Review Article

Immunotherapy for metastatic breast cancer

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Abstract: The development of immune-check inhibitors has resulted in a paradigm shift for immune oncology therapeutics in the past few decades. Blocking antibodies against programmed death 1 (PD-1)/programmed death ligand 1 (PD-L1) leads to robust local tumor control and a durable response in patients with various tumors that are refractory to standard treatments. Breast cancer was traditionally thought to be poorly immunogenic and yield a relatively lower mutation burden compared to 'inflamed' tumors, including melanoma and non-small cell lung carcinoma. Accumulated results have demonstrated that higher T lymphocyte infiltration was observed in triple-negative breast cancer (TNBC) and human epidermal growth factor receptor 2 (HER2) breast cancer compared to estrogen receptor-positive, HER2-negative luminal breast cancer. Among molecular subtypes, single agent cancer immunotherapy showed the most promising results in TNBC. The development of immuno-oncology combinations is required to increase the clinical benefit of immunotherapy against breast cancer. Response to the immunotherapy depends on the dynamic interaction between tumor and immune cells in the tumor microenvironment. In the era of precise medicine, exploration of actual immune response and development of biomarkers is required to maximize the clinical benefit of cancer immunotherapy.

Keywords: Breast cancer; triple-negative breast cancer (TNBC); immunotherapy; checkpoint inhibitor

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Introduction

The immune system has developed to protect the host from continuous exposure to micro-organisms. This biological defense mechanism cannot work well unless it can distinguish between body cells (self) and micro-organisms or infected cells (non-self). Cancer cells are derived from body cells that gain malignant characteristics by a genetic alteration. The 'foreignness' of malignant cells, however, can often evade the immune system and develop into a clinically significant mass. In the past decades, the field of cancer immunology has oscillated between pessimism and optimism regarding the existence of biological defenses in the elimination of cancer cells. However, blocking monoclonal antibodies (mAbs) targeting cytotoxic T-lymphocyte associated antigen-4 (CTLA-4), programmed death-1 (PD-1) and programmed death ligand-1 (PD-L1) can restore pre-existing anti-cancer immunity and achieve a durable clinical response in various types of solid and hematological tumors that are refractory to standard therapies (1-8). The success of these immune checkpoint inhibitors demonstrates the existence of anti-cancer immunity in patients with malignant tumors. Breast cancer was traditionally thought to be poorly immunogenic compared to highly immunogenic ('inflamed') cancers, including malignant melanoma and non-small cell lung carcinoma. However, previous studies of tumor-infiltrating lymphocytes (TILs) and cancer genome sequences have demonstrated that estrogen receptor (ER)-negative, human epidermal growth factor receptor 2 (HER2)-positive subtype and triple-negative breast cancer (TNBC) are more immunogenic than ER-positive-HER2-negative subtypes (9,10). TILs represent pre-existing immunity and are potent biomarkers for predicting prognosis and possibly response

to immunotherapy, especially for TNBC. In this review, we will discuss the basis of cancer immunity and current status of immune checkpoint therapy for metastatic breast cancer.

Adaptive immunity against breast cancer

Fifty years ago, in the history of cancer immunity, Burnet (11) proposed the immune surveillance theory that the body can eliminate transformed cells by immune cells, which are capable of recognizing and destroying the transformed cells before they become clinically significant tumors. Among immune cells, T lymphocytes are major players in adaptive immunity, and how to activate them has been a key issue in the development of cancer immunotherapy. However, the outcomes of conventional immunotherapies were not as satisfying as expected. In the past 15 years, previous studies elucidated that the function of immune surveillance was a part of anti-cancer immunity, and the new extended concept of immunoediting was proposed by Schreiber to understand the immunodynamics between tumors and the immune system (12,13). Immunoediting describes a series of interactions between tumor cells and immune cells which lead to cancer suppression and promotion in three phases: elimination, equilibrium, and escape (14,15). In the elimination phase, tumors have relatively high immunogenicity and innate immunity by natural killer (NK) cells, and the subsequent adaptive immunity by T cells can eliminate tumor cells before forming a clinically significant mass. In the equilibrium phase, some tumor cells have evolved with genetic and epigenetic changes to be resistant to attack by the immune system. Tumor cells become dormant as occult cancer, caught in the balance between anti-tumor and pro-tumor immunity in the tumor microenvironment. In the escape phase, tumor cells evolve to exhibit poor immunogenicity and proliferate to form clinically significant masses. For tumor cells, the absence of antigens, the loss of major histocompatibility complex (MHC), acquired anti-apoptosis and increased survival leads to immuno-evasion. In the tumor microenvironment, expression of immune checkpoints increased the frequency of immunosuppressive cells including regulatory T cells, myeloid-derived suppressor cells (MDSC), and immunomodulatory factors, including tumor growth factor (TGF) and vascular endothelial growth factor (VEGF), which also cause peripheral tolerance. Currently, how to release the brake on the immune system is a key issue in the reactivation of anti-tumor immunity (16).

Tumor mutation burden (TMB)

Innate immunity recognizes conserved structures in pathogens via Toll-like receptors (pattern recognition), whereas acquired immunity recognizes approximately 10 peptides derived from pathogens in context with MHC class I and class II (17-19). Naïve T cells are primed and activated by professional antigen presenting cells (dendritic cells). Primed T cells differentiate into antigen -specific T cells, and clonally expanded T cells can eliminate tumor cells in the tumor microenvironment. Cancer cells originate from normal cells with viral infections or gene mutations. Driver mutations are critical for malignant transformation and shared across any given type of cancer. Therefore, these mutated genes are ideal targets for drug treatments. Recent cancer genomic analyses have demonstrated that tumor cells accumulate gene mutations during development, and the altered proteins can be recognized by the immune system (20). These neoantigens are related to malignant transformations (driver mutations) or the products of increasing genetic instability (passenger mutations). Differences in TMB are observed among malignant tumors, and higher somatic mutations (10/Mb) are found in melanoma and non-small cell lung cancer (21). Breast cancer yields a rather low mutation rate of 1/Mb, which might cause relatively low immunogenicity. Some missense mutations yield mutational epitopes (neoepitope) in context with the patient's MHC class I and class II.

The study using The Cancer Genome Atlas has demonstrated that TMB is associated with local anti-tumor immunity (22). Melanoma, non-small cell lung cancer and miss match repair deficiency colon cancer (Lynch syndrome) are thought to be immunogenic ('inflamed') tumor due to high genetic instability (23-26). For breast cancer, TNBC and HER2-enriched breast cancer yield relatively higher TMB and more T cell infiltration compared to the luminal subtype. As TMB is reversely correlated with prognosis in the luminal subtype (27), TMB may not reflect immunogenicity in this subtype.

Tumor-infiltrating lymphocyte (TIL)

TILs represent pre-existing immunity, and lymphocyte-predominant breast cancer (LPBC) has more than 50–60% lymphocyte infiltration in the stroma. Incidence of LPBC is 20% for TNBC, 16% for HER2 subtype and 6% for

ER-positive luminal subtype (28). Since Aaltomaa (29) reported that TIL was an independent prognostic factor for highly proliferative breast cancer, accumulating results demonstrate that high frequencies of TIL were associated with a favorable prognosis and a better clinical response to chemotherapy in TNBC. In an adjuvant setting, the BIG 02-98 trial, Loi (9) reported that every 10% increase of stromal TIL was associated with a risk reduction of TNBC recurrence (HR =0.85, P=0.025), and a better clinical outcome was observed in LPBC compared to non LPBC (HR =0.30, P=0.018). When combined, the results of the ECOG2197 and ECOG119 trials demonstrated that every 10% increment of stromal TIL was associated with better disease-free survival (DFS) and overall survival (OS) in TNBC (30). For the neoadjuvant setting, TIL was considered to be a reliable biomarker for predicting pathological complete response (pCR) for breast cancer. According to a recent meta-analysis that included 3,771 patients, a high frequency of stromal TIL was a good biomarker for predicting pCR in all molecular subtypes (31). Better clinical outcomes were observed for LPBC in HER2-positive breast cancer for adjuvant and neoadjuvant settings (32-35). However, TIL was associated with a shorter overall survival for HER2-negative luminal subtype (31,36). These results indicated that the biology of TIL was different in the subtypes of breast cancer.

In the subset analyses of TILs, increased frequencies of CD8-positive effector T cells (37) and CD4-positive Th1 cells were associated with a better prognosis, while an increased frequency of CD4-positive Th2 cells was negatively associated (38,39). The prognostic impact of regulatory T cell (Treg) infiltration in breast cancer remains controversial. A recent meta-analysis reported that a high infiltration of Treg was associated with a poor prognosis in breast cancer (40). However, the clinical impact of Treg infiltration was varied in the subtypes of breast cancer. The association with a favorable prognosis was observed in ER-negative HER2-positive breast cancer but not ER-positive luminal breast cancer (41). The CD8/FoxP3 was significantly associated with OS (40) and residual tumor was a significant parameter to predict a better DFS in residual TNBC after neoadjuvant chemotherapy (42). Standardization of TIL assessment in residual cancer is now under way.

Analyses of the molecular profiles of tumors and tumor-associated cells have demonstrated that plasma cell signatures were associated with a favorable prognosis, while tumor-associated neutrophil signatures were associated with the opposite in breast cancer (43). Collectively, evaluation of both frequencies and composition of TIL is required to understand the immunogenicity of breast cancer and dynamic immune response in the tumor microenvironment.

Immunogenic cell death

In the cancer immunity cycle (44), the release of cancer cell antigens is the first step in initiating the anti-tumor response. Chemotherapy was traditionally thought to suppress immune response. However, there is a growing consensus that anti-cancer agents can induce immunogenic cell death (ICD) to modulate anti-tumor immunity (45). When tumor cells are exposed to chemotherapeutics, they release 'danger signals' including extracellular ATP, calreticulin (CRT), high mobility group box 1 (HMGB1) and heat shock proteins (HSPs). For example, expression of CRT, HSP70, and HSP90 on tumor cells can promote the up-take of dying cells by dendritic cells to present the tumor antigen to T cells ('eat-me' signal). The release of HMGB1 promotes the synthesis of pro-inflammatory factors including type I interferon (IFN), interleukin (IL)-1, IL-12 and tumor necrosis factor (TNF). The secretion of type I IFNs from dying cells also promotes the synthesis of chemokine CXCL10 which mediates chemotactic effects on T cells. These danger signals lead to the priming of the adaptive immune response against tumor cells (*Table 1*). Moreover, cyclophosphamide, docetaxel and gemcitabine are known to decrease the immunosuppressive immune cells including Treg and MDSC (46,47). These immune modulations are the rationale for the combination of chemotherapy with checkpoint inhibitors.

It is well known that radiation therapy achieves local tumor control, but sometimes leads to tumor shrinkage at a distant site out of the radiation field. This abscopal effect is thought to be associated with ICD-releasing danger signals from irradiated tumor cells. Recent studies have demonstrated that CTLA-4 blockade following radiation therapy broadens the T cell receptor (TCR) repertoire, and a diverse TCR repertoire is required to elicit tumor rejection (48,49). The combination of radiotherapy with immunotherapy can increase the abscopal effect, which leads to systemic acquired T cell responses.

Single agent immunotherapy

TNBC is known to be the most immunogenic subtype; therefore, most of the trials using immune checkpoint

Table 1 Danger signal and induction of adaptive immune response

Danger signals	Receptor	Immunomodulation
CRT	LRP1	Anthracycline induces CRT on tumor cells. Dendritic cells expressing LRP1 can efficiently uptake dying cells ('eat-me signal') and presenting tumor antigens to T cells
HSP70, HSP90	LRP	HSPs promote the uptake of chemotherapy-driven dying cells
ATP	P2RX7	Extracellular ATP from dying cells recruits and activates dendritic cells ('find-me' signal)
HMGB1	TLR4	Danger signal of HMGB1 from tumor cells promotes the secretion of type I IFNs from dendritic cells and elicits anti-tumor immunity
Type I IFN	IFN receptors	Anthracycline activates TLR3 and stimulates the release of intrinsic type I IFNs from dying cancer cells in autocrine and paracrine manners
CXCL10	CXCR3	The secretion of CXCL10 induce by type I IFNs mediates chemotactic effects on T cells

CRT, calreticulin; LRP1, low density lipoprotein receptor-related protein 1; HSP, heat shock protein; ATP, adenosine triphosphate; P2RX7, P2X purinergic receptor 7; HMGB1, high mobility group box 1; TLR, Toll-like receptor; IFN, interferon; CXCL10, C-X-C motif chemokine ligand 10; CXCR3, C-X-C motif chemokine receptor 3.

inhibitors have been focused on metastatic TNBC. In the KEYNOTE-012 phase Ib trial, monotherapy with the anti-PD-1 inhibitor, pembrolizumab, was explored in 32 patients with PD-L1-positive metastatic TNBC (50). PD-L1 positivity was defined as ≥1% on tumor cells or expression in the stroma by immunohistochemistry using 22C3 mAb. Pembrolizumab achieved a durable response in the patients with heavy prior treatment with anthracycline, taxanes and platinum. Among the 27 patients who were evaluated for tumor response, the objective response rate (ORR) reached 18.5%, the median time to response was 17.9 weeks (range, 7.3 to 32.4 weeks), and the median duration of response was not yet reached. The median treatment with pembrolizumab was five doses (range, 1 to 36 doses), and 15.6% of patients experienced at least one grade 3 or 4 adverse event. In the KEYNOTE-086 phase II trial, there were three cohorts categorized by PD-L1 expression and the history of metastatic treatment (51). TNBC patients were assigned to cohort A regardless of PD-L1 expression. Cohort B contained patients who were PD-L1-positive on tumor cells or in the stroma and who had no prior metastatic treatment. Cohort C involved patients with PD-L1 positivity after prior metastatic treatment. For 170 patients assigned to cohort A, 4.7% of ORR was observed (one CR and seven PRs). The median progression free survival (PFS) and duration of response (DOR) was 2 and 6.3 months, respectively. Fifty-two patients in cohort B experienced a higher ORR of 23.1% (two CRs, ten PRs) and the median DOR was 8.4 months. In the KEYNOTE-028 study, 25 heavily pretreated patients with ER-positive and HER2-negative disease, with PD-L1 expression in the stroma or in ≥1% tumor cells, were evaluated for tumor response (52). With a median duration of follow-up of 7.3 months, the ORR was 12%, and the clinical benefit rate was 20%. Progression of disease was observed in 5 (60%) patients. Overall, 16% of patients experienced at least one grade 3 or 4 adverse event.

The clinical efficacy of targeted therapy against PD-L1 was also investigated for patients with metastatic TNBC. Atezolizumab, the human IgG1 PD-L1 targeting mAb, was tested in 115 patients with metastatic TNBC (53). For the 113 patients who were evaluated for response, the ORR reached 10% (three CRs, eight PRs) according to regular RECIST criteria, and 13% according to immuno-related RECIST criteria. The ORR of 26% was observed in patients who received first line therapy and 11% in those who received second or further line therapies. The median DOR reached 21.1 months. The 1-year overall survival rate was 41%, and the 2-year survival rate was 22% in all patients. However, 1- and 2-year survival rates for responders was 100% compared to 38% and 11% for non-responders. The results indicated that a small group of patients who responded to atezolizumab monotherapy experienced a longer clinical outcome.

In this study, PD-L1 expression was assessed by immunohistochemistry using clone SP142 mAb. The definition of PD-L1 positivity was ≥5% [immune cell (IC) 2/3] on immune cells in the stroma as opposed to <5% (IC 1/0), and the positive rate was 34%. The ORR for PD-L1 IC 2/3 and IC 1/0 patients were 17% and 8%, respectively. The one-year OS for patients with IC2/3 was 45% *vs.* 37% for those with IC0/1. There was a trend toward favorable

response for patients with higher PD-L1 expression, but patients with lower PD-L1 expression also benefited from atezolizumab. Exploratory analysis revealed that higher response rates seemed to be associated with higher levels of TIL (≥10%) and CD8 T cell infiltration (≥1.35%).

In the JAVELIN trial, another anti-PD-L1 mAb, avelumab, was tested in 168 patients with all subtypes of metastatic breast cancer in (54). PD-L1 expression was determined by 22C3 mAb and the definition of positivity was ≥1% on tumor cells or ≥10% in stromal immune cells. The ORR was 3.0% (1 CR, 7 PRs) in all patients, and the ORR reached 5.2% in 58 patients with TNBC. There was a trend toward a higher ORR in patients with PD-L1 expression (16.7% *vs.* 1.6% for all patients, 22.2% *vs.* 2.6% for TNBC).

Immuno-oncology combinations

In breast cancer, combination therapy with chemotherapy was intensively investigated. Adams *et al.* (55) reported the clinical efficacy of atezolizumab combined with nab-paclitaxel in patients with metastatic TNBC. It is still unclear whether or not steroids during chemotherapy may reduce the effectiveness of immunotherapy; however, nab-paclitaxel does not require steroids as opposed to conventional taxane. Thirty-two patients were assigned, and 87% underwent prior treatment with taxane. Twenty-four out of 32 patients were evaluated for treatment efficacy, and the ORR reached 42% at a median follow-up of 5.2 months. The confirmed ORR was 67% in the first line, 25% in the second line, and 29% in the third or further lines. There was a trend towards a better response to combined treatment in patients whose tumors expressed PD-L1. The following phase III IMpassion 130 trial is investigating the combination of atezolizumab plus nab-paclitaxel in patients with previously untreated metastatic TNBC (56).

To evaluate eribulin combined with pembrolizumab, the ENHANCE-1/KEYNOTE-150 phase Ib/II study was considered for patients with metastatic TNBC (57). In evaluable 106 patients, the ORR with the combination increased to 26.4% (95% CI: 16.8–35.4). Patients with no prior metastatic treatment (n=65) had the ORR of 29.2% (95% CI: 18.6–41.8) whereas those with one or two prior treatments (n=41) had the ORR of 22.0% (95% CI: 10.6–37.6). This combination therapy was superior to monotherapy with eribulin in the historical control (ORR 10–20%). Clinical response was observed regardless of PD-L1 expression. The ORR with combination treatment was 30.6% for PD-L1-positive patients (n=49) and 22.4% for PD-L1-negative patients (n=49). The median PFS and the median OS was 4.2 months (95% CI: 4.1–5.6) and 17.7 months, (95% CI: 13.5– not estimable), respectively.

Future direction of immunotherapy

Cancer immunotherapy achieved a durable clinical response in patients with advanced cancer that was refractory to the standard of care. Approximately 20% of patients experience a long-lasting life prolongation whereas more than half of patients are still non-responders to checkpoint blockade. Clarifying the reasons why checkpoint inhibitors still cannot restore anti-tumor immunity in these non-responders is a key issue for future immuno-oncology research.

Other immunosuppressive molecules are targeted to increase the activity of immune checkpoint inhibitors. Indoleamine 2,3 dioxygenase (IDO) induces immunosuppression through degradation of tryptophan, which is an important regulator of innate and adaptive immunity (58). Adenosine is a mediator of immunosuppression, and tumors can generate adenosine through CD73 in response to anti-PD-1/ani-PD-L1 (59). The adenosine-A2A receptor on NK and T cells is another target to release the brake on anti-tumor activity. The IDO-1 inhibitor (epacadostat) (60) and an oral antagonist of the adenosine-A2A receptor (CPI-444) (61) were investigated in combination with anti-PD-1 and anti-PD-L1 mAb for advanced solid tumors. The preliminary data demonstrated that these combination therapies were safe and their outcomes were promising. Arginase is a key immunosuppressive enzyme secreted from MDSC. The arginase inhibitors of CB-1158 can relive immune suppression and lead to high serum arginine. A tumor microenvironment with high arginine can activate effector cells, including NK and T cells. The phase I study showed that oral CB-1158 combined with anti-PD1 therapy was well tolerated, and peripheral NK cells and T cells were in the activation state (62).

Accumulating evidence has demonstrated that metabolic energetics of the tumor and the tumor microenvironment play an important role in the regulation of tumor immune response. Tumor cells can obtain energy from aerobic glycolysis (Warburg effect) whereas immune cells tend to starve in hypoxic microenvironments. Metformin, a type 2 diabetes drug, can inhibit oxygen consumption in tumor cells resulting in reduced intra-tumoral hypoxia. Combination therapy with metformin and PD-1 blockade

Table 2 Trials of immunotherapy for metastatic breast cancer

Subtype	Trial	N	Treatment	PD-L1 positivity	ORR (%)	Prognosis (months)	References
Single agent							
TNBC	KEYNOTE-012	32	Pembrolizumab	≥1% tumor or positive in stroma	18.5	mDOR: NR	Nanda (50)
TNBC Cohort A: all Cohort B: PD-L1+	KEYNOTE-086	170 (cohort A); 52 (cohort B)	Pembrolizumab	≥1% CPS*	4.7 (cohort A); 23.1 (cohort B)	Cohort A: mDOR, 6.3; mPFS, 2.0 Cohort B: mDOR, 8.4	Adams (51)
ER+ HER2- PD-L1+	KEYNOTE-028	25	Pembrolizumab	≥1% tumor positive in stroma	12	CBR: 20%	Rugo (52)
TNBC	NCT01375842	115	Atezolizumab	IC 2/3	10	mDOR: 21.1	Schmid (53)
All subtype	JAVELIN	168 (TNBC 58)	Avelumab	≥1% tumor; ≥10% IC	3 (TNBC 5.2)	mPFS (TNBC): 5.9 (5.9); mDOR (TNBC): 28.0 (31.0); mOS: (TNBC): 8.1 (9.2)	Dirix (54)
Combination							
TNBC	NCT01633970.	32	Atezolizumab + nab-paclitaxel	≥1% tumor cell or IC	42.0; 67 (1st line); 25 (2nd line); 29 (>3rd line)		Adams (55)
TNBC	ENHANCE-1/ KEYNOT150	106	Pembrolizumab + eribulin	≥1 CPS	26.4; 29.2 (1st line); 22.0 (≥2nd line); 30.6 (PD-L1+); 22.4 (PD-L1-)	mPFS: 4.2; mOS: 17.7; mDOR: 8.3; CBR: 36.8	Tolaney (57)

*CPS, combined positive score = (number of PDL1 staining cells including tumor cells and immune cells/total number of viable tumor cells) ×100. PD-L1, programmed death-ligand 1; ORR, objective response rate; TNBC, triple-negative breast cancer; mDOR, median duration of response; NR, not reported; mPFS, median progression free survival; CBR, clinical benefit rate; IC, immune cells; mOS, median overall survival.

has improved intra-tumoral T-cell function (63). Mitochondrial activity in cytotoxic T cells is associated with the anti-tumor activity of PD-1 blockade. In the mouse model, bezafibrate, an antilipemic agent, improves mitochondrial dysfunction and synergizes with the anti-tumor activity of anti-PD-1 therapy through expansion of CTLs in the tumor microenvironment (64).

Recent studies have reported that the intestinal microbiome is related to the response to checkpoint blockade (65-67). In each study, specific bacterial strains in stool were associated with response to immunotherapy. A relative abundance of *Bifidobacterium*, *Akkermans* and *Ruminococcus* was observed in responders, whereas the *Bacteroides* strain was dominant in non-responders. Cytotoxic T cells in the tumor microenvironment were observed in the submucosa of the colon. The effects of the microbiome can cross the mucosal barrier, but it is still unclear why specific strains of intestinal flora can induce systemic anti-tumor activity. These data raise important questions regarding limiting use of antibiotics and considering diet or pro-biotic intake to augment the anti-tumor activity of checkpoint blockade.

Taken together, triple-negative breast cancer is the most immunogenic subtype of breast cancer and some populations can be eliminated by re-activating anti-tumor immunity. The results of clinical trials descried in this review are summarized in *Table 2*. Accumulating results demonstrate that TMB and TIL represent antigenicity and pre-existing immunity. Recent studies have demonstrated that the efficacy of immunotherapy is also associated with

cellular metabolism and host physiology. The development of these intrinsic and extrinsic biomarkers is required to maximize the clinical benefits of immunotherapy in patients with breast cancer.

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Footnote

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