Immunotherapy and targeted therapy combinations in metastatic breast cancer

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Immunotherapy is emerging as a new treatment modality in breast cancer. After long-standing use of endocrine therapy and targeted biological therapy, improved understanding of immune evasion by cancer cells and the discovery of selective immune checkpoint inhibitors have created novel opportunities for treatment. Single-drug therapies with monoclonal antibodies against programmed death-1 (PD-1) and programmed death ligand-1 (PD-L1) have shown little efficacy in patients with metastatic breast cancer, in part because of the low number of tumour-infiltrating lymphocytes in most breast cancers. There is growing interest in the development of combinations of immunotherapy and molecularly targeted therapies for metastatic breast cancer. In this Personal View, we review the available data and ongoing efforts to establish the safety and efficacy of immunotherapeutic approaches in combination with HER2-targeted therapy, inhibitors of cyclin-dependent kinases 4 and 6, angiogenesis inhibitors, poly(ADP-ribose) polymerase inhibitors, as well as chemotherapy and radiotherapy.

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Introduction

The 2018 Nobel Prize in Physiology or Medicine was awarded to Dr James P Allison and Dr Tasuku Honjo for their respective discoveries of the immune checkpoint proteins cytotoxic-T-lymphocyte-associated antigen 4 (CTLA-4) and programmed death-1 (PD-1).[1] The discovery of immune checkpoints, which regulate immune activation, and their successful inhibition with monoclonal antibodies has led to a surge in new investigational therapies for the treatment of solid tumours and haematological malignancies.[2,3] Although patient survival has improved over the past two decades, metastatic breast cancer is still incurable with the currently available treatment modalities, including surgery, radiotherapy, chemotherapy, endocrine therapy, and targeted biological therapy. In patients with breast cancer, novel approaches that harness the immune system are showing promising results in combination with other therapies. Hormone receptor (HR)-positive breast cancer, the most common subtype, is a predominantly immunologically cold tumour because of the low number of tumour-infiltrating lymphocytes (TILs) associated with it and its low mutational burden. Tumours positive for HER2 (also known as receptor tyrosine-protein kinase erbB-2) respond well to HER2-targeted therapies, and this effect is partly mediated by immune effector mechanisms.[4] Triple-negative breast cancer , which is considered to be the most immunogenic subtype, is emerging as a major area of development for immuno-oncology therapeutics. Ongoing clinical trials are testing many immunotherapies and targeted therapeutic combinations in all subtypes of breast cancer with the goal of improving survival rates and ultimately curing this devastating disease.

High TIL counts and immune-related gene expression signatures in the primary tumour are consistently associated with better survival in triple-negative breast cancer, HER2-positive, and high-risk HR-positive breast cancers, irrespective of whether the patient received systemic adjuvant therapy.[5–9] Additionally, high TIL counts are associated with greater sensitivity of the tumour to chemotherapy, as shown by the high pathological complete response to preoperative chemotherapy in patients with primary breast cancers that have a high immune cell infiltrate.[10–12] These studies also showed that triple-negative breast cancer has the highest immune cell infiltration when compared with the other breast cancer subtypes. Although infiltration varies between individuals, low-grade, HR-positive cancers have the lowest TIL counts. The advent of effective immunotherapies allows testing the causal relationships behind these associations. Five large randomised trials are currently assessing the efficacy of drugs targeting PD-1 (NCT03036488 and NCT02954874) and programmed death ligand-1 (PD-L1) (NCT03197935, NCT03281954, and NCT02926196), in combination with standard neo-adjuvant (preoperative) or adjuvant (postoperative) chemotherapies in early-stage triple-negative breast cancer.

The contributions of the immune micro-environment to the clinical course of metastatic breast cancers are less clear and less extensively studied, owing to the small number of metastatic specimens available. However, several small studies reported lower TIL counts[13,14] and PD-L1 expression[15,16] in metastatic lesions compared with primary tumours, except for lymph node metastases, which have significantly higher TIL counts and PD-L1 expression than visceral metastases.[17] A study that paired primary and metastatic breast cancers also reported lower expression of immunotherapy drug targets, chemotactic and immune-cell-activating cytokines, and decreased antigen-presenting machinery, along with upregulation of immunosuppressive molecules in metastatic breast cancer tissues.[18] These results suggest that metastatic breast cancers are more immune-depleted and inert to immune activation than primary tumours. This inert micro-environment in metastatic lesions is consistent with the immunity-editing model of cancer progression,[19] which postulates that cancers evolve through continuous interaction with the immune system, leading to eventual escape from immune control. Indeed, an inverse relationship between higher expression of genes related

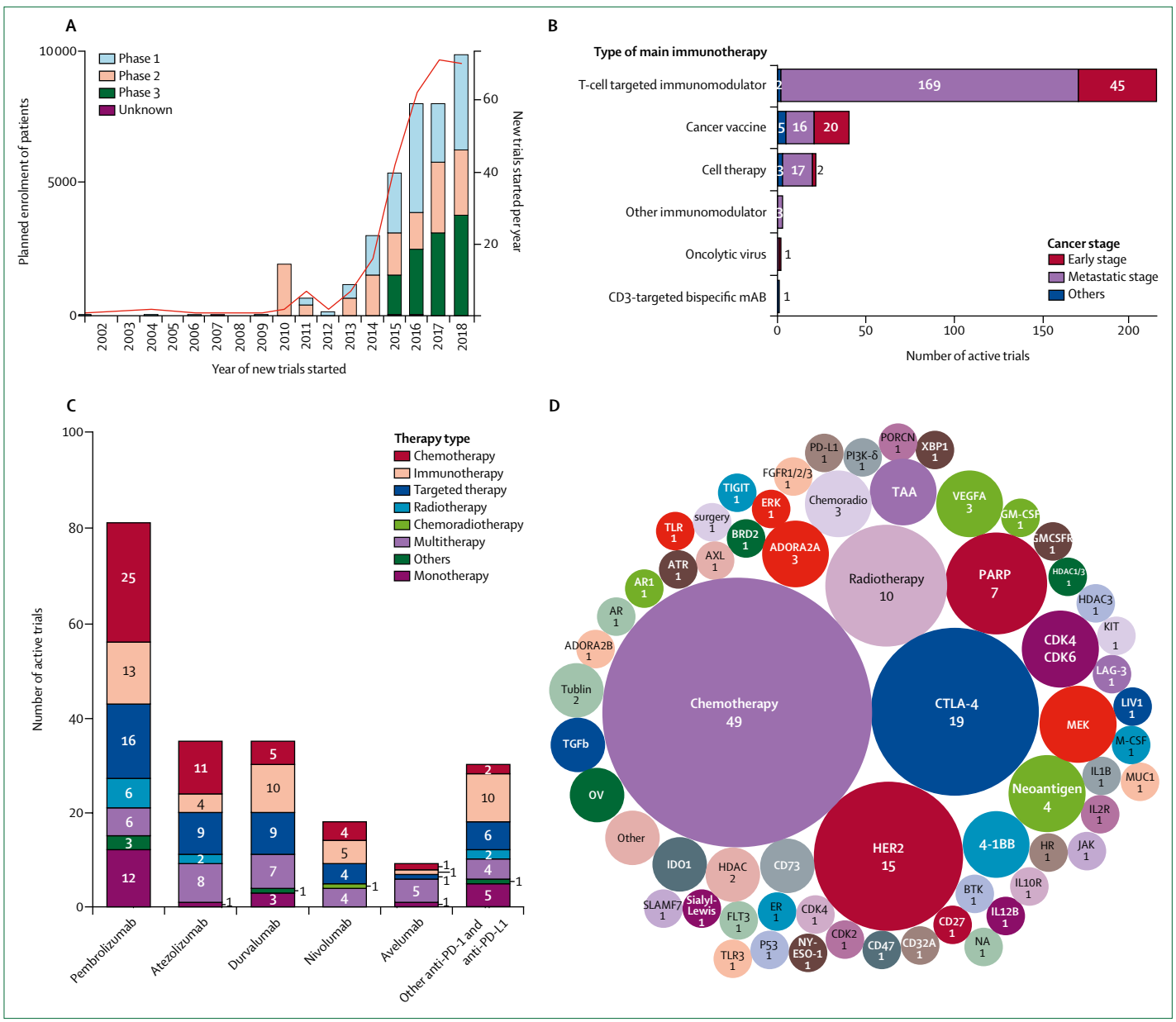

Figure 1: **Immuno-oncology trials in breast cancer**
(A) Bars are numbers of patients, red line shows numbers of trials. Growth of the number of immunotherapy trials in breast cancer over time. (B) The landscape of trials by disease stage and immunotherapy category by cancer stage. (C) Immuno-oncology combination trials by therapy type. 208 trials involve targeting of PD-1 and PD-L1, including 185 combination trials. (D) Trials of anti-PD-1 and anti-PD-L1 compounds in combination with other therapies. The number in each bubble indicates the number of active PD-1 and PD-L1 combination trials testing each target. Multitherapy refers to trials that evaluate three or more drugs together, including one immune checkpoint inhibitor and at least two drugs from any other drug classes. mAb=monoclonal antibody. PD-1=programmed death-1. PD-L1= programmed death ligand-1.

to immunity, and lower clonal heterogeneity and mutational load in primary triple-negative breast cancer has been demonstrated.[20,21] This relationship can be explained by an active antitumour immune surveillance system in immune-rich primary breast cancers that continuously eliminates cancer cells, leading to lower clonal heterogeneity and simpler genomes in the surviving cancer cells. This notion predicts that metastatic lesions, which have escaped immune surveillance at the primary tumour site, will have lower immunogenicity and greater clonal heterogeneity than the corresponding primary tumour. Another reason for the lower immunogenicity of metastatic disease in clinical trials assessing metastatic cancers could be the fact that immune-activated tumours have a better response to primary chemotherapy, so that the remaining immuno-

logically cold tumours are enriched in metastatic clones that have been treated previously.

The landscape of immunotherapy breast cancer trials

The development of immunotherapy drugs in breast cancer has been slower than in other cancers, largely because initial phase 1 trials noted remarkable responses in melanoma and lung cancer that led to rapid extension studies in these cancer types, so that breast cancer became a lower priority for drug development. Also, despite extensive research on the prognostic value of immune cells in early-stage breast cancer, several vaccine and cytokine studies in the 1980–90s did not identify any prognostic factors, leading to the assumption that breast cancer is immune-inert.[22,23] However, the number of clinical trials of immunotherapies for breast cancer and other solid-tumour cancers has grown rapidly in the past 6 years. The main reason for this increase is the potential of immunotherapy as a general novel approach to cancer treatment, particularly with the outlook towards metastatic breast cancer, which is incurable with conventional treatments. As of September, 2018, 285 trials open to patients with breast cancer are assessing some form of immunotherapy, either as monotherapy or in combination with other treatments (figure 1). Overall, these trials aim to recruit 38 424 patients, and most target patients with metastatic cancer. However, many trials recruit patients with other types of cancer and therefore the total number of 38 424 patients could overestimate the planned recruitment number of patients with breast cancer. 216 (76%) of the 284 studies are testing T-cell-targeted immunomodulators, predominantly drugs targeting PD-1 and PD-L1 (figure 1).[24] Of 208 trials evaluating anti-PD-1 and anti-PD-L1 compounds, most (n=185) are combination trials with other cancer therapies, such as chemotherapies (n=48), immunotherapy drugs (n=43), and targeted therapies (n=45; figure 1). Although trials are predominantly investigating drugs that target PD-1 and PD-L1, 281 trials are testing a total of 37 different immune targets (figure 2). The leading targets are PD-1, PD-L1, HER2, and tumour-associated antigens. Only a few immunotherapy trials of breast cancer have reported preliminary or final results.

Monotherapies targeting PD-1 and PD-L1

The various anti-PD-1 and anti-PD-L1 antibodies used in clinical trials have subtle differences in their molecular structure. For example, pembrolizumab is a humanised IgG4 monoclonal antibody, whereas nivolumab is a fully human monoclonal IgG4; durvalumab is a fully human monoclonal IgG1-κ, whereas avelumab is a fully human monoclonal IgG1-λ. To what extent these differences translate into different profiles of clinical efficacy and toxicity is unknown. Overall, the data available to date suggest broadly similar efficacy and toxicity with all antibodies targeting the PD-1 axis.

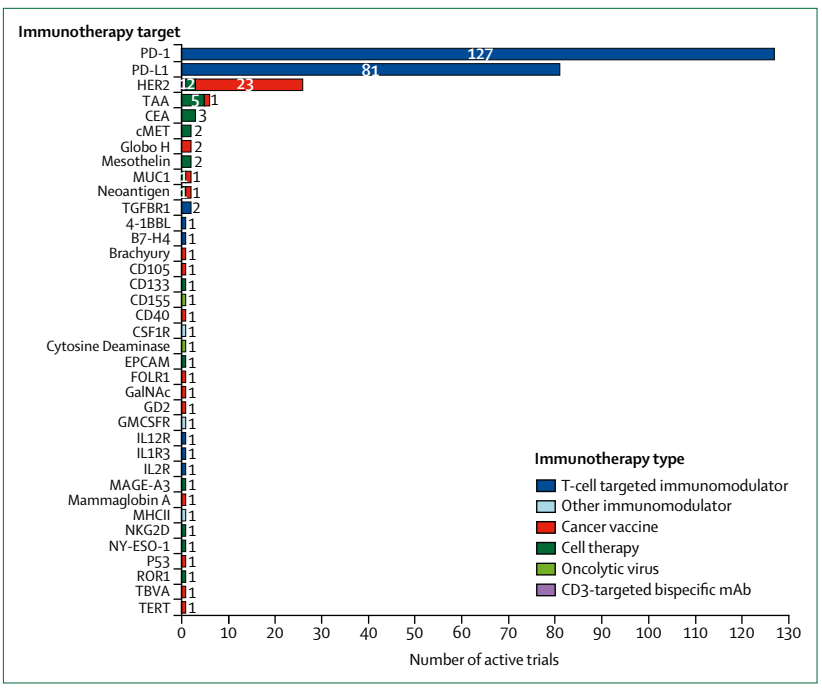

Figure 2: **Immune targets of all immunotherapy trials in breast cancer**
mAb=monoclonal antibody. PD-1=programmed death-1. PD-L1=programmed death ligand-1. TAA=tumour-associated antigens.

Five phase 1 and phase 2 trials reported preliminary results on the activity of anti-PD-1 or anti-PD-L1 drugs as monotherapy in different subtypes of breast cancer.[25–29] The proportions of patients who achieved an objective response ranged from 5% to 24%, which is similar to what is observed in other cancers, with many of the responses long-lasting.[25–27] The KEYNOTE-012 trial assessed the safety and efficacy of pembrolizumab (anti-PD-1 antibody) in metastatic triple-negative breast cancer with PD-L1 expression on at least 1% of either immune or tumour cells, as detected by immunohistochemistry with the anti-PD-L1 22C3 antibody.[25] In 27 patients who were evaluable for efficacy assessment, five (19%) achieved an overall response. Conversely, the KEYNOTE-086 trial examined the safety of pembrolizumab in previously treated metastatic triple-negative breast cancer, irrespective of PD-L1 expression (cohort A, n=170), and reported that a substantially lower proportion of patients (nine [5%]) than those in the KEYNOTE-012 trial achieved an overall response.[26,30] Cohort B of the KEYNOTE-086 trial assessed pembrolizumab as a first-line therapy for patients with PD-L1-positive metastatic triple-negative breast cancer.[28,31] Four (3%) of 84 enrolled patients achieved a complete objective response and 14 (7%) achieved a partial response, so total objective response was 21%. As seen in other immunotherapy trials, some responses were durable, compared with the transient responses usually seen with chemotherapy. The median duration of the response was 10·4 months (range from 4·2 to 19·2), with some responses still ongoing at data cutoff. The KEYNOTE-028

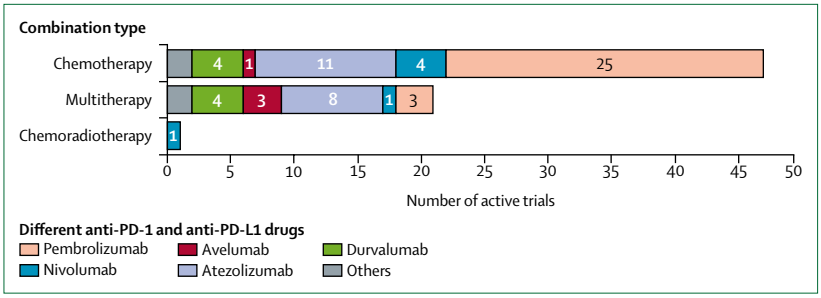

Figure 3: **Combinations of anti-PD-1 and anti-PD-L1 drugs and chemotherapy in clinical trials of breast cancer**
Multitherapy refers to trials that evaluate three or more drugs, including an immune checkpoint inhibitor and at least one chemotherapy drug. PD-1=programmed death-1. PD-L1=programmed death ligand-1.

trial assessed pembrolizumab in PD-L1-positive, metastatic, HR-positive breast cancers and reported a partial response in three (12%) of 25 patients who were evaluable for efficacy.[27] Additionally, results are pending from KEYNOTE-119 (NCT02555657), a large, ongoing, randomised trial comparing pembrolizumab as monotreatment with chemotherapy chosen by the physician as a second-line or further treatment in metastatic triple-negative breast cancer.

A phase 1 trial tested the efficacy and safety of atezolizumab (anti-PD-L1) in metastatic triple-negative breast cancer.[32] 11 (10%) of 115 evaluable patients achieved an overall response, which varied depending on the number of previous chemotherapy treatments. Five (24%) of 21 patients who received atezolizumab as a first-line therapy had an overall response, but only six (6%) of 94 patients did if they received the antibody as second-line or further treatment. Patients with at least 1% expression of PD-L1 on immune cells as detected by the SP142 antibody (11 [12%] of 91) responded better and survived longer (median overall survival of 10 months) than those with cancers negative for PD-L1 (none of 21, median overall survival of 6 months). In this study, expression of PD-L1 in tumour cells had no predictive value towards response to treatment or survival. However, high numbers of immune cells in tumours were independently associated with higher overall response and longer overall survival, whereas high tumour burden and liver metastasis were associated with a smaller treatment benefit.

The JAVELIN study included all breast cancer subtypes regardless of their PD-L1 status and tested the antitumour activity of avelumab (anti-PD-L1).[33] In the triple-negative breast cancer cohort (n=58), three (5%) patients achieved an objective response. In the oestrogen and progesterone receptor-positive, HER2-negative (n=72) cohort, two (3%) patients responded to treatment but none (0%) did in the HER2-positive (n=26) cohort. A significant relationship between PD-L1 positivity and a better objective response could not be established. In this study, estimating PD-L1 expression was based on the percentages of tumour cells expressing PD-L1 at 1% and 5% thresholds with any staining intensity and a 25% threshold with moderate-to-high staining. In addition, dense aggregates of tumour-associated immune cells (identified as non-malignant cells based on morphology) adjacent to tumour cells were evaluated using a defined threshold of 10% of immune cells expressing PD-L1 at any staining intensity.

Overall, these results show modest single-drug activity for all three antibodies in metastatic breast cancer and suggest greater activity in triple-negative breast cancer, in PD-L1-positive cancers, and in the first-line therapy setting.

Targeting PD-1 and PD-L1 in combination with chemotherapy

The modest activity of monotherapy approaches in early studies led to the initiation of clinical trials that added immune checkpoint inhibitors to commonly used chemotherapies. Despite the fact that chemotherapy results in an immunocompromised state in patients, strong preclinical rationale support the investigation of such combinations.[34] Preclinical in-vivo studies in animal models and clinical studies showed complex drug-dependent and dose-dependent interactions between chemotherapy and the immune system that could be used to induce synergy between cytotoxic drugs and immunotherapy. In cancer models, cellular injury induced by doxorubicin and cyclophosphamide elicits a strong cytotoxic immune response that partially mediates the response to treatment.[35] Paclitaxel also induces pro-inflammatory cytokine secretion in macrophages, leading to recruitment and activation of dendritic cells, natural killer cells, and T cells.[36] Chemotherapy can also inhibit myeloid-derived suppressor cells and FOXP3 regulatory T cells.[37] Paclitaxel, cisplatin, and doxorubicin can induce upregulation of mannose-6-phosphate receptors on tumour cells, leading to increased permeability for granzyme B.[38] Several chemotherapies induce NKG2-D type II integral membrane protein (also known as killer cell lectin-like receptor K1) ligand expression on tumour cells and therefore enhance their susceptibility to lysis mediated by natural killer cells.[39] Perhaps not surprisingly, the most common combination trials of immune checkpoint inhibitors involve chemotherapy. In 2018, 77 breast cancer trials were evaluating combinations of chemotherapy plus one or more immunotherapies, and 69 of these trials combined anti-PD-1 and anti-PD-L1 antibodies with chemotherapy (figure 3). Pembrolizumab is the most common drug tested in chemotherapy combination trials. Only a few have reported outcomes to date.

Atezolizumab in combination with nanoparticle albumin-bound paclitaxel (nab-paclitaxel) was evaluated in a phase 1 study (n=33) of metastatic triple-negative breast cancer.[40] 13 (39%) of the 33 patients achieved an overall response; 13 patients received the drug combination as a first-line treatment and seven (54%) of them achieved an overall response, which is somewhat higher than historical response rates (around 30–40%)[41] with first-line nab-paclitaxel alone. In the pivotal phase 3

trial IMpassion130,[42] 902 patients with triple-negative breast cancer were randomly assigned to atezolizumab plus nab-paclitaxel or placebo plus nab-paclitaxel as a first-line therapy for metastatic disease. Each group enrolled 451 patients and median follow-up was 12·9 months. In patients with PD-L1-positive tumours, median progression-free survival was 7·5 (95% CI 6·7–9·2) months for the atezolizumab treatment group and 5·0 (3·8–5·6) months for the placebo group (hazard ratio 0·62 [95% CI 0·49–0·78]). In these patients, median overall survival for the atezolizumab group was 25·0 (22·6 to not estimable) months and for the placebo group it was 15·5 (13·1–19·4) months (hazard ratio 0·62 [0·45–0·86]). No new adverse effects were identified. However, more patients had to discontinue therapy because of side-effects in the atezolizumab group than in the placebo group (72 [16%] of the 452 patients who ended up receiving atezolizumab plus nab-paclitaxel and were evaluable, and 36 [8%] of the 438 who received placebo plus nab-paclitaxel and were evaluable). The IMpassion130 trial represents an important advance in the management of metastatic triple-negative breast cancer. Preliminary data suggest that the overall survival rate was improved in the PD-L1-positive subgroup but the final overall survival results are not yet available. IMpassion131 (NCT03125902), is currently recruiting patients who will be randomly assigned to atezolizumab plus paclitaxel versus placebo plus paclitaxel as a first-line therapy for metastatic triple-negative breast cancer.

The phase 1b/2 ENHANCE-1 trial assessed the activity of pembrolizumab combined with eribulin in 104 patients with metastatic triple-negative breast cancer.[43] Of the 82 evaluable patients, 21 (26%) achieved an objective response, as did 12 (25%) of 48 patients who had received no previous chemotherapy for metastatic disease and nine (27%) of the 34 who had been previously exposed to chemotherapy. Objective response was achieved by nine (26%) of 35 patients with PD-L1-positive cancers. This response was higher than that recorded for patients with PD-L1-negative tumours (nine [25%] of 36). Pembrolizumab is also being evaluated in an ongoing KEYNOTE-355 (NCT02819518) trial in patients with metastatic previously untreated triple-negative breast cancer who are randomly assigned to pembrolizumab or placebo in combination with one of three different chemotherapies (paclitaxel, nab-paclitaxel, or gemcitabine with carboplatin). Results from this trial have not yet been announced. Collectively, results from IMpassion130, IMpassion131, and KEYNOTE-355 will establish the clinical utility of atezolizumab and pembrolizumab as a first-line combination therapy for metastatic triple-negative breast cancer.

Targeting PD-1 and PD-L1 in combination with molecularly targeted therapies

In addition to acting synergistically with chemotherapies, immune checkpoint inhibitors might enhance the efficacy of inhibitors of molecular targets. Currently, 45 clinical trials are investigating immunotherapy plus targeted therapy combinations in breast cancer (summarised in tables 1 and 2), and several have already reported preliminary results.

Anti-PD-1 or anti-PD-L1 drug with secondary therapy	Combination therapy	Trial registration number
HER2 inhibitors		
Durvalumab with trastuzumab	..	NCT02649686
Atezolizumab with pertuzumab	Trastuzumab	NCT03417544
Atezolizumab with PRS-343	..	NCT03650348
Atezolizumab with trastuzumab	Chemotherapy	NCT02605915, NCT03595592
Atezolizumab with trastuzumab and pertuzumab	Chemotherapy	NCT03125928
Atezolizumab with trastuzumab emtansine	..	NCT02924883
Nivolumab with trastuzumab deruxtecan	..	NCT03523572
Pembrolizumab with trastuzumab emtansine	Cetuximab	NCT02318901
Pembrolizumab with HER2-BATs	..	NCT03272334
Pembrolizumab with trastuzumab	Chemotherapy	NCT03199885
Pembrolizumab with trastuzumab	Ganitumab	NCT01042379
Pembrolizumab with trastuzumab emtansine	..	NCT03032107
Poly(ADP-ribose) polymerase inhibitors		
Durvalumab with olaparib	..	NCT02734004, NCT03167619, NCT03544125, and NCT03594396
Durvalumab with olaparib	Cediranib	NCT02484404
Atezolizumab with olaparib	..	NCT02849496
Pembrolizumab with niraparib	..	NCT02657889
Cyclin-dependent kinase inhibitors		
Avelumab with palbociclib	Fulvestrant	NCT03147287
Avelumab with palbociclib	Tamoxifen	NCT03573648
Pembrolizumab with abemaciclib	..	NCT02779751
Pembrolizumab with dinaciclib	..	NCT01676753
Pembrolizumab with palbociclib	..	NCT02778685
Other anti-PD-1 or anti-PD-L1 with ribociclib	..	NCT03294694
Angiogenesis inhibitors		
Durvalumab with bevacizumab	..	NCT02802098
Atezolizumab with bevacizumab	Cobimetinib	NCT03280563
Atezolizumab with cobimetinib	Bevacizumab	NCT03395899
Avelumab with bevacizumab	ALT-803	NCT03175666
Avelumab with cancer vaccine	Bevacizumab	NCT03387085
Histone deacetylase inhibitors		
Atezolizumab with entinostat	..	NCT02708680
Pembrolizumab with entinostat	..	NCT02909452
Pembrolizumab with vorinostat	..	NCT02395627
Other anti-PD-1 or anti-PD-L1 with panobinostat	LCL161	NCT02890069

PD-1=programmed death-1. PD-L1=programmed death ligand-1. HER2-BATs=anti-HER2 bispecific antibody-armed activated T cells.

Table 1: **Ongoing trials evaluating anti-PD-1 and anti-PD-L1 drugs in combination with molecular targeted therapies and chemotherapy**

HER2-targeting therapies

Trastuzumab, a humanised anti-HER2 antibody was initially developed to inhibit HER2 signalling in patients with breast cancers with amplification or overexpression of the *HER2* (*ERBB2*) gene.[52] Subsequently, it has become

Trial name or registration number	Immune checkpoint inhibitor	Combination therapy	Number of evaluable patients	Phase	Key results
Chemotherapy					
NCT01633970[40]	Atezolizumab	Nab-paclitaxel	33	1b	Objective response from 13 (39%) patients overall and from 7 (54%) of 13 who received atezolizumab as first-line treatment
NCT02425891 (IMpassion130)[42]	Atezolizumab	Nab-paclitaxel	902	3	Median progression-free survival was 7.2 months (95% CI 5·6–7·5) and median overall survival was 21.3 months (95% CI 17·3–23·4) for all patients; median overall survival in the PD-L1-positive subgroup was 25.0 months (95% CI 22·6–not established)
NCT02753595 (ENHANCE-1)[43]	Pembrolizumab	Eribulin	83	1b/2	Objective response from 21 (26%) of 83 evaluable patients overall, 12 (25%) of 48 with no previous chemotherapy, and 9 (26%) of 35 with PD-L1-positive cancers
HER2 inhibitors					
NCT02129556 (KEYNOTE-014/ PANACEA)[44]	Pembrolizumab	Trastuzumab	58	1b/2	Objective response from 15% of patients with PD-L1-positive cancers and 39% if their TIL counts were above 5%, disease control achieved in 25% of patients in the PD-L1-positive subgroup
NCT02649686 (CCTGIND.229)[45]	Durvalumab	Trastuzumab	15	1	No objective response; best outcome was stable disease at week 6 in 4 (29%) of 14 patients with PD-L1-negative cancers
Poly(ADP-ribose) polymerase inhibitors					
NCT02734004 (MEDIOLA)[46]	Durvalumab	Olaparib	288	1/2	Objective response was achieved by 67% of patients in the first-line setting group
NCT02657889 (KEYNOTE-162/ TOPACIO)[47]	Pembrolizumab	Niraparib	45	1/2	Objective response was achieved by 13 (29%) of 45 evaluable patients overall, 8 (67%) of 12 patients with g*BRCA* mutations, 33% of patients with PD-L1-positive cancers, and 15% of patients with PD-L1-negative cancers
Cyclin-dependent kinase inhibitors					
NCT02779751[48]	Pembrolizumab	Abemaciclib	28	1b	Objective response was achieved in 14% of patients with HR-positive, HER-negative metastatic breast cancer
Anti-PD-1 and anti-PD-L1 combinations					
NCT02536794[49]	Durvalumab	Tremelimumab	17	1	Objective response was achieved by 3 (17%) patients overall; all 3 had triple-negative breast cancer, so objective response for this group was 43% (n=7)
Radiotherapy					
NCT01497808[50]	Ipilimumab	Radiotherapy	22	1	Objective response was achieved by 4 (18%) of patients
NCT02730130[51]	Pembrolizumab	Radiotherapy	17	2	Partial response was achieved by 3 (33%) of 9 evaluable patients

Nab-paclitaxel=nanoparticle albumin-bound paclitaxel. PD-L1=programmed death ligand-1. TIL= tumour-infiltrating lymphocytes. HR=hormone receptor. PD-1=programmed death-1.

Table 2: **Trials evaluating immune checkpoint inhibitors in combination with molecular therapies or chemotherapy that have reported preliminary results**

clear that part of the antitumour activity of trastuzumab is immune-mediated.[4,53] Several studies showed increases in TIL counts after trastuzumab treatment and induction of antibody-dependent cellular cytotoxicity in patients.[54–56] These observations make trastuzumab an attractive drug for combination immunotherapy therapies. The phase 1b/2 KEYNOTE-014/PANACEA trial (NCT02129556) evaluated pembrolizumab in combination with trastuzumab in patients with HER2-positive metastatic breast cancers that had progressed after previous HER2-targeted therapies. Seven (15%) patients in the PD-L1-positive cohort (n=46) and none in the PD-L1-negative cohort (n=12) achieved an overall response.[44] The median duration of response was 11·2 months. Overall response correlated with TIL counts in patients with cancers with at least 5% TILs; 39% responded compared with 5% of patients whose TIL counts were lower than 5%, suggesting that quantification of TILs could help to identify patients who are most likely to benefit from this treatment. However, the presence of TILs was generally low in these metastatic biopsies, in which median TIL count was 1%. Of note, five (11%) patients in the PD-L1-positive cohort continued to have no disease progression at the time of reporting (2018).

The CCTGIND.229 (NCT02649686) phase 1 trial[45] tested the combination of durvalumab (anti-PD-L1) and trastuzumab in 15 patients with metastatic HER2-positive breast cancer who had received extensive previous HER2-targeted therapies. Dose-limiting toxicities or objective responses were not observed; the best response documented in the trial was stable disease at week 6 of treatment in four (29%) of 14 evaluable patients. All patients had PD-L1 expression lower than 1% on tissue archival or pre-study biopsy.

Combining antibody–drug conjugates with immunotherapy is a novel approach to cancer treatment based on

the theoretical potential of targeted chemotherapy to generate neo-antigens resulting in T-cell trafficking to solid tumours.[57,58] Trastuzumab emtansine showed synergy with antibodies blocking CTLA-4 and PD-L1 in mouse models of HER2-positive breast cancer.[59] Based on these data, the randomised KATE2 trial[60] evaluated the safety and efficacy of atezolizumab in combination with trastuzumab emtansine, compared with trastuzumab emtansine alone, in patients with HER2-positive metastatic breast cancer previously treated with trastuzumab and a taxane. Safety and efficacy data[60] presented at the 2018 San Antonio Breast Cancer Symposium showed no significant improvement in time to progression after the addition of atezolizumab to trastuzumab emtansine, although the combination was well tolerated. Nevertheless, exploratory analyses showed improved time to progression in patients in the trastuzumab emtansine with atezolizumab group who had PD-L1-positive tumours.

Poly(ADP-ribose) polymerase (PARP) inhibitors

PARP inhibitors olaparib and talazoparib are approved in the USA as monotherapies for patients with metastatic breast cancers with deleterious germline mutations in the *BRCA1* and *BRCA2* genes (g*BRCA*), which encode breast cancer type 1 and type 2 susceptibility proteins. It has been hypothesised that cancers with deficiency in DNA repair due to the combined effects of *BRCA* loss of function (causing impaired homologous recombination) and inhibition of PARP (causing impaired repair of nucleotides and base excision) will be highly susceptible to genomic instability, which will generate DNA fragments capable of activating the intracellular stimulator of interferon genes pathway.[61] This genomic instability will also give rise to many protein-altering mutations that create immunogenic neo-antigens. PARP inhibition was also shown to upregulate PD-L1 expression in breast cancer cell lines and animal models.[61] Based on these rationales, several trials are investigating combinations of PARP inhibitors and immunotherapy agents. The MEDIOLA trial (NCT02734004) is a phase 1/2 open-label basket study of olaparib and durvalumab in patients with different types of solid tumours. Results from patients in the HER2-negative, g*BRCA*-positive metastatic cohort have already been presented in an abstract.[46] Eligible patients could not have previously received a PARP inhibitor or immunotherapy (eg, inhibitors against PD-1, PD-L1, or PD-L2, or anti-CTLA-4 therapy) but previous treatment with anthracycline, taxane, and platinum was allowed. Patients received 300 mg olaparib daily for 4 weeks as monotherapy, then 1·5 g intravenous durvalumab was administered once every 4 weeks in addition to olaparib. Data from the first 25 of the 30 enrolled patients showed that 13 (52%) had HR-positive disease and 12 (48%) had triple-negative breast cancer. Additionally, six (67%) of nine patients who had received no previous therapy achieved an overall response, compared with six (67%) of nine patients with one previous therapy, one (20%) of five patients with two previous therapies, and no patients with three or more previous therapies.[62]

The KEYNOTE-162/TOPACIO (NCT02657889) trial is testing the combination of niraparib and pembrolizumab in patients with metastatic triple-negative breast cancer who have received up to two previous lines of therapy. 12 (22%) of 54 patients enrolled in the trial had known deleterious g*BRCA* mutations when preliminary results were reported.[47] 45 patients were evaluable overall, and 13 (29%) achieved either a complete (three) or partial (ten) objective response. Patients with g*BRCA* mutations had higher objective responses (eight [67%]) than the rest of the cohort. Finally, patients with PD-L1-positive cancers responded better than those with PD-L1-negative ones (33% *vs* 15%).

Inhibitors of cyclin-dependent kinase 4 (CDK4) and CDK6

The inhibitors of CDK4 and CDK6 palbociclib, ribociclib, and abemaciclib improved the progression-free survival in patients with oestrogen-receptor-positive, HER2-negative[63] and HR-positive, HER2-negative metastatic breast cancers.[64,65] Inhibitors of CDK4 and CDK6 work primarily by suppressing retinoblastoma phosphorylation in cancer cells, which stops the cell cycle and inhibits cell proliferation. However, these inhibitors can also modulate kinase signalling and cellular senescence and can enhance tumour immunogenicity. Inhibitors of CDK4 and CDK6 have been shown to inhibit the proliferation of immunosuppressive regulatory T cells to a greater extent than that of other T-cell types, and therefore could shift the local immune balance in favour of an antitumour immune response.[66] Findings from preclinical studies[66–68] also showed that inhibitors of CDK4 and CDK6 can increase the presentation of neo-antigen by MHC class 1 molecules in cancer cells through increasing the expression of endogenous retroviral sequence fragments, as well as induce fibroblasts to release proinflammatory cytokines.

These results suggest that clinically useful synergy might exist between inhibitors of CDK4 and CDK6 and immune checkpoint inhibitors. Indeed, in a murine cancer model, abemaciclib monotherapy delayed tumour growth and increased the tumour's T-cell inflammatory signature, while combination with anti-PD-L1 therapy led to complete tumour regression.[69] All three inhibitors of CDK4 and CDK6 approved by the US Food and Drug Administration (FDA) are now in clinical trials in combination with immune checkpoint inhibitors (table 2). The first phase 1/2 study that reported preliminary results[48] tested abemaciclib plus pembrolizumab in 28 patients with HR-positive metastatic breast cancer and four (14%) showed an objective response at 24 weeks. The investigators pointed out that this response is somewhat higher than the response seen with abemaciclib monotherapy at corresponding early time points (11% of patients) in the MONARCH 1 study.[70]

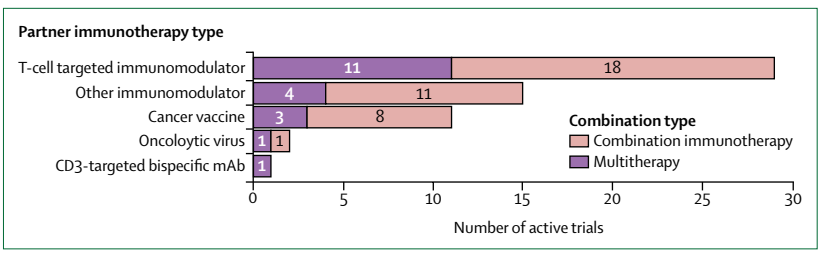

Figure 4: **Anti-PD-1 and anti-PD-L1 drugs with other immunotherapy combinations in clinical trials of breast cancer**
Combination immunotherapy trials are those investigating drugs targeting PD-1 or PD-L1, combined with one or more other immunotherapy. Multitherapy refers to trials that include an anti-PD-1 or anti-PD-L1 drug, together with one or more immunotherapy and at least one other therapy type (ie, chemotherapy, radiotherapy, or targeted therapy). mAb=monoclonal antibody. PD-1=programmed death-1. PD-L1=programmed death ligand-1.

Angiogenesis inhibitors

Altered vasculature in the tumour micro-environment could contribute to immunosuppression of the tumour by reducing the trafficking and activation of effector T cells and promoting the expansion of regulatory T cells and myeloid-derived suppressor cells in solid tumours.[71] Various combinations of angiogenesis inhibitors and PD-1 and PD-L1 inhibitors are in clinical development for patients with advanced solid tumours, with promising preliminary results reported for renal cell carcinoma, melanoma, and glioblastoma.[72–74] Whether this approach will lead to improved clinical outcomes in patients with metastatic breast cancer is unknown, especially because of the low efficacy of bevacizumab or small molecule inhibitors of VEGF receptors in breast cancer. Currently, five trials are exploring angiogenesis inhibitors in combination with immunotherapy in breast cancer (tables 1 and 2) but none have reported results to date.

Targeting PD-1 and PD-L1 in combination with other immunotherapies

The multiplicity of positive and negative feedback loops and intertwined regulatory mechanisms of the immune system offer a large number of potential strategies to augment the modest single-drug activity of antibodies targeting PD-1 and PD-L1. Conceptually, these strategies fall into categories of increasing cancer antigen presentation, enhancing immune cell infiltration into the tumour micro-environment, and augmenting the activity of various effector cells.[75] Practical implementation of these strategies ranges from developing vaccines, targeting chemokines that regulate immune trafficking, activating co-stimulatory molecules, and inhibiting suppressor components of the T-cell receptor signalling axis, to genetically engineering T cells and multifunctional molecules. Several reviews have been published on various combination immunotherapy strategies and their rationales.[76–79]

In the clinic, combining anti-CTLA-4 and anti-PD-1 drugs to treat metastatic melanoma proved to be highly effective, but more toxic than administering the drugs individually, and provided the proof of principle for successful combination of immunotherapy agents. Extensive efforts are ongoing to combine drugs targeting PD-1 and PD-L1 with multiple other immunological agents. There are 58 ongoing breast cancer trials of combination immunotherapy (figure 4), but only a small pilot study[49] has reported outcomes to date. This study evaluated the combination of durvalumab (anti-PD-L1) and tremelimumab (anti-CTLA-4) in patients with metastatic triple-negative breast cancer and endocrine-receptor-positive cancer. 18 patients were enrolled (seven with metastatic triple-negative breast cancer and 11 with endocrine-receptor-positive cancer) and three (17%) achieved an overall response. However, they were all patients with triple-negative breast cancer and the overall response for that group was therefore 43%.

Inhibitors of PD-1 and PD-L1 and radiotherapy

Radiation-induced DNA damage results in the release of molecular signals called damage-associated molecular patterns, which increase cytokine release, promote antigen presentation, and stimulate T-cell responses.[80] These effects make radiotherapy a modality potentially synergistic with immunotherapy.

Results from randomised clinical trials have shown improvement in the survival of patients with high-risk early-stage breast cancer who are receiving post-mastectomy radiotherapy. It has been assumed that radiation improves survival because it eradicates local micrometastatic disease, thereby eliminating a potential source of future metastasis. However, evidence is emerging that radiation might have broader systemic effects. The literature on radiotherapy describes that irradiating a metastatic lesion can occasionally induce responses in distant metastatic sites, known as the abscopal effect, presumably through immune mechanisms.[81,82] The abscopal effect was also observed in trials that combined radiation with immune checkpoint therapy in cancer. In a seminal, single-arm clinical trial of 22 patients who each had multiple metastases from melanoma, hypofractionated radiation to a single index lesion, followed by administration of ipilimumab, led to a partial reduction in the sizes of non-irradiated metastatic sites in four (18%) patients.[50] The American Society for Radiation Oncology, the Society for Immunotherapy of Cancer, and the US National Cancer Institute recognised the potential of this strategy and organised a collaborative scientific workshop in 2017 to discuss the incorporation of immunotherapy into radiotherapy.[83]

Ten clinical trials are studying the effects of radiotherapy with immunotherapy in patients with breast cancer (figure 1). Preliminary results were presented from a trial that combined radiotherapy with pembrolizumab in patients with metastatic triple-negative breast cancer, regardless of PD-L1 expression.[51] All patients had two or fewer measurable sites of metastatic disease, with at least one site requiring palliative radiotherapy as routine care.

Pembrolizumab was given within 3 days of the first radiation fraction, then once every 3 weeks. The primary endpoint was an overall reduction of non-irradiated lesions at week 13. Of the 17 women enrolled, nine were evaluable for response at week 13 and three (33%) achieved a partial response. These results are promising, since the single-drug efficacy of pembrolizumab in patients with heavily pretreated, metastatic triple-negative breast cancer tumours not selected for PD-L1 expression is low (about 5%), as shown by results from cohort A in the KEYNOTE-086 trial.[26]

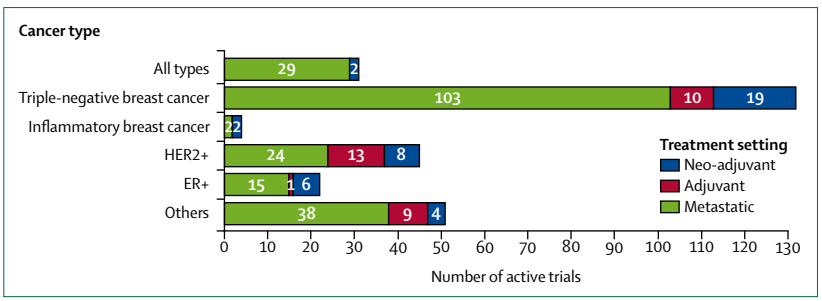

Figure 5: **Disease subtypes and clinical settings of immunotherapy trials of breast cancer**
Most immunotherapies are tested in patients with metastatic disease. All types=all breast cancers regardless of receptor status. HER2+=HER2-positive cancers regardless of oestrogen receptor status. ER+=oestrogen-receptor-positive, HER2-negative cancers. Others=all other less prevalent histological or genetic markers.

Conclusion

Immunotherapy is now established as the fifth treatment modality for cancer, complementing surgery, radiotherapy, chemotherapy, and molecularly targeted therapies. Five different immune checkpoint inhibitors are now approved by the FDA to treat ten different cancer types, and pembrolizumab is also approved for microsatellite instability-high tumours of any histology. However, immunotherapy for the treatment of breast cancer is only just beginning to advance. No immunotherapy was approved by the FDA for breast cancer as of Feb 1, 2019, although pembrolizumab could be used in microsatellite instability-high breast cancers. This situation will probably change after results from large randomised trials are evaluated by regulatory agencies.

This Personal View shows several important trends in the development of immunotherapy in patients with breast cancer. We observed a very rapid growth in the number of clinical trials that test immunotherapies. More than 70 new trials opened in 2018 alone (figure 1), which reflects the interest in the potential impact of these drugs on clinical outcomes, particularly the unprecedentedly long duration of positive responses seen across patients with other types of cancer. Justifiably, most of these trials examine how to integrate immunotherapy drugs into existing treatment modalities including chemotherapy, radiotherapy, and drugs targeting oncogenic molecules including but not limited to HER2, hormone receptors, CDKs, and PARP. However, the rapid and uncoordinated increase in the number of trials also creates challenges. In the best-case scenario, positive results from many trials could yield a confusing array of equally reasonable treatment choices with no clear superiority of any particular approach. The worst-case scenario is that many trials will not recruit enough patients due to inter-trial competition for recruitment, and the ones that do might generate obsolete results because of rapid shifts in standards of care during the study. Additionally, many trials address very similar questions in identical patient populations. Single-agent immunotherapy trials are running in parallel with combination immunotherapy and immuno-chemotherapy trials. Positive survival results from a large immuno-chemotherapy trial in the first-line metastatic setting, followed by an FDA approval, would substantially slow enrolment in monotherapy and combination immunotherapy trials in the same clinical setting. Rapid adoption of an immunotherapy agent for the initial therapy of patients with metastatic triple-negative breast cancer would also shift the clinical question to what therapy is appropriate for patients whose cancer progresses after an initial response or who have never responded to initial immunotherapy. It is concerning that most of the trials currently open exclude patients with previous exposure to immune checkpoint inhibitors.

Another remarkable development is that metastatic and adjuvant or neo-adjuvant trials are conducted in parallel (figure 5). The first phase 1/2 neo-adjuvant trial in breast cancer (NCT02489448) opened for recruitment in July, 2015, a year before the results of the first phase 1 metastatic trial were even published.[25] In 2018, the first large, randomised trial of metastatic triple-negative breast cancer, IMpassion130,[42] has reported results. The first large, randomised, neo-adjuvant trial of triple-negative breast cancer has also completed recruitment and at least four other large, neo-adjuvant and adjuvant trials are ongoing. This rapid, parallel development in the curative (ie, adjuvant and neo-adjuvant) and metastatic settings were possible in part because of the extensive safety data and clinical experience that have accumulated about these drugs from studies of other cancer types. However, their long-term safety in otherwise healthy individuals, who are the typical patients in adjuvant trials, is still unknown.

It is probable that immunotherapies will increasingly play a role in the treatment of breast cancer and that along with therapeutic advances new challenges will arise. The clinical utility of immunotherapies will probably vary by disease subtype and enrichment strategies for recruitment of patients will need to be developed to minimise exposure to potentially toxic and very expensive therapies of those who are unlikely to benefit. Another challenge in the conduct of immunotherapy trials (eg, IMpassion130) and the implementation of their results in routine clinical practice is the wide range of different antibodies that are used to immunohistochemically test for PD-L1 positivity, the varied definitions of positivity, and the different ways

Search strategy and selection criteria

We searched PubMed and Google Scholar for articles published in English using the search terms "immune checkpoint inhibitors" or "immunotherapy" and "breast cancer" and "clinical trial". Articles that included these terms and were published on PubMed between Jan 1, 2009, and Oct 31, 2018, were included for review. Because of the scarcity of published results from clinical trials with immune-oncology drugs in breast cancer, trials presented at the annual meetings of the American Society of Clinical Oncology, the American Association for Cancer Research, and the 2017 and 2018 San Antonio Breast Cancer Symposia were also included. We also searched the ClinicalTrials.gov database on Sept 5, 2018, for studies that included the common immunotherapy drugs in the search box for interventions and breast cancer for conditions.

in which pathologists score PD-L1 tumour expression. However, new data[84,85] suggest that several of the antibodies commonly used for the treatment of lung cancer (E1L3N, 22C3, 28-8, SP263) have similar sensitivity to each other in detecting the expression of PD-L1, with the exception of SP142, which has lower sensitivity. In conclusion, many immunotherapy and targeted therapy combinations are being tested in patients with advanced breast cancer. Finding the optimal combination for individual patients will require validating predictive biomarkers in the tumour and its surrounding micro-environment, understanding the pharmacokinetics and pharmacodynamics of these drug combinations, and optimising dosage to improve clinical outcomes, not only in patients with metastatic cancers, but also in patients at earlier disease stages who are at high risk of relapse.

Contributors

Individual sections of the manuscript were written with contributions from all authors. Figures were prepared by VMH-L and JT. All authors reviewed and approved the final version.

Declaration of interests

FJE reports institutional grants from Genentech Roche, Novartis, Merrimack, Celgene, Pfizer, and Eli Lilly; and personal fees from Celltrion Healthcare, Seattle Genetics, Pfizer, Nanostring, Genentech Roche, Novartis, AstraZeneca, Merrimack, Celgene, Spectrum, and Eli Lilly, outside the submitted work. LP reports personal consultation fees from AstraZeneca, Merck, Seattle Genetics, Novartis, Genentech Roche, Pieris, Eisai, Almac, Syndax, Immunomedics, Celgene, and Boehringer Ingelheim, outside the submitted work. VMH-L and JT declare no competing interests.

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