Current Breast Cancer Reports (2019) 11:259–271
https://doi.org/10.1007/s12609-019-00345-z

IMMUNO-ONCOLOGY (S TOLANEY, SECTION EDITOR)

Immunotherapy in Triple-Negative Breast Cancer: Present and Future

Isaac Kim[1] · Katherine Sanchez[1] · Heather L. McArthur[2] · David Page[1]

Published online: 20 November 2019
© The Author(s) 2019

Abstract

Purpose of Review Immunotherapy is emerging as an effective treatment option for metastatic triple-negative breast cancer. In this review, we summarize clinical data of immunotherapy in triple-negative breast cancer and comment on future directions in the field.

Recent Findings IMpassion130 was a phase III trial that demonstrated progression-free survival benefit, and potentially overall survival benefit, of first-line chemotherapy (nab-paclitaxel) plus anti-programmed death ligand 1 (PD-L1) atezolizumab, among PD-L1-positive metastatic triple-negative breast cancers. Studies are ongoing to evaluate other combination therapies with immune checkpoint blockade in TNBC, and to evaluate efficacy in PD-L1-negative tumors and in later lines of therapy.

Summary Immunotherapy is now a standard option in the treatment of triple-negative breast cancer. Ongoing trials may expand the degree of clinical benefit. Further work is ongoing to identify novel predictive biomarkers, which in the future may enable a personalized approach of combination immunotherapy.

Keywords Breast cancer · Immunotherapy · Triple-negative · Immune checkpoint blockade · PD-L1 · IMpassion130

Introduction

Biologically, TNBCs are highly heterogeneous and until recently had no informative biomarkers for targeted therapy [1], leaving cytotoxic chemotherapy as the only available systemic approach.

However, among breast cancer subtypes, TNBC has characteristics that may make it more responsive to treatment with immunotherapy. These characteristics include a higher mutational burden, where despite a high range of variance within each tumor type, TNBC was found to have more median mutations than HER2-positive or luminal-type hormone receptor positive tumors [2]. Higher tumor mutational burden can lead to a higher frequency of immunogenic mutations [3, 4], and has been described as a marker of improved survival following immunotherapy across multiple tumor types [5, 6]. TNBC also exhibits higher mean quantities of tumor infiltrating lymphocytes (TILs) relative to other breast cancer subtypes. In early-stage TNBC, TIL count is associated with improved survival, reduced recurrence risk, and increased likelihood of response to neoadjuvant chemotherapy [7–9]. TIL count has also been described as a potential biomarker of immunotherapy response [10].

TNBC also has a higher rate of programmed death ligand 1 (PD-L1) expression relative to other breast cancer subtypes, providing a potential therapeutic target with antibody inhibitors of programmed death 1 (PD-1) or PD-L1 [11, 12]. Recently, the Food and Drug Administration (FDA) approved atezolizumab (anti-PD-L1) for use in combination with nab-paclitaxel for PD-L1-positive advanced TNBC. While immune checkpoint blockade is the most studied form of immunotherapy for TNBC, other modalities are also being evaluated. In this review, we aim to examine the current role of immunotherapy in TNBC, present the modalities of immunotherapy currently being evaluated, and discuss the future of immunotherapy in the clinical management of TNBC.

Immune Checkpoint Blockade

A number of anti-PD-1/L1 antibodies have been evaluated in metastatic TNBC as monotherapy (Table 1). These agents are generally well tolerated and may induce durable responses; however, responses appear restricted to a minority of patients. For example, KEYNOTE-012 was a phase Ib study of pembrolizumab monotherapy in PD-L1-positive patients

This article is part of the Topical Collection on *Immuno-oncology*

✉ David Page
David.page2@providence.org

[1] Earle A. Chiles Research Institute, Providence Cancer Institute, 4805 NE Glisan St. Suite 2N35, Portland, OR 97213, USA

[2] Cedars-Sinai Medical Center, Los Angeles, CA, USA

Table 1 Reported trials of immune checkpoint blockade monotherapy and in combination with chemotherapy in the metastatic setting for TNBC

Trial/phase	Treatment groups	ORR	ORR PD-L1+	ORR PD-L1-	Percent PD-L1+	Median PFS	Median OS	Reference
First-line setting trials								
NCT01375842; first-line subgroup[1]; phase Ia	Atezolizumab	24%	12%	0%	78%	1.4 months	17.6 months	Emens et al. [13]
NCT01633970; first-line subgroup; phase Ib[2]	Atezolizumab +Nab-paclitaxel	53.8%	41.4%	33.3%	50% of evaluable patients	8.6 months	21.9 months	Adams et al. [14]
ENHANCE/KEYNOTE-150; first-line cohort[3]; phase Ib/II	Pembrolizumab + eribulin	29.2%[3]	30.6%	22.4%	46.2%	4.2 months	17.7 months	Tolaney et al. [15]
KEYNOTE-086, Cohort B; phase II	Pembrolizumab	23.1%	23.1% (all PD-L1+)	n/a	100%	2.1 months	18.0 months	Adams et al. [16]
IMpassion130; phase III	Atezolizumab + Nab-paclitaxel vs. placebo + Nab-paclitaxel	56% vs. 45.9%	58.9% vs. 42.6%	n/a	40.9%	ITT: 7.2 months vs. 5.5 months; PD-L1+: 7.5 months vs. 5.0 months	ITT: 21.3 months vs. 17.6 months; PD-L1+: 25.0 months vs. 15.5 months	Schmid et al. [17]
Second-line+ and non-differentiated setting trials								
NCT01375842; second-line subgroup[1]; phase Ia	Atezolizumab	6%	12%	0%	78%	1.4 months	8.9 months	Emens et al. [13]
NCT01633970; second-line subgroup; phase Ib[2]	Atezolizumab + Nab-paclitaxel	30%	41.4%	33.3%	50% of evaluable patients	4.1 months	11.4 months	Adams et al. [14]
ENHANCE/KEYNOTE-150; second-line cohort[3]; phase Ib/II	Pembrolizumab + eribulin	22%	30.6%	22.4%	46.2%	4.2 months	17.7 months	Tolaney et al. [15]
KEYNOTE-012, TNBC cohort; phase Ib	Pembrolizumab	18.5%	18.5% (all PD-L1+)	n/a	100%	1.9 months	11.2 months	Nanda et al. [18]
JAVELIN; phase Ib[4]	Avelumab	5.2%	16.7%	1.6%	62.5%	PD-L1+: 1.4 months; PD-L1-: 1.4 months	PD-L1+: 6.5 months; PD-L1-: 8.3 months	Dirix et al. [19]
KEYNOTE-086, cohort A; phase II	Pembrolizumab	4.7%	4.8%	4.7%	61.8%	2.0 months	8.9 months	Adams et al. [20]

[1] Response by PD-L1 status and median PFS were evaluated in the combined cohort. Median survival in combined cohort was 8.9 months, but 17.6 months in the first-line subgroup

[2] ORR for the combined cohort was 39.4%. ORR by PD-L1 listed for combined cohort. Median PFS was 5.5 months; median OS was 14.7 months for the combined cohort

[3] ORR for the combined cohort was 26.4%. Response by PD-L1 status and median OS/PFS were evaluated in the combined cohort

[4] Data presented from the TNBC cohort. ORR in the combined group of metastatic breast cancer was 3.0%

ORR, objective response rate; *PD-L1*, programed death-ligand 1; *PFS*, progression-free survival; *OS*, overall survival; *ITT*, intention-to-treat

(PD-L1 ≥ 1% by IHC), which demonstrated an objective response rate (ORR) = 18.5%, with 3 responders with ongoing response ≥ 1 year. Notably, the population was heavily pre-treated with a median number of prior lines of systemic therapy in the metastatic setting of 2, with 25% of patients having received ≥ 5 lines [18]. A follow-up phase II study evaluated pembrolizumab according to PD-L1 status and line of therapy, with cohort A including pre-treated patients ($n = 170$, 62% PD-L1-positive), and cohort B including first-line PD-L1-positive patients ($n = 84$). Cohort A reported a 5.3% ORR (5.7% among PD-L1-positive), whereas cohort B reported a 21.4% ORR. Responses were durable, with a median duration of response of 10.4 months in cohort B (not reached in cohort A) [16•, 20]. Of note, a phase III comparison of 2nd/3rd-line pembrolizumab monotherapy versus investigator's choice chemotherapy (capecitabine, eribulin, gemcitabine, or vinorelbine) failed to meet its primary endpoint of overall survival [21]. The pembrolizumab data suggest that anti-PD-1/L1 is more effective in earlier lines of therapy. This finding was confirmed in the phase Ib evaluation of atezolizumab monotherapy, whereby first-line ORR was 24% ($n = 21$), versus 6% in later lines ($n = 94$) [13]. In this trial, response also appeared dependent on PD-L1 status, with PD-L1-positive tumors having an ORR of 12%, versus 0% for PD-L1-negative tumors. This trial also confirmed the durability of response, with median response in the first-line setting lasting 21 months.

Immune Checkpoint Blockade with Chemotherapy

Chemotherapy is known to have various immunomodulatory effects, with growing evidence that the restoration of host immunosurveillance may be part of the benefit seen with conventional chemotherapies [22–24]. IMpassion130 was a registrational phase III trial that evaluated chemotherapy (nab-paclitaxel) plus atezolizumab versus placebo in first-line metastatic/advanced TNBC [17•]. In the intention-to-treat (ITT) analysis at a median follow-up of 12.9 months, median PFS was 7.2 months in the atezolizumab plus nab-paclitaxel arm ($n = 451$) versus 5.5 months in the placebo plus nab-paclitaxel arm ($n = 451$) ($p = 0.0025$). In a pre-specified subgroup analysis, improvements were more pronounced in PD-L1-positive tumors (PFS, 7.5 vs. 5.0 months). In the ITT overall survival (OS) analysis, OS was 21.3 months in the atezolizumab arm versus 17.6 months in the chemotherapy alone arm, which did not meet statistical significance ($p = 0.08$). Among PD-L1-positive tumors, the median OS was 25.0 months versus 15.5 months. The hazard ratio was reported of 0.62 (95% CI 0.45–0.86); however, formal p value testing could not be performed because it was not designated as a pre-specified primary outcome in the setting of the ITT OS being non-significant. This data led to the FDA approval for atezolizumab plus nab-paclitaxel only for PD-L1-positive tumors. Updated OS data from the second interim analysis at median follow-up of 18.0 months showed an OS of 21.0 months in the atezolizumab plus nab-paclitaxel arm versus 18.7 months in the nab-paclitaxel alone arm ($p = 0.0777$) in the ITT population. In the PD-L1-positive subgroup, OS was 25.0 months versus 18.0 months, for a hazard ratio of 0.71. This updated data showed persistent benefit in overall survival in the PD-L1-positive population [25].

IMpassion130 was the first randomized trial to demonstrate improved outcomes of anti-PD-1/L1; however, the trial leaves a number of questions unanswered. No atezolizumab monotherapy arm was included in the study, and it is unknown whether patients could equally benefit from a sequential approach of atezolizumab followed by nab-paclitaxel at progression. One appealing future direction might be to employ biomarkers or risk stratification factors (LDH, visceral disease) to identify a subset of patients most likely to benefit from monotherapy. In the monotherapy trials, responses have been worse in patients who have had multiple prior lines of therapy. In addition, the inclusion of patients with elevated LDH was thought to have played a role in the response rate seen in KEYNOTE-012/086, with no responses noted in patients with elevated LDH in KEYNOTE-012 [18]. Investigators commented on the possibility that these patients had more aggressive, rapidly growing tumors compared with patients with lower baseline LDH. Other poor prognostic factors included the presence of visceral disease and liver metastases. However, in the IMpassion130 subgroup analysis, subjects with liver metastases had a similar improvement in benefit with the addition of atezolizumab to nab-paclitaxel [17•].

A second unanswered question is whether patients with early relapse could benefit from combination therapy. The ongoing IMpassion132 trial will evaluate patients who would have been IMpassion130 ineligible, enrolling subjects with relapse ≤ 12 months from receipt of curative-intent chemotherapy to receive atezolizumab or placebo with investigator choice gemcitabine + carboplatin or capecitabine. The primary endpoint will be overall survival [26]. In the ongoing KEYNOTE-355 study, subjects with de novo metastatic disease or relapse > 6 months from receiving curative-intent therapy receive investigator's choice chemotherapy (gemcitabine/carboplatin or nab-paclitaxel or paclitaxel) with or without pembrolizumab [27]. In a recent phase Ib trial, patients with early relapse < 12 months from curative-intent chemotherapy experienced 38% ORR ($n = 3/8$) to capecitabine plus pembrolizumab [28].

A third unanswered question is whether alternative chemotherapy backbones will be safe or effective. Chemotherapies have varied mechanisms of immunomodulation and have been the subject of extensive review [22–24]. Phase III trials of various combinations are ongoing, including the aforementioned KEYNOTE-355 study of investigator's choice chemotherapy (gemcitabine/carboplatin or nab-paclitaxel or paclitaxel) with or without pembrolizumab and a number of small

phase Ib/II trials have demonstrated safety and encouraging activity of combinations including capecitabine, eribulin, doxorubicin, cisplatin, and paclitaxel, among others (Table 1). The TONIC study aimed to compare the immunomodulatory effects of induction chemotherapy followed by nivolumab (anti-PD-1). The overall ORR = 20%, with the highest response rates noted in the cisplatin (ORR = 23%) and doxorubicin (ORR = 35%) cohorts. Analysis of initial and post-induction biopsies showed upregulation of immune-related genes in PD-1/PD-L1 and T cell cytotoxicity pathways in the doxorubicin and cisplatin cohorts [29•]. In addition to exhibiting immunostimulatory effects, one must also consider the long-term lymphodepleting effects of chemotherapy. In a recent comparison of pembrolizumab plus capecitabine or paclitaxel, both chemotherapy backbones were associated with sustained and profound decay of T cell populations over time, including CD4+ and CD8+ T cell populations [28]. Lymphodepletion has been hypothesized as one potential mechanism to explain the decline in anti-PD-1/L1 efficacy in later lines of therapy in TNBC.

Immune Checkpoint Blockade in the Neoadjuvant and Adjuvant Settings

Preliminary neoadjuvant studies of anti-PD-1/L1 have been encouraging, with combination approaches being well tolerated and associated with increases in pathologic complete response (pCR) rate, a known surrogate of overall survival in TNBC (Table 2) [32]. KEYNOTE-173 was a phase Ib study of pembrolizumab with various regimens/dosings of platinum and taxanes as neoadjuvant therapy, followed by 4 cycles of doxorubicin + cyclophosphamide prior to surgery in patients with stage II-III TNBC [33]. In this small study, pCR rates of various chemotherapy/pembrolizumab combinations ranged from 60 to 80%, with the best responses observed in carboplatin-containing cohorts. These data provide rationale for a phase III trial evaluating curative-intent chemotherapy (carboplatin + "ACT" doxorubicin, cyclophosphamide, paclitaxel) + placebo versus pembrolizumab (KEYNOTE-522) [34]. I-SPY2 is an adaptive phase II study that evaluated pCR rates of ACT with or without pembrolizumab. A total of 69 subjects were randomized to combination therapy and showed a 40% increase in the estimated pCR of 60% from 20% in the chemotherapy only control. There are concerns that the pCR rate in the control group was lower than expected; however, the study design permitted investigators to switch therapy or advance to surgery in the setting of clinical non-response, and these subjects were considered treatment failures per the ITT analysis. Of note, dramatic increases in pCR were observed with pembrolizumab even in the context of potential immunosuppressive effects of steroids administered with paclitaxel [30]. Additional neoadjuvant studies are ongoing (Table 3).

One unanswered question is whether treatment sequencing can modulate response. GeparNuevo was a phase II study randomizing subjects to neoadjuvant chemotherapy plus durvalumab (anti-PD-L1) versus placebo [31]. Approximately 117 patients received a 2-week durvalumab/placebo induction prior to commencing chemotherapy. The study failed to meet its endpoint, with 53.4% of patients in the durvalumab arm ($n = 88$) and 44.2% in the placebo arm ($n = 86$) achieving pCR ($p = 0.287$). However, in an unplanned analysis, subjects treated with induction therapy had greater difference in pCR rate (61% vs. 41.4%), highlighting the possibility that anti-PD-L1 pretreatment may enhance response. A related question is whether anti-PD-1/L1 would be effective in the adjuvant setting. In murine models, neoadjuvant immunotherapy was superior to adjuvant immunotherapy in reducing metastatic lesions [35•]. However, treating in the adjuvant setting affords the opportunity to select patients at higher risk of recurrence based upon

Table 2 Reported trials of immune checkpoint blockade in the neoadjuvant setting in TNBC

Trial/phase	Treatment groups	pCR in ICB	pCR in control	Reference
I-SPY2; phase II	Pembrolizumab + paclitaxel followed by AC vs. placebo + paclitaxel followed by AC	62.4%[1]	22.3%[1]	Nanda et al. [30]
GeparNuevo; phase II	Durvalumab + Nab-paclitaxel vs. placebo + Nab-paclitaxel	ITT 53.4%; window 61.0%	ITT 44.2%; window 41.4%	Loibl et al. [31]
KEYNOTE-173; phase Ib	Pembrolizumab + chemotherapy by cohort followed by AC: Cohort A: nab-paclitaxel 125 mg/m^2 weekly Cohort B: nab-paclitaxel 100 mg/m^2 weekly plus carboplatin AUC6 every 3 weeks Cohort C: nab-paclitaxel 125 mg/m^2 weekly plus carboplatin AUC5 every 3 weeks Cohort D: nab-paclitaxel 125 mg/m^2 weekly plus carboplatin AUC2 weekly Cohort E: paclitaxel 80 mg/m^2 weekly plus carboplatin AUC5 every 3 weeks Cohort F: paclitaxel 80 mg/m^2 weekly plus carboplatin AUC2 weekly	Cohort A: 60% Cohort B: 80% Cohort C: 80% Cohort D: 60% Cohort E: 30% Cohort F: 50%	No control arm	Schmid et al. [33]

[1] Estimated pCR rates reported for the TNBC cohort

pCR, pathologic complete response; *ORR*, objective response rate; *AC*, doxorubicin + cyclophosphamide; *ITT*, intention-to-treat; *AUC*, area under curve

Table 3 List of notable ongoing trials evaluating combination therapies with immune checkpoint blockade in TNBC

Trial	Phase/(enrollment)	Intervention/comparison	Setting	Primary endpoint
Chemotherapy				
IMpassion131, NCT03125902	III (600 participants)	Atezolizumab + paclitaxel vs. placebo + paclitaxel	Locally advanced/metastatic	PFS in PD-L1+; PFS in ITT
IMpassion132, NCT03371017	III (350 participants)	Atezolizumab vs. placebo with investigator choice gemcitabine + carboplatin or capecitabine	Locally advanced/metastatic	OS
KEYNOTE-355, NCT02819518	III (882 participants)	Part 1: pembrolizumab with Nab-paclitaxel or paclitaxel or gemcitabine + carboplatin; part 2: pembrolizumab + chemotherapy vs. placebo + chemotherapy	Locally advanced/metastatic	Part 1: safety; part 2: PFS in PD-L1+, OS in all participants, OS in PD-L1+
KEYSTONE, NCT03777579	III (375 participants)	JS001 (anti-PD-1 antibody) + Nab-paclitaxel vs. placebo + Nab-paclitaxel	Metastatic	PFS
IMpassion 031, NCT03197935	III (324 participants)	Atezolizumab + Nab-paclitaxel followed by AC vs. placebo + Nab-paclitaxel followed by AC	Neoadjuvant	pCR; pCR in PD-L1+
NeoTRIPaPDL1, NCT02620280	III (278 participants)	Atezolizumab + carboplatin + Nab-paclitaxel vs. carboplatin + Nab-paclitaxel	Neoadjuvant	EFS
NSABP B-59, NCT03281954	III (1520 participants)	Neoadjuvant: atezolizumab + paclitaxel + carboplatin vs. placebo + paclitaxel + carboplatin; adjuvant: atezolizumab + AC vs. placebo + AC	Neoadjuvant; adjuvant	pCR; EFS
KEYNOTE-522, NCT03036488	III (1174 participants)	Neoadjuvant: pembrolizumab + paclitaxel + carboplatin followed by AC or EC vs. placebo + paclitaxel + carboplatin followed by AC or EC; adjuvant: pembrolizumab vs. placebo	Neoadjuvant; adjuvant	pCR; EFS
IMpassion 030, NCT03498716	III (2300 participants)	Atezolizumab + paclitaxel with ddAC or ddEC vs. paclitaxel followed by ddAC or ddEC	Adjuvant	iDFS
Radiotherapy				
NCT02730130	II (17 participants)	Pembrolizumab + radiotherapy	Locally advanced/metastatic	ORR
NCT02499367	II (84 participants)	Nivolumab + induction with radiotherapy or chemotherapy	Metastatic	PFS
AZTEC, NCT03464942	II (52 participants)	Atezolizumab + single dose SABR vs. atezolizumab + fractionated dose SABR	Metastatic	PFS
NCT03366844	I (30 participants)	Pembrolizumab + radiotherapy	Neoadjuvant	Safety/tolerability; change in TIL
PANDoRA, NCT03872505	II (140 participants)	Durvalumab + carboplatin + paclitaxel vs. durvalumab + carboplatin + paclitaxel + radiation	Neoadjuvant	pCR
Cryoablation				
NCT03546686	II (150 participants)	Ipilimumab + nivolumab + cryoablation + breast surgery + post-surgery nivolumab vs. breast surgery	Neoadjuvant; adjuvant	EFS
MEK inhibitors				
NCT03106415	I/II (38 participants)	Pembrolizumab + binimetinib	Locally advanced/metastatic	Phase I: maximum tolerated dose; phase II: ORR
InCITe, NCT03971409	II (150 participants)	Avelumab + binimetinib or utomilumab or anti-OX40 antibody	Locally advanced/metastatic	ORR
PARP inhibitors				
DORA, NCT03167619	II (60 participants)	Durvalumab + olaparib vs. olaparib alone	Metastatic	PFS
NCT03801369	II (28 participants)	Durvalumab + olaparib	Metastatic	ORR
Androgen receptor				
NCT02971761	II (29 participants)	GTX-024 (selective androgen receptor modulator) + pembrolizumab	Metastatic	Safety; response rate: CR or PR
NCT03650894	II (138 participants)	Bicalutamide + nivolumab + ipilimumab	Locally advanced/metastatic	Clinical benefit rate
Vaccines				
NCT03362060	I (20 participants)	Pembrolizumab + PVX-410 vaccine vs. vaccine alone	Metastatic	Immune response (T cell activation)
NCT02432963	I (19 participants)	Pembrolizumab + p53MVA vaccine	Unresectable solid tumors	Safety

Table 3 (continued)

Trial	Phase/(enrollment)	Intervention/comparison	Setting	Primary endpoint
NCT03761914	I/II (15 participants in TNBC arm)	Pembrolizumab + galinpepimut-S	Residual disease after chemotherapy	Safety; ORR
NCT02826434	I (20 participants)	Durvalumab + PVX-410 vaccine vs. vaccine alone	Post-standard of care	Safety
NCT03199040	I (24 participants)	Durvalumab + neoantigen DNA vaccine vs. vaccine alone	Post-standard of care	Safety
NCT03606967	II (70 participants)	Durvalumab + Nab-paclitaxel + neoantigen vaccine	Metastatic	PFS
NCT03289962	I (770 participants)	Atezolizumab + RO7198457, dose escalation study	Locally advanced/metastatic	Safety/dosing

PFS, progression-free survival; *PD-L1*, programmed death-ligand 1; *ITT*, intention-to-treat; *OS*, overall survival; *PD-1*, programmed cell death protein 1; *pCR*, pathologic complete response; *EFS*, event-free survival; *AC*, doxorubicin + cyclophosphamide; *EC*, epirubicin + cyclophosphamide; *ddAC/ddEC*, dose dense doxorubicin + cyclophosphamide or epirubicin + cyclophosphamide; *iDFS*, invasive disease-free survival; *ORR*, objective response rate; *SABR*, stereotactic ablative radiotherapy; *TIL*, tumor infiltrating lymphocytes; *CR*, complete response; *PR*, partial response

suboptimal chemotherapy response. A number of adjuvant anti-PD-1/L1 studies are ongoing, including IMpassion030, a phase III study of adjuvant atezolizumab versus placebo in combination with chemotherapy (NCT03498716), and SWOG 1418, a phase III study of adjuvant pembrolizumab versus placebo for subjects experiencing non-pCR following neoadjuvant chemotherapy (NCT02954874).

Dual Immune Checkpoint Blockade

There is interest in evaluating the combination of anti-PD-1/L1 with antibodies against other checkpoints such as cytotoxic T lymphocyte antigen 4 (CTLA-4). Ipilimumab (anti-CTLA-4) is FDA-approved in combination with anti-PD-1 (nivolumab) for the indication of melanoma [36, 37], lung cancer, and renal cell carcinoma, and is thought to enhance response by blocking suppressive CTLA-4 signaling on T cells, and/or by depleting CTLA-4-expressing T-regulatory cells [38]. Studies in breast cancer with dual immune checkpoint blockade have been limited, though preclinical studies have shown promise with this combination in TNBC [39]. In a pilot study of anti-PD-L1 (durvalumab) plus anti-CTLA-4 (tremelimumab), the estimated ORR for TNBC was 43% ($n = 4/7$) [40]. A study of dual immune checkpoint blockade using nivolumab and ipilimumab with androgen receptor blockade in metastatic HR-positive and TNBC is currently enrolling [41]. Furthermore, a number of early phase studies are ongoing to evaluate the safety and efficacy of anti-PD-1/L1 with other immune checkpoint agents, including modulators of macrophage or natural killer cell activity.

Immunotherapy with Locoregional Therapy

Radiation is frequently employed in TNBC, either in the adjuvant setting following surgical resection or in the metastatic setting to palliate symptoms. The abscopal effect has long been described in radiotherapy whereby regression of non-radiated lesions occurs following local radiotherapy, a phenomenon thought to be related to a systemic anti-tumor immune response [42]. The synergistic effect of radiotherapy with immunotherapy may be related to release of tumor antigens and DAMPs that can activate an immune response [43]. In a phase II study, pembrolizumab plus radiotherapy in metastatic TNBC was well tolerated in a heavily pre-treated population. Eight of the 17 patients enrolled could not be evaluated for response due to rapid tumor progression and death; however, 33% of evaluable patients ($n = 3/9$) experienced partial response, with one response up to 31 weeks, and another ongoing at 22 weeks, for an ORR = 17.6% [44]. Multiple trials are underway to evaluate the combination of immunotherapy with radiotherapy in TNBC, in the metastatic setting with pembrolizumab (NCT02730130) and nivolumab (NCT02499367), and in pre-operative settings with pembrolizumab (NCT03366844) and

durvalumab (NCT03872505). Studies are also underway comparing single versus multi-fraction stereotactic ablative body radiotherapy with atezolizumab in advanced TNBC (NCT03464942). In addition to radiotherapy, cryoablation has been evaluated in combination with immunotherapy. A pilot study of pre-operative cryoablation and single-dose of ipilimumab found to be safe and associated with intratumoral and systemic immune effects [45]. A phase II study of peri-operative ipilimumab + nivolumab + cryoablation after taxane-based neoadjuvant chemotherapy in resectable TNBC is ongoing (NCT03546686).

Immunotherapy with Other Targeted Therapies

Approximately 15% of TNBCs have alterations in the Ras/MAPK pathway. In preclinical TNBC models, MEK inhibiters were clinically active in combination with anti-PD-1/L1 inhibitors, and associated with MHC and PD-L1 upregulation [46]. The phase II COLET study evaluated the MEK 1/2 inhibitor cobimetinib with atezolizumab plus taxane in TNBC, with an ORR = 34% (11/32•) with paclitaxel, and an ORR = 29% (9/31) with nab-paclitaxel. Higher response rates were noted in PD-L1-positive tumors (ORR 44%/33%) [47]. Additional studies are underway (NCT03106415, NCT03971409).

BRCA1/2 mutations are found in 20–30% of TNBCs [48••, 49], causing deficiency in DNA repair and sensitivity to DNA-targeting cytotoxic agents (cisplatin/carboplatin) and inhibitors of the poly(ADP-Ribose) polymerase 1 enzyme (PARP) [48••, 50, 51]. Furthermore, PARP inhibitors can upregulate PD-L1 and enhance cancer-associated immunosuppression, and therefore, combination with anti-PD-1/L1 agents is of interest [52]. TOPACIO/KEYNOTE-162 was a study combining the PARP inhibitor niraparib with pembrolizumab in TNBC, with initial data on 45 patients showing an ORR = 29%, with a disease control rate of 49%. Among BRCA mutant patients, the ORR was 67% with a disease control rate of 75% [53]. Studies with durvalumab plus olaparib in metastatic TNBC are also ongoing (NCT03167619, NCT03801369).

The androgen receptor has also been identified as a potential target for TNBC, with approximately 55% of TNBC having some degree of upregulation of the androgen receptor [54]. Early trials have shown tolerability and clinical benefit with androgen receptor blockade in TNBC, with a clinical benefit rate of 35% at 16 weeks in one study, with higher rates noted in patients who were positive for an androgen-related gene signature [55]. Preclinical studies have shown that androgen receptor blockade may augment thymic production of T cells, leading to interest in combination with immunotherapy [56]. A phase II study of pembrolizumab with GTx-024, a nonsteroidal selective androgen receptor modulator, showed that treatment was well tolerated, with 2 partial responses and 2 with stable disease out of 16 patients, and is ongoing [57]. Additionally, a phase II study combining bicalutamide with dual immune checkpoint blockade with nivolumab plus ipilimumab is currently underway (NCT03650894) [41].

More than 3000 next-generation immunomodulatory agents are in clinical development either as monotherapy or in combination with anti-PD-1/L1 to increase efficacy. As of this review, no phase II/III studies have confirmed clinical benefit of this approach in TNBC. However, going forward, next-generation biomarkers (such as DNA/RNA deep sequencing and multispectral TIL analysis) may provide more nuanced understanding of immune profiles and pharmacodynamic effects of immunotherapy agents, and could ultimately be used to personalize combination therapy and improve likelihood of clinical benefit.

Cellular Therapy

T cells have been identified as key players in potentiating anti-tumor immunity, and are being investigated in TNBC. Adoptive cell therapy is a form of immunotherapy that involves isolating T cells from a patient, enriching for tumor-specific clones (sometimes by selecting for reactivity against mutated proteins), expanding and activating these cells ex-vivo, and then autologously administrating them back to the patient [58, 59]. Recently, a subject with chemo-refractory hormone receptor–positive breast cancer experienced a complete response with this approach, and evaluation is ongoing in TNBC patients [60]. An alternative adoptive cell therapy is chimeric antigen receptor (CAR) T cell therapy, whereby T cells are genetically engineered to express receptors against a specific target (such as CD19 for B cell malignancies). Target selection is critical for the success and safety of this approach, as even low target expression on non-malignant tissue can lead to substantial toxicity. Severe allergic reactions, cytokine release syndrome, and neurologic toxicities have been documented with cellular therapies, often requiring inpatient administration and monitoring [61]. Potential targets under investigation for cellular therapy in TNBC include MUC1 [62], NKG2D [63], AXL [64], TEM8/ANTXR1 [65], FRα [66], mesothelin (NCT02892114), and ROR1 (NCT02706392). Preliminary data of 4 TNBC patients treated with ROR1+ CAR-T cells has been presented, with 2 patients demonstrating stable disease (one at 15 weeks, one at 19 weeks), and one patient with partial response after a 2nd infusion [67].

Vaccines

A number of cancer vaccines are in development for TNBC, and aim to facilitate anti-tumor immunity by directing the immune response against tumor-associated antigens (NCT03674827, NCT03387085, NCT02593227, NCT03012100) [68]. In addition, there is great interest in the combination of vaccines with immune checkpoint blockade to enhance the ability of vaccines

to elicit a T cell response (NCT03362060, NCT02432963, NCT03761914, NCT02826434, NCT03199040, NCT03606967, NCT03289962). Early data on peptides as vaccines for metastatic cancer showed low response rates, with a 2.9% ORR in a combined evaluation of 381 patients with metastatic cancer receiving peptide vaccines [69]. More encouraging results have come from personalized peptide vaccination (PPV), a type of vaccine where antigens are selected from a pool of different peptides based on pre-existing host immunity. Currently, a maximum of 4 peptides are selected among a group of 31 different HLA class-I peptide candidates, based on HLA typing and pre-existing immune responses to each candidate. Early trials in multiple different tumor types have shown a response rate of 9.9% and a disease control rate of 42.9% in a group of 500 patients with advanced cancer [70]. PPV has been evaluated in TNBC and was safe and potentially effective, with a median PFS of 7.5 months and median OS of 11.1 months in metastatic TNBC, with 1 complete response and 1 partial response noted in a cohort of 18 patients [71]. Additional small studies are ongoing in TNBC (NCT02427581). Dendritic cell (DC) vaccines have also been found to be an effective strategy in multiple cancer types, most notably prostate, and evaluation in TNBC has found safety and potential efficacy as well [72, 73].

Autophagy-based vaccines are being developed for TNBC. In short, cancer cells are manipulated ex vivo with proteasome inhibitors and other modulators of autophagy to create a vaccine against tumor-associated proteins as well as short-lived proteins and organelle fragments that would be otherwise degraded [74]. Autophagy-based vaccines have been shown preclinically to be effective not only in the autologous setting where the vaccine was made from a host's own tumor but also in an allogenic setting whereby the vaccine is made by other tumor cell lines, allowing for creation of an "off-the-shelf" vaccine that could be used for many different patients [74]. Based upon preclinical efficacy in mammary carcinomas, a trial of vaccine + anti-PD-1 + T cell agonist (anti-OX40) is underway (NCT02737475).

The Role of Biomarkers in Immunotherapy for Breast Cancer

The results of the IMpassion130 highlight the need for immune-based biomarkers in TNBC, as larger improvements in PFS and OS were noted in the PD-L1-positive subgroup compared with the ITT population. However, it must be noted that PD-L1 positivity may vary according to assay and cutoff and has not been consistently predictive

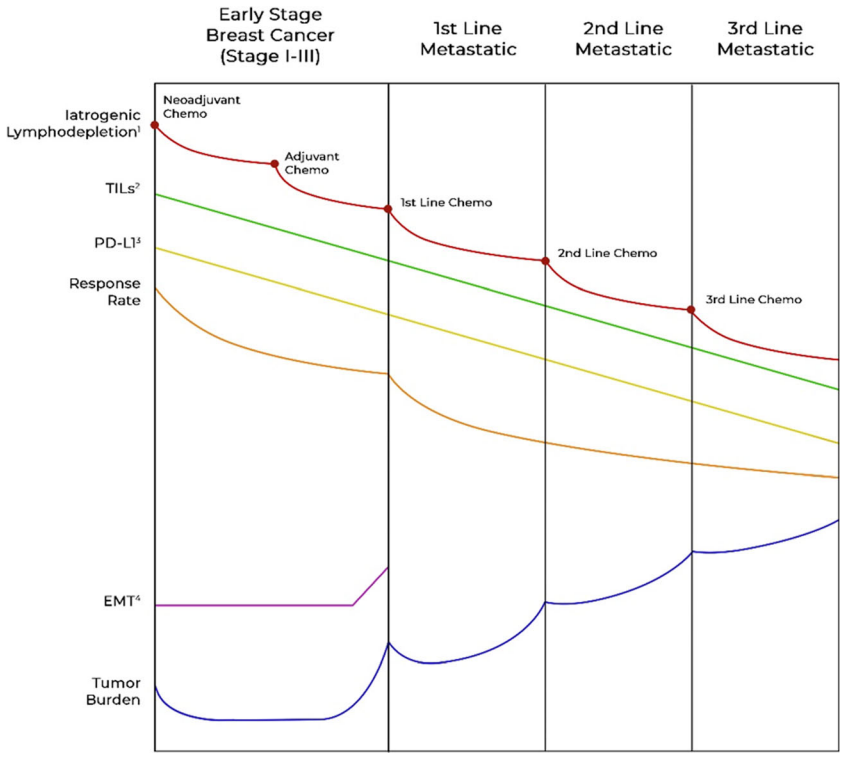

Fig. 1 Overview of potential biologic changes in TNBC over the natural history of the disease. The changes in iatrogenic lymphodepletion, TILs, and PD-L1 expression over the natural history of TNBC may impact the timing and effectiveness of immunotherapies. A trend towards decreased PD-L1 expression in metastatic versus early breast cancer has been seen, though a clear correlation has not been established. Epithelial-to-mesenchymal transition is being evaluated as a predictive marker for immunotherapy outcomes. TILs: tumor-infiltrating lymphocytes; PD-L1: programmed death-ligand 1; EMT: epithelial-mesenchymal transition. [1] Page et al. [27]. [2] Ogiya et al. [79]. [3] Manson et al., Tawfik et al. [86, 87]. [4] Terry et al. [88]

for anti-PD-1/L1 response [75]. One unanswered question is whether it is more relevant to test PD-L1 on tumor cells or immune cells or both, with preclinical data supporting the relevance of both [76]. IMpassion130 used a definition of PD-L1 positivity of ≥ 1% PD-L1 expression on immune cells, whereas other clinical trials employ combined tumor/immune cell scores or other approaches. Various PD-L1 assays (Dako 22C3, SP142, SP263) may provide incongruous results on the same tumor [77•]. In particular, the SP142 assay used in the IMpassion130 trial appears to be less sensitive than Dako 22C3 and SP263, with a lower percentage of tumors testing PD-L1-positive. It is unknown whether tumors that are PD-L1-negative by SP142 but PD-L1-positive by other assays are biologically distinct entities, and whether they would benefit from anti-PD-1/L1.

Another biomarker of interest is the stromal TIL score, which is known to be prognostic and predictive in the setting of curative-intent chemotherapy. In the IMpassion130 analysis, stromal TIL score or CD8+ T cell count did not independently predict benefit to atezolizumab [78]. There are some considerations as to why stromal TILs were unable to correlate with benefit. TILs have been noted to be decreased in metastatic compared with early breast cancers [79]. There also appears to be a scarcity of stroma in metastatic breast cancer specimens and whether this could contribute to an inability to detect an association between stromal TILs and OS benefit is a consideration. Assessment of KEYNOTE-086 patients who received pembrolizumab monotherapy in metastatic TNBC showed higher TILs could identify patients who were more likely to respond to therapy. TIL level was higher in the previously untreated population, and higher TIL levels were associated with significantly improved ORR and disease control rates [80].

Tumor mutational burden (TMB) has been correlated with response to immunotherapy in multiple cancer types; however, high TMB is uncommon in breast cancer [5]. In one study, only 3.1% of breast carcinomas had high TMB (> 20 mutations/Mb), compared with 39.7% of melanoma and up to 24.3% of lung carcinomas. In another study of TMB in 3689 breast cancer samples, 4.2% of TNBC samples were found to have a high TMB [81]. TMB may be a useful biomarker in TNBC patients with high TMB, but may also exclude patients who may benefit from immunotherapy. Extensive research is ongoing to develop pipelines for identifying specific mutations that may be associated with anti-tumor immune response [5, 6]. Mismatch repair deficiency is also predictive of benefit with anti-PD-1 therapy, and pembrolizumab is FDA-approved for solid tumors with high microsatellite instability or mismatch repair deficiency [82]. However, these defects are uncommon in TNBC, with one analysis estimating 0.7% in TNBC [83, 84]. A number of novel biomarkers are being explored in immunotherapy, such as serum proteins, peripheral blood immune cells, and host genomic factors [85].

Conclusions

Immunotherapy has emerged as a promising treatment modality for TNBC. Initial trials have established a role for anti-PD-L1 in the first-line metastatic setting in combination with chemotherapy. Ongoing trials will clarify the role of anti-PD-1/L1 as monotherapy, in later lines of therapy, or in combination with other therapies. Additional work is ongoing to identify clinical factors (LDH, liver metastases) and biomarkers to optimize use of these agents. Multiple trials are also underway evaluating which combination of therapies may work best with immune checkpoint blockade including combinations with chemotherapy, radiotherapy, cryotherapy, vaccines, and other targeted agents (Table 3**)**.

With factors such as TILs and PD-L1 expression decreased in metastatic versus early TNBC, and with the impact of iatrogenic lymphodepletion over the course of the disease and treatment for it, immunotherapy benefit may be more pronounced in earlier settings (14, 15, 17, 25, 76) (Fig. 1). Initial studies of immunotherapy in early TNBC have been encouraging (Table 2), and results of further studies evaluating the role of immunotherapy in early breast cancer are highly anticipated.

Compliance with Ethical Standards

Conflict of Interest Heather McArthur has consulted for Amgen, Bristol-Myers Squibb, Celgene, Eli Lilly, Genentech/Roche, Immunomedics, Merck, OBI Pharma, Pfizer, Puma, Spectrum Pharmaceuticals, Syndax Pharmaceuticals, Peregrine, Calithera, and TapImmune. The author has research supported by Bristol-Myers Squibb; MedImmune, LLC/AstraZenica; and Merck. David Page reports personal fees from Genentech, grants and personal fees from Merck, personal fees from Novartis, personal fees from Puma, personal fees from Nanostring, personal fees from Nektar, personal fees from Syndax, grants and personal fees from Brooklyn Immunotherapeutics, and grants and personal fees from Bristol Myers-Squibb outside the submitted work. Isaac Kim and Katherine Sanchez declare no conflicts of interest relevant to this manuscript.

Human and Animal Rights and Informed Consent All reported studies/experiments with human or animal subjects performed by the authors have been previously published and complied with all applicable ethical standards (including the Helsinki declaration and its amendments, institutional/national research committee standards, and international/national/institutional guidelines).

Open Access This article is distributed under the terms of the Creative Commons Attribution 4.0 International License (http://creativecommons.org/licenses/by/4.0/), which permits unrestricted use, distribution, and reproduction in any medium, provided you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made.

References

Papers of particular interest, published recently, have been highlighted as:
- • Of importance
- •• Of major importance

1. Denkert C, Liedtke C, Tutt A, von Minckwitz G. Molecular alterations in triple-negative breast cancer - the road to new treatment strategies. Lancet. 2017;389(10087):2430–42. https://doi.org/10.1016/S0140-6736(16)32454-0.
2. Narang P, Chen M, Sharma AA, Anderson KS, Wilson MA. The neoepitope landscape of breast cancer: implications for immunotherapy. BMC Cancer. 2019;19(1):200. https://doi.org/10.1186/s12885-019-5402-1.
3. Budczies J, Bockmayr M, Denkert C, Klauschen F, Lennerz JK, Györffy B, et al. Classical pathology and mutational load of breast cancer - integration of two worlds. J Pathol Clin Res. 2015;1(4):225–38. https://doi.org/10.1002/cjp2.25.
4. Luen S, Virassamy B, Savas P, Salgado R, Loi S. The genomic landscape of breast cancer and its interaction with host immunity. Breast. 2016;29:241–50. https://doi.org/10.1016/j.breast.2016.07.015.
5. Samstein RM, Lee C-H, Shoushtari AN, Hellmann MD, Shen R, Janjigian YY, et al. Tumor mutational load predicts survival after immunotherapy across multiple cancer types. Nat Genet. 2019;51(2):202–6. https://doi.org/10.1038/s41588-018-0312-8.
6. Goodman AM, Kato S, Bazhenova L, Patel SP, Frampton GM, Miller V, et al. Tumor mutational burden as an independent predictor of response to immunotherapy in diverse cancers. Molecular Cancer Therapeutics. 2017:molcanther.0386.2017. https://doi.org/10.1158/1535-7163.Mct-17-0386.
7. Loi S, Michiels S, Salgado R, Sirtaine N, Jose V, Fumagalli D, et al. Tumor infiltrating lymphocytes are prognostic in triple negative breast cancer and predictive for trastuzumab benefit in early breast cancer: results from the FinHER trial. Ann Oncol. 2014;25(8):1544–50. https://doi.org/10.1093/annonc/mdu112.
8. Salgado R, Denkert C, Demaria S, Sirtaine N, Klauschen F, Pruneri G, et al. The evaluation of tumor-infiltrating lymphocytes (TILs) in breast cancer: recommendations by an International TILs Working Group 2014. Ann Oncol. 2015;26(2):259–71. https://doi.org/10.1093/annonc/mdu450.
9. • Denkert C, von Minckwitz G, Darb-Esfahani S, Lederer B, Heppner BI, Weber KE, et al. Tumour-infiltrating lymphocytes and prognosis in different subtypes of breast cancer: a pooled analysis of 3771 patients treated with neoadjuvant therapy. Lancet Oncol. 2018;19(1):40–50. https://doi.org/10.1016/S1470-2045(17)30904-X **This study was an analysis of 3771 patients using the standardized methods in the guidelines of the International TIL Working Group that showed TILs were predictive for response to neoadjuvant chemotherapy in all molecular subtypes of breast cancer assessed, and were also associated with survival benefit in HER2-positive and TNBC.**
10. Stanton SE, Adams S, Disis ML. Variation in the incidence and magnitude of tumor-infiltrating lymphocytes in breast cancer subtypes: a systematic review. JAMA Oncology. 2016;2(10):1354–60. https://doi.org/10.1001/jamaoncol.2016.1061.
11. Beckers RK, Selinger CI, Vilain R, Madore J, Wilmott JS, Harvey K, et al. Programmed death ligand 1 expression in triple-negative breast cancer is associated with tumour-infiltrating lymphocytes and improved outcome. Histopathology. 2016;69(1):25–34. https://doi.org/10.1200/JCO.2018.36.15_suppl.1010.
12. Mittendorf EA, Philips AV, Meric-Bernstam F, Qiao N, Wu Y, Harrington S, et al. PD-L1 expression in triple-negative breast cancer. Cancer Immunol Res. 2014;2(4):361–70. https://doi.org/10.1158/2326-6066.Cir-13-0127.
13. Emens LA, Cruz C, Eder JP, Braiteh F, Chung C, Tolaney SM, et al. Long-term clinical outcomes and biomarker analyses of atezolizumab therapy for patients with metastatic triple-negative breast cancer: a phase 1 study. JAMA Oncol. 2019;5(1):74–82. https://doi.org/10.1001/jamaoncol.2018.4224.
14. Adams S, Diamond JR, Hamilton E, Pohlmann PR, Tolaney SM, Chang C-W, et al. Atezolizumab plus nab-paclitaxel in the treatment of metastatic triple-negative breast cancer with 2-year survival follow-up: a phase 1b clinical trial. JAMA Oncol. 2019;5(3):334–42. https://doi.org/10.1001/jamaoncol.2018.5152.
15. Tolaney S, Kalinsky K, Kaklamani V, Savulsky C, Olivo M, Aktan G, et al. Abstract PD6-13: Phase 1b/2 study to evaluate eribulin mesylate in combination with pembrolizumab in patients with metastatic triple-negative breast cancer. Cancer Res. 2018;78(4 Supplement):PD6-13-PD6. https://doi.org/10.1158/1538-7445.Sabcs17-pd6-13.
16. • Adams S, Loi S, Toppmeyer D, Cescon DW, De Laurentiis M, Nanda R, et al. Pembrolizumab monotherapy for previously untreated, PD-L1-positive, metastatic triple-negative breast cancer: cohort B of the phase II KEYNOTE-086 study. Annals of Oncology. 2018;30(3):405–11. https://doi.org/10.1093/annonc/mdy518 **This study, along with reference [20], is the data from the 2 cohorts of KEYNOTE-086 showing that pembrolizumab monotherapy for metastatic triple-negative breast cancer was more efficacious as first-line therapy and with PD-L1 positivity.**
17. •• Schmid P, Adams S, Rugo HS, Schneeweiss A, Barrios CH, Iwata H, et al. Atezolizumab and nab-paclitaxel in advanced triple-negative breast cancer. New England Journal of Medicine. 2018;379(22):2108–21. https://doi.org/10.1056/NEJMoa1809615 **The IMpassion130 study demonstrated increased overall survival in metastatic TNBC in a PD-L1 expressing subgroup. This trial led to the FDA approval for atezolizumab + nab-paclitaxel for PD-L1-positive metastatic TNBC.**
18. Nanda R, Chow LQM, Dees EC, Berger R, Gupta S, Geva R, et al. Pembrolizumab in patients with advanced triple-negative breast cancer: phase Ib KEYNOTE-012 study. J Clin Oncol. 2016;34(21):2460–7. https://doi.org/10.1200/JCO.2015.64.8931.
19. Dirix LY, Takacs I, Jerusalem G, Nikolinakos P, Arkenau H-T, Forero-Torres A, et al. Avelumab, an anti-PD-L1 antibody, in patients with locally advanced or metastatic breast cancer: a phase 1b JAVELIN Solid Tumor study. Breast Cancer Res Treat. 2018;167(3):671–86. https://doi.org/10.1007/s10549-017-4537-5.
20. Adams S, Schmid P, Rugo HS, Winer EP, Loirat D, Awada A, et al. Pembrolizumab monotherapy for previously treated metastatic triple-negative breast cancer: cohort a of the phase II KEYNOTE-086 study. Ann Oncol. 2018;30(3):397–404. https://doi.org/10.1093/annonc/mdy517.
21. Winer EP, Dang T, Karantza V, Su S-C. KEYNOTE-119: A randomized phase III study of single-agent pembrolizumab (MK-3475) vs single-agent chemotherapy per physician's choice for metastatic triple-negative breast cancer (mTNBC). J Clin Oncol. 2016;34(15_suppl):TPS1102-TPS. https://doi.org/10.1200/JCO.2016.34.15_suppl.TPS1102.
22. Wang Y-J, Fletcher R, Yu J, Zhang L. Immunogenic effects of chemotherapy-induced tumor cell death. Genes Dis. 2018;5(3):194–203. https://doi.org/10.1016/j.gendis.2018.05.003.
23. Bracci L, Schiavoni G, Sistigu A, Belardelli F. Immune-based mechanisms of cytotoxic chemotherapy: implications for the design of novel and rationale-based combined treatments against cancer. Cell Death Differ. 2014;21(1):15–25. https://doi.org/10.1038/cdd.2013.67.
24. Zitvogel L, Galluzzi L, Smyth Mark J, Kroemer G. Mechanism of action of conventional and targeted anticancer therapies: reinstating immunosurveillance. Immunity. 2013;39(1):74–88. https://doi.org/10.1016/j.immuni.2013.06.014.

25. Schmid P, Adams S, Rugo HS, Schneeweiss A, Barrios CH, Iwata H, et al. IMpassion130: updated overall survival (OS) from a global, randomized, double-blind, placebo-controlled, Phase III study of atezolizumab (atezo) + nab-paclitaxel (nP) in previously untreated locally advanced or metastatic triple-negative breast cancer (mTNBC). J Clin Oncol. 2019;37(15_suppl):1003. https://doi.org/10.1200/JCO.2019.37.15_suppl.1003.
26. Dent R, Andre F, Goncalves A, Kummel S, Martin M, Schmid P, et al. IMpassion132: A double-blind randomized phase 3 trial evaluating chemotherapy (CT) ± atezolizumab (atezo) for early progressing locally advanced/metastatic triple-negative breast cancer (mTNBC). J Clin Oncol. 2018;36(15_suppl):TPS1115-TPS. https://doi.org/10.1200/JCO.2018.36.15_suppl.TPS1115.
27. Cortés J, Guo Z, Karantza V, Aktan G. KEYNOTE-355: randomized, double-blind, phase III study of pembrolizumab (pembro) + chemotherapy (chemo) vs placebo (PBO) + chemo for previously untreated, locally recurrent, inoperable or metastatic triple-negative breast cancer (mTNBC). J Clin Oncol. 2018;36(5_suppl):TPS18-TPS. https://doi.org/10.1200/JCO.2018.36.5_suppl.TPS18.
28. Page DB, Chun B, Pucilowska J, Kim I, Sanchez K, Redmond WL, et al. Pembrolizumab (pembro) with paclitaxel (taxol) or capecitabine (cape) as early treatment of metastatic triple-negative breast cancer (mTNBC). J Clin Oncol. 2019;37(15_suppl):1015. https://doi.org/10.1200/JCO.2019.37.15_suppl.1015.
29.• Voorwerk L, Slagter M, Horlings HM, Sikorska K, van de Vijver KK, de Maaker M, et al. Immune induction strategies in metastatic triple-negative breast cancer to enhance the sensitivity to PD-1 blockade: the TONIC trial. Nat Med. 2019;25(6):920–8. https://doi.org/10.1038/s41591-019-0432-4 **The TONIC study is an adaptive multigroup trial evaluating nivolumab with various induction regimens. Higher responses were noted with doxorubicin and cisplatin inductions. Potential mechanisms for this were explored with analysis of baseline and post-induction biopsies.**
30.• Nanda R, Liu MC, Yau C, Asare S, Hylton N, Veer LVT, et al. Pembrolizumab plus standard neoadjuvant therapy for high-risk breast cancer (BC): Results from I-SPY 2. J Clin Oncol. 2017;35(15_suppl):506. https://doi.org/10.1200/JCO.2017.35.15_suppl.506 **The I-SPY 2 trial has an adaptive multigroup trial whose design which evaluates novel neoadjuvant therapies. A 40% increase in pCR was noted with pembrolizumab + paclitaxel.**
31. Loibl S, Untch M, Burchardi N, Huober JB, Blohmer JU, Grischke E-M, et al. Randomized phase II neoadjuvant study (GeparNuevo) to investigate the addition of durvalumab to a taxane-anthracycline containing chemotherapy in triple negative breast cancer (TNBC). J Clin Oncol. 2018;36(15_suppl):104. https://doi.org/10.1200/JCO.2018.36.15_suppl.104.
32. Cortazar P, Zhang L, Untch M, Mehta K, Costantino JP, Wolmark N, et al. Pathological complete response and long-term clinical benefit in breast cancer: the CTNeoBC pooled analysis. Lancet. 2014;384(9938):164–72. https://doi.org/10.1016/S0140-6736(13)62422-8.
33. Schmid P, Park Y, Muñoz-Couselo E, Kim S-B, Sohn J, Im S-A, et al. Abstract PD5–01: KEYNOTE-173: Phase 1b multicohort study of pembrolizumab (Pembro) in combination with chemotherapy as neoadjuvant treatment for triple-negative breast cancer (TNBC). Cancer Res. 2019;79(4 Supplement):PD5–01-PD5. https://doi.org/10.1158/1538-7445.Sabcs18-pd5-01.
34. Schmid P, Cortes J, Bergh JCS, Pusztai L, Denkert C, Verma S, et al. KEYNOTE-522: Phase III study of pembrolizumab (pembro) + chemotherapy (chemo) vs placebo + chemo as neoadjuvant therapy followed by pembro vs placebo as adjuvant therapy for triple-negative breast cancer (TNBC). J Clin Oncol. 2018;36(15_suppl):TPS602-TPS. https://doi.org/10.1200/JCO.2018.36.15_suppl.TPS602.
35.• Liu J, Blake SJ, Yong MCR, Harjunpää H, Ngiow SF, Takeda K, et al. Improved efficacy of neoadjuvant compared to adjuvant immunotherapy to eradicate metastatic disease. Cancer Discovery. 2016;6(12):1382–99. https://doi.org/10.1158/2159-8290.Cd-16-0577 **This preclinical study in murine models found neoadjuvant immunotherapy was superior to adjuvant therapy in reducing metastatic lesions. Multiple clinical trials are underway evaluating immunotherapy in the neoadjuvant setting.**
36. Larkin J, Chiarion-Sileni V, Gonzalez R, Grob JJ, Cowey CL, Lao CD, et al. Combined nivolumab and ipilimumab or monotherapy in untreated melanoma. N Engl J Med. 2015;373(1):23–34. https://doi.org/10.1056/NEJMoa1504030.
37. Wolchok JD, Chiarion-Sileni V, Gonzalez R, Rutkowski P, Grob J-J, Cowey CL, et al. Overall survival with combined nivolumab and ipilimumab in advanced melanoma. N Engl J Med. 2017;377(14):1345–56. https://doi.org/10.1056/NEJMoa1709684.
38. Pardoll DM. The blockade of immune checkpoints in cancer immunotherapy. Nat Rev Cancer. 2012;12(4):252–64. https://doi.org/10.1038/nrc3239.
39. Crosby EJ, Wei J, Yang XY, Lei G, Wang T, Liu C-X, et al. Complimentary mechanisms of dual checkpoint blockade expand unique T-cell repertoires and activate adaptive anti-tumor immunity in triple-negative breast tumors. Oncoimmunology. 2018;7(5):e1421891-e. https://doi.org/10.1080/2162402X.2017.1421891.
40. Santa-Maria CA, Kato T, Park J-H, Kiyotani K, Rademaker A, Shah AN, et al. A pilot study of durvalumab and tremelimumab and immunogenomic dynamics in metastatic breast cancer. Oncotarget. 2018;9(27):18985–96. https://doi.org/10.18632/oncotarget.24867.
41. Page DB, Kim IK, Chun B, Redmond WL, Martel M, Mori M, et al. A phase II study of dual immune checkpoint blockade (ICB) plus androgen receptor (AR) blockade to enhance thymic T-cell production and immunotherapy response in metastatic breast cancer (MBC). J Clin Oncol. 2019;37(15_suppl):TPS1106-TPS. https://doi.org/10.1200/JCO.2019.37.15_suppl.TPS1106.
42. Liu Y, Dong Y, Kong L, Shi F, Zhu H, Yu J. Abscopal effect of radiotherapy combined with immune checkpoint inhibitors. J Hematol Oncol. 2018;11(1):104. https://doi.org/10.1186/s13045-018-0647-8.
43. Demaria S, Formenti SC. Radiation as an immunological adjuvant: current evidence on dose and fractionation. Front Oncol. 2012;2:153. https://doi.org/10.3389/fonc.2012.00153.
44. McArthur HL, Barker CA, Gucalp A, Lebron-Zapata L, Wen YH, Phung A, et al. A single-arm, phase II study assessing the efficacy of pembrolizumab (pembro) plus radiotherapy (RT) in metastatic triple negative breast cancer (mTNBC). J Clin Oncol. 2018;36(5_suppl):14. https://doi.org/10.1200/JCO.2018.36.5_suppl.14.
45. McArthur HL, Diab A, Page DB, Yuan J, Solomon SB, Sacchini V, et al. A pilot study of preoperative single-dose ipilimumab and/or cryoablation in women with early-stage breast cancer with comprehensive immune profiling. Clin Cancer Res. 2016;22(23):5729–37. https://doi.org/10.1158/1078-0432.CCR-16-0190.
46. Loi S, Dushyanthen S, Beavis PA, Salgado R, Denkert C, Savas P, et al. RAS/MAPK activation is associated with reduced tumor-infiltrating lymphocytes in triple-negative breast cancer: therapeutic cooperation between MEK and PD-1/PD-L1 immune checkpoint inhibitors. Clin Cancer Res. 2016;22(6):1499–509. https://doi.org/10.1158/1078-0432.CCR-15-1125.
47. Brufsky A, Kim S-B, Zvirbule Z, Dirix LY, Eniu AE, Carabantes F, et al. Phase II COLET study: atezolizumab (A) + cobimetinib (C) + paclitaxel (P)/nab-paclitaxel (nP) as first-line (1L) treatment (tx) for patients (pts) with locally advanced or metastatic triple-negative breast cancer (mTNBC). J Clin Oncol. 2019;37(15_suppl):1013. https://doi.org/10.1200/JCO.2019.37.15_suppl.1013.
48.•• Robson M, Im S-A, Senkus E, Xu B, Domchek SM, Masuda N, et al. Olaparib for metastatic breast cancer in patients with a germline BRCA mutation. New England Journal of Medicine. 2017;377(6):523–33. https://doi.org/10.1056/NEJMoa1706450 **This study of the PARP inhibitor olaparib led to the first**

FDA-approved treatment for germline BRCA mutated HER2-negative breast cancer. In the study population, 50% were TNBC.

49. Greenup R, Buchanan A, Lorizio W, Rhoads K, Chan S, Leedom T, et al. Prevalence of BRCA mutations among women with triple-negative breast cancer (TNBC) in a genetic counseling cohort. Ann Surg Oncol. 2013;20(10):3254–8. https://doi.org/10.1245/s10434-013-3205-1.
50. Litton JK, Rugo HS, Ettl J, Hurvitz SA, Gonçalves A, Lee K-H, et al. Talazoparib in patients with advanced breast cancer and a germline BRCA mutation. N Engl J Med. 2018;379(8):753–63. https://doi.org/10.1056/NEJMoa1802905.
51. Farmer H, McCabe N, Lord CJ, Tutt ANJ, Johnson DA, Richardson TB, et al. Targeting the DNA repair defect in BRCA mutant cells as a therapeutic strategy. Nature. 2005;434(7035):917–21. https://doi.org/10.1038/nature03445.
52. Jiao S, Xia W, Yamaguchi H, Wei Y, Chen M-K, Hsu J-M, et al. PARP inhibitor upregulates PD-L1 expression and enhances cancer-associated immunosuppression. Clin Cancer Res. 2017;23(14):3711–20. https://doi.org/10.1158/1078-0432.CCR-16-3215.
53. Vinayak S, Tolaney SM, Schwartzberg LS, Mita MM, McCann GA-L, Tan AR, et al. TOPACIO/Keynote-162: niraparib + pembrolizumab in patients (pts) with metastatic triple-negative breast cancer (TNBC), a phase 2 trial. J Clin Oncol. 2018;36(15_suppl):1011. https://doi.org/10.1200/JCO.2018.36.15_suppl.1011.
54. Gucalp A, Traina TA. Targeting the androgen receptor in triple-negative breast cancer. Curr Probl Cancer. 2016;40(2–4):141–50. https://doi.org/10.1016/j.currproblcancer.2016.09.004.
55. Traina TA, Miller K, Yardley DA, O'Shaughnessy J, Cortes J, Awada A, et al. Results from a phase 2 study of enzalutamide (ENZA), an androgen receptor (AR) inhibitor, in advanced AR+ triple-negative breast cancer (TNBC). J Clin Oncol. 2015;33(15_suppl):1003. https://doi.org/10.1200/jco.2015.33.15_suppl.1003.
56. Velardi E, Tsai JJ, Holland AM, Wertheimer T, Yu VWC, Zakrzewski JL, et al. Sex steroid blockade enhances thymopoiesis by modulating Notch signaling. J Exp Med. 2014;211(12):2341–9. https://doi.org/10.1084/jem.20131289.
57. Lee-Bitar JS, Frankel PH, Yost SE, Synold TW, Martinez N, Tang A, et al. A phase II clinical trial of pembrolizumab and selective androgen receptor modulator GTx-024 in patients with advanced androgen receptor-positive triple-negative breast cancer. Journal of Clinical Oncology. 2019;37(15_suppl):1069. https://doi.org/10.1200/JCO.2019.37.15_suppl.1069.
58. Dudley ME, Rosenberg SA. Adoptive-cell-transfer therapy for the treatment of patients with cancer. Nat Rev Cancer. 2003;3(9):666–75. https://doi.org/10.1038/nrc1167.
59. Rosenberg SA, Restifo NP. Adoptive cell transfer as personalized immunotherapy for human cancer. Science. 2015;348(6230):62–8. https://doi.org/10.1126/science.aaa4967.
60. Zacharakis N, Chinnasamy H, Black M, Xu H, Lu Y-C, Zheng Z, et al. Immune recognition of somatic mutations leading to complete durable regression in metastatic breast cancer. Nat Med. 2018;24(6):724–30. https://doi.org/10.1038/s41591-018-0040-8.
61. Brudno JN, Kochenderfer JN. Toxicities of chimeric antigen receptor T cells: recognition and management. Blood. 2016;127(26):3321–30. https://doi.org/10.1182/blood-2016-04-703751.
62. Zhou R, Yazdanifar M, Roy LD, Whilding LM, Gavrill A, Maher J, et al. CAR T cells targeting the tumor MUC1 glycoprotein reduce triple-negative breast cancer growth. Front Immunol. 2019, 10;(1149). https://doi.org/10.3389/fimmu.2019.01149.
63. Han Y, Xie W, Song D-G, Powell DJ Jr. Control of triple-negative breast cancer using ex vivo self-enriched, costimulated NKG2D CAR T cells. J Hematol Oncol. 2018;11(1):92. https://doi.org/10.1186/s13045-018-0635-z.
64. Wei J, Sun H, Zhang A, Wu X, Li Y, Liu J, et al. A novel AXL chimeric antigen receptor endows T cells with anti-tumor effects against triple negative breast cancers. Cell Immunol. 2018;331:49–58. https://doi.org/10.1016/j.cellimm.2018.05.004.
65. Byrd TT, Fousek K, Pignata A, Szot C, Samaha H, Seaman S, et al. TEM8/ANTXR1-specific CAR T cells as a targeted therapy for triple-negative breast cancer. Cancer Res. 2018;78(2):489–500. https://doi.org/10.1158/0008-5472.CAN-16-1911.
66. Song D-G, Ye Q, Poussin M, Chacon JA, Figini M, Powell DJ Jr. Effective adoptive immunotherapy of triple-negative breast cancer by folate receptor-alpha redirected CAR T cells is influenced by surface antigen expression level. J Hematol Oncol. 2016;9(1):56. https://doi.org/10.1186/s13045-016-0285-y.
67. Specht J, Lee S, Turtle C, Berger C, Balakrishnan A, Srivastava S, et al. Abstract P2-09-13: a phase I study of adoptive immunotherapy for ROR1+ advanced triple negative breast cancer (TNBC) with defined subsets of autologous T cells expressing a ROR1-specific chimeric antigen receptor (ROR1-CAR). Cancer Res 2019;79(4 Supplement):P2-09-13-P2-09-13. https://doi.org/10.1158/1538-7445.Sabcs18-p2-09-13.
68. Curigliano G, Viale G, Ghioni M, Jungbluth AA, Bagnardi V, Spagnoli GC, et al. Cancer–testis antigen expression in triple-negative breast cancer. Ann Oncol. 2010;22(1):98–103. https://doi.org/10.1093/annonc/mdq325.
69. Rosenberg SA, Yang JC, Restifo NP. Cancer immunotherapy: moving beyond current vaccines. Nat Med. 2004;10(9):909–15. https://doi.org/10.1038/nm1100.
70. Sasada T, Noguchi M, Yamada A, Itoh K. Personalized peptide vaccination: a novel immunotherapeutic approach for advanced cancer. Hum Vaccin Immunother. 2012;8(9):1309–13. https://doi.org/10.4161/hv.20988.
71. Takahashi R, Toh U, Iwakuma N, Takenaka M, Otsuka H, Furukawa M, et al. Feasibility study of personalized peptide vaccination for metastatic recurrent triple-negative breast cancer patients. Breast Cancer Res. 2014;16(4):R70-R. https://doi.org/10.1186/bcr3685.
72. Zhang P, Yi S, Li X, Liu R, Jiang H, Huang Z, et al. Preparation of triple-negative breast cancer vaccine through electrofusion with day-3 dendritic cells. PLoS One. 2014;9(7):e102197-e. https://doi.org/10.1371/journal.pone.0102197.
73. O'Shaughnessy J, Roberts LK, Smith JL, Levin MK, Timis R, Finholt JP, et al. Safety and initial clinical efficacy of a dendritic cell (DC) vaccine in locally advanced, triple-negative breast cancer (TNBC) patients (pts). J Clin Oncol. 2016;34(15_suppl):1086. https://doi.org/10.1200/JCO.2016.34.15_suppl.1086.
74. Page DB, Hulett TW, Hilton TL, Hu H-M, Urba WJ, Fox BA. Glimpse into the future: harnessing autophagy to promote anti-tumor immunity with the DRibbles vaccine. J Immunother Cancer. 2016;4:25. https://doi.org/10.1186/s40425-016-0130-4.
75. Stovgaard ES, Dyhl-Polk A, Roslind A, Balslev E, Nielsen D. PD-L1 expression in breast cancer: expression in subtypes and prognostic significance: a systematic review. Breast Cancer Res Treat. 2019;174(3):571–84. https://doi.org/10.1007/s10549-019-05130-1.
76. Tang F, Zheng P. Tumor cells versus host immune cells: whose PD-L1 contributes to PD-1/PD-L1 blockade mediated cancer immunotherapy? Cell Biosci. 2018;8(1):34. https://doi.org/10.1186/s13578-018-0232-4.
77.• Tsao MS, Kerr KM, Kockx M, Beasley M-B, Borczuk AC, Botling J, et al. PD-L1 Immunohistochemistry comparability study in real-life clinical samples: results of Blueprint Phase 2 Project. J Thoracic Oncol. 2018;13(9):1302–11. https://doi.org/10.1016/j.jtho.2018.05.013 **The PD-L1 Blueprint Project is evaluating whether PD-L1 assays can be used interchangeably in a safe manner. This article presents their phase 2 project evaluating 5 different commercially available PD-L1 assays for sensitivity, reliability, and interchangeability.**

78. Emens L, Loi S, Rugo H, Schneeweiss A, Diéras V, Iwata H, et al. Abstract GS1-04: IMpassion130: Efficacy in immune biomarker subgroups from the global, randomized, double-blind, placebo-controlled, phase III study of atezolizumab + nab-paclitaxel in patients with treatment-naïve, locally advanced or metastatic triple-negative breast cancer. Cancer Research. 2019;79(4 Supplement):GS1-04-GS1. https://doi.org/10.1158/1538-7445.Sabcs18-gs1-04.
79. Ogiya R, Niikura N, Kumaki N, Bianchini G, Kitano S, Iwamoto T, et al. Comparison of tumor-infiltrating lymphocytes between primary and metastatic tumors in breast cancer patients. Cancer Sci. 2016;107(12):1730–5. https://doi.org/10.1111/cas.13101.
80. Loi S, Adams S, Schmid P, Cortés J, Cescon DW, Winer EP, et al. LBA13Relationship between tumor infiltrating lymphocyte (TIL) levels and response to pembrolizumab (pembro) in metastatic triple-negative breast cancer (mTNBC): results from KEYNOTE-086. Ann Oncol. 2017;28(suppl_5). https://doi.org/10.1093/annonc/mdx440.005.
81. Barroso-Sousa R, Jain E, Kim D, Partridge AH, Cohen O, Wagle N. Determinants of high tumor mutational burden (TMB) and mutational signatures in breast cancer. J Clin Oncol. 2018;36(15_suppl):1010. https://doi.org/10.1200/JCO.2018.36.15_suppl.1010.
82. Le DT, Uram JN, Wang H, Bartlett BR, Kemberling H, Eyring AD, et al. PD-1 blockade in tumors with mismatch-repair deficiency. N Engl J Med. 2015;372(26):2509–20. https://doi.org/10.1056/NEJMoa1500596.
83. Anbazhagan R, Fujii H, Gabrielson E. Microsatellite instability is uncommon in breast cancer. Clin Cancer Res. 1999;5(4):839–44.
84. Obeid E, Ellerbrock A, Handorf E, Goldstein L, Gatalica Z, Arguello D, et al. Abstract PD6-03: Distribution of microsatellite instability, tumor mutational load, and PD-L1 status in molecularly profiled invasive breast cancer2018. PD6-03 p. https://doi.org/10.1158/1538-7445.SABCS17-PD6-03
85. Spencer KR, Wang J, Silk AW, Ganesan S, Kaufman HL, Mehnert JM. Biomarkers for immunotherapy: current developments and challenges. American Society of Clinical Oncology Educational Book. 2016;36:e493–503. https://doi.org/10.1200/edbk_160766.
86. Tawfik O, Kimler BF, Karnik T, Shehata P. Clinicopathological correlation of PD-L1 expression in primary and metastatic breast cancer and infiltrating immune cells. Hum Pathol. 2018;80:170–8. https://doi.org/10.1016/j.humpath.2018.06.008.
87. Manson QF, Schrijver WAME, ter Hoeve ND, Moelans CB, van Diest PJ. Frequent discordance in PD-1 and PD-L1 expression between primary breast tumors and their matched distant metastases. Clinical & Experimental Metastasis. 2019;36(1):29–37. https://doi.org/10.1007/s10585-018-9950-6.
88. Terry S, Savagner P, Ortiz-Cuaran S, Mahjoubi L, Saintigny P, Thiery J-P, et al. New insights into the role of EMT in tumor immune escape. Mol Oncol. 2017;11(7):824–46. https://doi.org/10.1002/1878-0261.12093.

Publisher's Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.