patients with metastatic breast cancer either in remission or with stable disease after first-line treatment (n = 1,028) using this vaccine (STn-KLH; TheratopeTM, Biomira, Inc., Edmonton, AB, Canada) has been completed. Although the vaccine produced strong humoral responses, there was no DFS or overall survival (OS) benefit, except for the ER+ subgroup in which a significant OS benefit was observed [44].

A further peptide-derived vaccine based on a 20-amino acid domain from the extracellular portion of MUC-1 fused to oxidized mannan to improve antigen presentation and induce both cytokine and humoral responses has also been evaluated in a small randomized pilot study. Despite the small numbers (n = 31), the vaccine significantly reduced the recurrence rate (60.5 vs. 12%; p = 0.002) [45, 46].

A new vaccine, PANVAC™ (Therion Biologics Corp., Cambridge, MA, USA), targeting both MUC-1 and CEA, uses viral vectors to deliver the antigen together with 3 human T-cell co-stimulatory molecules [47]. In early clinical trials in heavily pretreated patients, the vaccine demonstrated some clinical activity, including a complete response [48, 49].

**Cancer/Testis Antigen Vaccines and Other Breast Cancer Target Antigens**

Cancer/testis antigens are characterized by their selective expression limited to germline cells. NY-ESO-1 and MAGE-A3 have been evaluated as vaccine candidates mostly in melanoma and lung cancer. MAGE-A3 and NY-ESO-1 in breast cancer are preferentially expressed in TNBC. They have been proven to be highly immunogenic, and immune response is associated with TILs and a favorable prognosis [50–53]. These antigens remain potential targets for breast cancer immunotherapy [37].

Other potential target antigens include NY-BR1, Mamoglobin-A, hTERT, and WT-1. Trials using DCs pulsed with hTERT-derived peptides have however failed to elicit anti-tumor immune responses.

**DNA-Based Vaccines**

DNA-based vaccines rely on the uptake of the DNA encoding the selected TAA by antigen-presenting cells (APCs) and its subsequent translation into protein, followed by intracellular processing and presentation. DNA vaccines can be administered as naked DNA, complexed with other molecules, as liposomal formu-
lations, or incorporated into nanoparticles. There is sound evidence that DNA-based vaccines can elicit a coordinated immune response involving humoral, cellular, as well as innate immune effectors. Such a response resembling a ‘physiologic’ immune response is now regarded as the most effective way to achieve tumor rejection. DNA vaccines could be produced on a large scale; however, naked DNA seems to be rather inefficient. Finding suitable vectors remains a challenge. Currently, delivery of TAA-encoding plasmids using electroporation is being investigated and has provided encouraging results [54–56].

**Vaccines Based on Viral Vectors for Antigen Delivery**

Viral vectors, including poxvirus family members, measles, and adenoviral vectors, generally produce longer lasting and broader immunity than either naked DNA or peptide vaccines [37]. They also offer the opportunity to deliver co-stimulatory molecules at the same time to enhance the immune response. In addition to PANVAC™, PROSTVAC™ (Bavarian Nordic, Kvistgaard, Denmark) directed against PSA and containing 3 additional co-stimulatory molecules (TRICOM™: ICAM-1, B7.1, LFA-3) is currently being tested in phase III trials for prostate cancer.

**Autologous Dendritic Cell Vaccines**

DCs constitute the most important APCs and are characterized by high levels of MHC molecules as well as co-stimulatory molecules. One of the major advantages is that DC-based vaccines are not HLA restricted and can stimulate both class I and II responses. For clinical applications, DCs can be generated from blood mononuclear cells, e.g. via apheresis. They can then be loaded with proteins, peptides, and cell lysates, or transfected with TAA-carrying vectors which can further be modified to co-express co-stimulatory molecules. This vaccine platform has been successfully implemented for the treatment of castration-resistant prostate cancer. Sipuleucel-T (Provenge™, Dendreon, Seattle, WA, USA) was the first T-cellular vaccine to receive FDA approval. It consists of autologous APCs, pulsed with prostatic acid phosphatase fused to GM-CSF, which are then reinfused into the patient. In a randomized phase III trial, it significantly prolonged OS by 4 months [57]. An experimental DC-based HER2-targeted version of Sipuleucel-T (Lapuleucel-T; Neuvenge™, Dendreon) has demonstrated limited activity in breast cancer. Several HER2-pulsed DC-based vaccines have been investigated in a variety of settings including ductal carcinoma in situ [58–61]. However, DC-based vaccines remain technically challenging with regards to large-scale manufacturing including in vitro expansion, maturation, and activation [38].

**Adaptive T-Cell Therapy**

Another avenue of cancer immunotherapy is adaptive T-cell transfer which has shown promising results in a variety of tumor entities. The basic principal is to obtain T-cells either from peripheral blood or the tumor itself and to reinfuse them after expansion and activation (e.g. TILs) or genetic modification to create a specificity for the targeted TAAs, like chimeric antigen receptor (CAR) and T-cell receptor (TCR) engineered T-cells. TILs have demonstrated promising clinical activity mainly in melanoma but also in metastatic cervical cancer [62, 63]. TCR and CAR T-cells have demonstrated intriguing results in hematologic malignancies. First early trials which include metastatic breast cancer and target a variety of antigens (e.g. MUC-1, cMET, CEA, HER2) are currently ongoing. Using genetically engineered T-cells is particularly attractive because they are modified to overcome peripheral immune escape mechanisms.

**Potential Role of Neoantigens as Vaccines**

Recent technologic advances with the fast-paced evolution of new sequencing technologies and a rapid decline in sequencing costs have created completely new (as yet unrealized) opportunities to detect large numbers of unique tumor-specific antigens which carry the potential for a personalized vaccination strategy targeting individual alterations in the amino acid sequence of proteins caused by non-synonymous mutations. First clinical trials exploiting such personalized vaccination strategies based on individual patients’ neoantigens as RNA-based vaccines in combination with individual TAA-targeting vaccines are currently under way in primary TNBC (MERIT project: NCT02316457).

However, despite some encouraging early results and a favorable toxicity profile, the clinical efficacy of breast cancer vaccines is still limited. The efficacy of active immunization is mainly impaired by a wide range of tumor immune escape mechanisms which become more and more complex as the disease progresses. In situations of large tumor burden and widespread disease, it is conceivable that vaccines alone will not suffice to overcome the immune tolerance mechanisms of the tumor. Mechanisms which normally ensure self-tolerance, such as Tregs and immune checkpoints like CTLA-4 and PD1/PD-L1, are frequently exploited by cancer cells to escape the host immune system. These can be therapeutically targeted, and combinations of antibodies against immune checkpoints or therapies depleting Tregs with vaccines have a strong rationale and might create synergy. It has also become clear that cancer vaccines should tackle several components of the immune system to elicit a more complete’ immunologic response, which could lead to more effective tumor regression. In addition, for patients with advanced disease and a large tumor burden, the combination of vaccines with conventional systemic therapies might be required to achieve satisfying clinical results.

**Immune Checkpoint Inhibition in Breast Cancer**

The activation of T-cells requires 2 distinct signals. The first signal is delivered by the interaction of an antigen-specific TCR and the MHC/antigen complex. TAAs or neoantigens are released from...
dying cancer cells. After their uptake by APCs such as DCs, they are processed into small peptides which are presented on MHC class I and II molecules to CD8+ and CD4+ T-cells to elicit an anti-tumor immune response. The binding of B7 molecules on the APCs to CD28 on T-cells delivers the second positive signal for antigen-specific T-cell activation. CTLA-4, which is physiologically upregulated upon T-cell activation, provides feedback by delivering an inhibitory signal to the T-cell upon binding to B7 molecules. This serves as an important mechanism to control physiologic T-cell activity. At the APC/T-cell interface, an array of co-stimulatory and inhibitory molecules have been identified, which is important for controlling T-cell responses. This negative immune checkpoint has been successfully exploited as a therapeutic target for anti-CTLA-4 antibodies. Ipilimumab, an antagonist antibody against CTLA-4, was the first immune checkpoint inhibitor to be approved for the treatment of metastatic melanoma.

The PD-1/PD-L1/2 pathway constitutes a second major counter-regulatory pathway. The PD-1 receptor on T-cells binds to its cognate ligands, PD-L1 and PD-L2, which are expressed on the tumor as well as immune cells within the tumor microenvironment. The binding leads to a shutdown of T-cells within the tumor during the effector phase. Monoclonal antibodies directed against PD-1 as well as PD-L1, e.g. pembrolizumab and nivolumab, have been approved for the treatment of a variety of metastatic solid tumors such as melanoma and NSCLC, and clinical trials continue to provide evidence of efficacy in a growing number of tumor entities and hematologic malignancies. The remarkable results observed in melanoma and NSCLC have set the ground for a race in clinical development in most tumor entities. A lot of effort has been put into evaluating PD1 and PD-L1 as predictive biomarkers for benefit from PD-1/PD-L1 targeted therapies. The focus has been on PD-L1 expression, as PD1 expression can be found on a variety of different cell types, including CD4+, CD8+ T-cells, B-cells, Tregs, and natural killer cells, and its predictive value is considered limited.

However, there are several methodological concerns with regard to the determination of PD-L1 expression. First, so far, there are no standardized detection methods, and correlative biomarker studies in different clinical trials have used different antibodies for detection by IHC as well as variable cut-offs for positivity. In breast cancer, using the same cut-off for PD-L1 positivity, positivity rates within the same breast cancer subtype differ by as much as 30% (19.4 vs. 55.4%) [64]. This is paralleled by the observation that there is only limited concordance between IHC- and mRNA-based determination of PD-L1 expression. Furthermore, although in several cancer types significant association between PD-L1 expression and benefit from immune checkpoint inhibitors has been observed, in all of these studies some degree of benefit in tumors deemed PD-L1-negative has consistently been seen, currently limiting the use of IHC-based PD-L1 to select patients for immune checkpoint inhibitor therapies. The strongest PD-L1 expression can be found on infiltrating immune cells as opposed to cancer cells, and this seems to play the most important predictive role. Therefore, to regard PD-L1 expression on tumor cells as the main mechanism of immune escape is far too simplistic [3].

So far, preliminary results of 5 phase I trials of PD-1/PD-L1 inhibitors have been published in abstract form as well as 1 report from an anti-CTLA-4 trial. Preliminary results from early trials of immune checkpoint inhibitors are summarized in table 1.

Table 1. Preliminary results of early trials of PD-1/PD-L1 antagonists in metastatic breast cancer

<table>
<thead>
<tr>
<th>Target</th>
<th>Agent</th>
<th>Subtype</th>
<th>Selected for PD-L1 positivity (cut-off, %)</th>
<th>PD-L1 positive patients, %</th>
<th>Patients evaluable for efficacy, n</th>
<th>ORR, %</th>
<th>Reference</th>
</tr>
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<tr>
<td>Anti-PD-1</td>
<td>pembrolizumab</td>
<td>TNBC</td>
<td>yes (≥ 1%)</td>
<td>59</td>
<td>27</td>
<td>18.5</td>
<td>[66]</td>
</tr>
<tr>
<td></td>
<td>pembrolizumab</td>
<td>ER+/HER2-</td>
<td>yes (≥ 1%)</td>
<td>19</td>
<td>25</td>
<td>12</td>
<td>[64]</td>
</tr>
<tr>
<td>Anti-PD-L1</td>
<td>atezolizumab</td>
<td>TNBC</td>
<td>yes (≥ 5%)</td>
<td>21</td>
<td>24</td>
<td>24</td>
<td>[67]</td>
</tr>
<tr>
<td></td>
<td>atezolizumab+</td>
<td>TNBC</td>
<td>no</td>
<td>24</td>
<td>41.7 (confirmed)</td>
<td>70.8 (unconfirmed)</td>
<td>[71]</td>
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<tr>
<td></td>
<td>nab-paclitaxel</td>
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<td></td>
<td></td>
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</tr>
<tr>
<td></td>
<td>avelumab</td>
<td>all (100%)</td>
<td>no</td>
<td>168</td>
<td>4.8</td>
<td></td>
<td>[68]</td>
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<tr>
<td></td>
<td></td>
<td>TNBC</td>
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<td>58</td>
<td>8.6</td>
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<tr>
<td></td>
<td></td>
<td>ER+/HER2-</td>
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<td>72</td>
<td>2.8</td>
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<td></td>
<td></td>
<td>HER2+</td>
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<td>26</td>
<td>3.8</td>
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</tr>
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<td></td>
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<td></td>
<td></td>
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<tr>
<td></td>
<td>PD-L1 high (≥ 10% immune cells)</td>
<td>12</td>
<td>33.3</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>TNBC + PD-L1 high (≥ 10% immune cells)</td>
<td>9</td>
<td>44</td>
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</table>

ORR = Overall response rate; TNBC = triple-negative breast cancer; ER = estrogen receptor; HER2 = human epidermal growth factor receptor 2; PD-1 = programmed cell death-1; PD-L1 = programmed cell death ligand 1.
Anti-CTLA-4 Antibodies in Breast Cancer

So far, there is only very limited data on CTLA-4 blockade in breast cancer. Vonderheide et al. [65] published results from a phase I trial including 26 patients with ER-positive metastatic breast cancer treated with tremelimumab in combination with exemestane. Stable disease lasting for ≥12 weeks could be observed in 11 patients as the best overall response. Several trials using CTLA-4 antibodies either as single agents or in combination with PD-1/PD-L1 targeted therapies are currently ongoing.

Anti-PD-1 Antibodies

The safety and efficacy of single agent pembrolizumab, a monoclonal anti-PD-1 antibody, has been investigated within the phase Ib KEYNOTE-012 trial in patients with metastatic TNBC. The rationale for early trials of PD-1/PD-L1 blockade in breast cancer to focus on TNBC is based on the putative higher genetic instability with presumed higher mutational load and neoantigens. In addition, TNBCs contain larger numbers of TILs, and there is a huge unmet need in this subtype. Patients enrolled in the trial were selected for PD-L1 positivity by a threshold of ≥1% using the 22C3 antibody. 59% of the 111 screened patients were positive. Pembrolizumab was administered at 10 mg/kg every 2 weeks. In the 27 patients evaluable for efficacy, an objective response rate of 18.5% could be observed at the first report, including 1 complete response. An additional 26% of patients were reported to have had stable disease as their best response. The median duration of response had not been reached at the time of presentation of the results, reflecting the durability of responses observed within the trial. Treatment-related adverse events (AEs) were mostly mild and manageable and included 5 grade 3/4 events. However, 1 treatment-related death due to disseminated intravascular coagulation was also reported, as were the typical immune-related AEs [66].

Based on these results, the phase II KEYNOTE-086 is currently recruiting patients with metastatic TNBC, and a large phase III trial (KEYNOTE-119) is in preparation.

In addition, the phase Ib KEYNOTE-028 trial investigated the efficacy of pembrolizumab in patients with ER+/HER2-, PD-L1-positive metastatic breast cancer. Using the same antibody and cut-off for PD-L1 positivity, only 19% (n = 48) of the screened population (n = 248) tested PD-L1-positive, reflecting the differences in PD-L1 expression within the tumor and its environment between luminal disease and TNBC. The reported response rate within the 25 patients enrolled and evaluable was 12%. All of the 3 responders remained on study treatment for more than 26 weeks, with the median time of response not yet reached at the time of presentation of the study at the 2015 San Antonio Breast Cancer Symposium [64].

Anti-PD-L1 Antibodies

The monoclonal anti-PD-L1 antibody atezolizumab (MPDL3280A) has been evaluated for efficacy and safety in patients with PD-L1-positive metastatic TNBC. The trial at the time of reporting had enrolled 27 patients in the TNBC cohort, selected for PD-L1 positivity defined as IHC staining on at least 5% of immune cells using the SP142 antibody. Subsequently, patients unselected for PD-L1 expression were enrolled, but results for this cohort have not been presented. 69% of screened TNBC patients tested positive for PD-L1. Atezolizumab was administered at doses of 15 or 20 mg/kg or a fixed dose of 1,200 mg every 3 weeks. The 27 patients enrolled were heavily pretreated, with 85% of them having received more than 4 lines of prior systemic therapy. The trial reported a 24% response rate for the 21 evaluable patients, including 3 partial and 2 complete responses. Median duration of response had not been reached [67].

An additional phase I trial, which was recently reported in abstract form at the 2015 San Antonio Breast Cancer Symposium, investigated the combination of atezolizumab and nab-paclitaxel in metastatic TNBC unselected for PD-L1 expression. The trial included 32 patients, 24 of which were assessable for efficacy. The confirmed overall response rate (ORR) within the trial was 41.7% and the investigator-assessed unconfirmed ORR 70.8%. Considering that 87% of patients had received prior taxanes and that the response rate in patients treated in third or further line was still 28.6%, these early results are encouraging. A randomized phase III trial of nab-paclitaxel in combination with either atezolizumab or placebo as first-line therapy for patients with TNBC is currently ongoing (IMpassion130, NCT02425891).

The monoclonal anti-PD-L1 antibody avelumab has been evaluated for efficacy in patients with metastatic breast cancer unselected for subtype or PD-L1 expression within the expansion phase of the solid tumor phase Ib JAVELIN trial. The trial recruited 168 patients, including 34.5% patients with TNBC, 42.9% ER+/HER2- patients, and 15.5% HER2+ patients, as well as 7.1% with unknown subtype. Patients received single agent avelumab (10 mg/kg every 2 weeks) until disease progression. The ORR for the entire study cohort was only 4.8%. Within the specific subtypes, the ORR was 8.6% for TNBC patients, and 2.8 and 3.8% for ER+/HER2- and HER2+ patients, respectively. Exploratory analyses suggested higher efficacy in patients with PD-L1-positive infiltrating immune cells (cut-off >10%). However, this subgroup included only 12 out of 124 patients evaluable for PD-L1 expression [68].

To date, more than 50 clinical trials are ongoing or about to start investigating immune checkpoint inhibitors in breast cancer including combinatorial immune checkpoint blockade strategies, as well as novel agents (e.g. durvalumab), targets (e.g. anti-CSF1R, CD27 agonists, LAG3), and trials in the neoadjuvant setting [3].

Conclusion

Immune checkpoint inhibitors targeting PD-1 and PD-L1 have demonstrated clinical activity in metastatic breast cancer, with response rates ranging from 5 to 24%, varying by subtype and PD-L1 positivity. Durability of response reported in other tumor entities has also been observed in breast cancer. PD-L1 positivity might enhance the chance of benefiting; however, patients with PD-L1-negative tumors may also respond. Despite the encouraging signals from these early trials, most patients treated with single agent antibodies targeting the PD-1 pathway do not respond. Thus, addi-
tional predictive biomarkers are crucial to select patients for the best treatment strategies. In addition, intelligent combinatorial strategies have great potential to enhance efficacy. Such strategies include the addition of standard therapies (e.g. chemotherapy, radiotherapy) to PD-1/PD-L1 blockade in patients with larger tumor burden as well as the combination of different checkpoint inhibitors like CTLA-4 and PD-L1 antibodies, which has recently been tested for metastatic melanoma with remarkable efficacy compared to single agent CTLA-4 blockade alone [69, 70]. Further attempts to enhance the efficacy of immune checkpoint inhibition follow the strategy of increasing the number of TILs, like breast cancer vaccines and adoptive T-cell therapies, and of depleting or blocking immunosuppressive cells from the tumor microenvironment, and are currently under investigation.

**Disclosure Statement**

Dr. F. Marmé has received honoraria and travel grants from Roche, Novartis, AstraZeneca, PharmaMar, Amgen, Genomic Health, Pfizer, and Eisai.

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Borresen-Dale AL, Boyault S, Burkhardt B, Butler AP, 
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