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# Immunotherapy, an evolving approach for the management of triple negative breast cancer: Converting non-responders to responders

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ABSTRACT

Immunotherapy comprises a promising new era in cancer therapy. Immune checkpoint inhibitors targeting either the programmed death (PD)-1 receptor or its ligand PD-L1 were first approved by the Food and Drug Administration (FDA) for the management of metastatic melanoma in 2011. The approval of this class is being extended to include other types of immunogenic tumors. Although breast cancer (BC) was first categorized as non-immunogenic tumor type, there are certain subsets of BC that showed a high level of tumor infiltrating lymphocytes (TILs). Those subsets include the triple negative breast cancer (TNBC) and HER-2 positive breast tumors. Preliminary data from clinical trials presented promising outcomes for patients with advanced stage/metastatic TNBC. While the objective response rate (ORR) was relatively low, it is still promising because of the observation that the patients who respond to the treatment with immune checkpoint blockade have favorable prognosis and often show a significant increase in the overall survival. Therefore, the main challenge is to find ways to enhance the tumor response to such therapy and to convert the non-responders to responders. This will consequently bring new hopes for patients with advanced stage metastatic TNBC and help to decrease death tolls from this devastating disease. In the current review, we are highlighting and discussing the up-to-date strategies adopted at either the preclinical or the clinical settings to enhance tumor responsiveness to immunotherapy.

## 1. Introduction

The programmed death (PD)-1 receptor–PD-1ligand (PD-L1) interaction is a key immune checkpoint that is overridden by malignant tumors to escape from the immune surveillance (Zou et al., 2016). PD-1 receptors are normally expressed during the initial activation of T-cells to suppress the unnecessary or excessive immune response that can precipitate autoimmune reactions. The PD-1/PD-L1pathway is engaged by cancer cells to undergo immune evasion. PD-1 receptors suppress T-cell activation upon the interaction of PD-1 with PD-L1ligand proteins. PD-1 is expressed on activated T lymphocytes and myeloid cells, while PD-L1 is mostly expressed on antigen-presenting cells together with other hematopoietic, non-hematopoietic cells and some epithelial cells (Intlekofer and Thompson, 2013). Tumor immune evasion occurs because of the upregulated expression of PD-L1 on tumor cells and on other components of tumor microenvironment (Juneja et al., 2017). Monoclonal antibodies targeting PD-1/PD-L1 immune checkpoints are an evolving approach for the management of cancer. These immune checkpoint inhibitors include PD-1 antibodies such as pembrolizumab and nivolumab or PD-L1 antibodies as avelumab and atezolizumab (Emens et al., 2017). The favorable clinical outcomes in patients receiving PD-1/PD-L1 immune checkpoint inhibitors are mostly associated with upregulated expression of PD-L1. Nonetheless, some studies reported clinical outcomes in patients with tumors that lack PD-L1 expression (Herbst et al., 2014). Tumor infiltrating lymphocytes (TILs) enriched tumor microenvironment is a feature associated with higher response rates to immune checkpoint inhibitors (Wein et al., 2017). Such tumors are called hot or inflamed tumors. It is noteworthy that breast tumors, which exhibit poor prognostic criteria such as estrogen receptor (ER)-negative or progesterone receptor (PR) negative status and lymph node positivity were shown to have higher levels of TILs (Muraro et al., 2011; Wein et al., 2017). Outcomes from clinical trials highlighted that a higher percentage of CD8+ TILs is a feature associated with higher response rates to immune check point inhibitors in triple-negative breast cancer (TNBC) patients (Herbst et al., 2014; Tumeh et al., 2014). Thus, immunotherapy is considered a promising therapeutic option for TNBC which has poor response to conventional therapies and does not have any specific targeted therapy options. The

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response of TNBC to immunotherapy is higher than the response rate of ER- positive breast cancer (Dirix et al., 2015; Savas et al., 2016). Several subsets of TNBC harbor mutations such as ATM and TP53 mutations and alterations in PI3K/MEK and BRCA pathways. The higher incidence of mutations in the tumor cells of TNBC contributes to the increased immunogenicity and boosts the percentage of TILs in TNBC tumors (Anders et al., 2016; Cancer Genome Atlas, 2012). Since advanced TNBC is among the tumors that exhibit high recurrence rate and resistance to the common chemotherapies, clinical trials are in process to evaluate the potential merit of immune checkpoint inhibitors in an effort to find a successful approach for treatment of such hard to treat tumors. The objective response rate (ORR) to pembrolizumab (PD-1 antibody) in patients with advanced PD-L1 + /TNBC was 18.5% (n = 27) (Nanda et al., 2016). Avelumab (PD-L1 antibody) produced an ORR of 44.4% (n = 9) in PD-L1 + /TNBC patients (Dirix et al., 2015). The ORR in PD-L1 + /TNBC patients receiving atezolizumab (PD-L1 antibody) was 13% (n = 71) (Schmid et al., 2017).

While the response was relatively low, it is still promising because of the observation that the patients who respond to treatment with immune checkpoint blockade have favorable prognosis and often show a significant increase in the overall survival with extended anti-tumor immunity. Therefore, the main challenge is to find ways to enhance the tumor response to such therapy and to convert the non-responders to responders. This will consequently help to decrease deaths tolls and open new hopes for patients with advanced stage/metastatic TNBC. In the current review, we are discussing the up-to-date strategies adopted at either the preclinical or the clinical settings to enhance tumor responsiveness to immunotherapy.

## 2. Nab-paclitaxel

Taxanes exhibit distinctive modulatory effects leading to activation of several subsets of immune cells (Zagozdzon and Golab, 2001). Low–dose metronomic paclitaxel therapy induces TLR4-mediated activation and maturation of dendritic cells in mouse models. It can also promote the production of proinflammatory cytokines from CD4 + with subsequent priming of CD8 + tumor antigen specific T −cells (Machiels et al., 2001; Pfannenstiel et al., 2010). Evaluation of cellular immunity in breast cancer patients that received taxane treatment after surgery indicated that T-cell blastogenesis and natural killer cell cytotoxic function were enhanced in patients that received taxanes compared to patients that did not administer taxanes (Carson et al., 2004). Therefore, taxanes appeared to be promising candidates for immunotherapy combinations. Nab-paclitaxel (Abraxane®) is the agent of choice to be combined with immunotherapies. It does not require corticosteroid pretreatment because it is a solvent-free preparation that does not elicit hypersensitivity reactions (Robinson and Keating, 2006). Therefore, the patients' immunity will be preserved. Combined treatment of the PD-L1- antibody atezolizumab (840 mg every 2 weeks) with nab-paclitaxel (100 mg/m$^2$ weekly) resulted in a marked improvement in the ORR in patients with metastatic TNBC. The confirmed ORR was 66.7% in patients receiving the combined treatment (Adams et al., 2016). The enrolled patients were unselected for PD-L1 and did not receive prior treatment for advanced TNBC. Therefore, the combination was tested as a frontline therapy regardless to the status of PD-L1 in the tumor microenvironment. Improved antitumor responses were reported in patients with or without PD-L1 expression (Adams et al., 2016). Based on these promising results, a phase III double-blinded, randomized clinical trial IMpassion 130 was initiated to study the effect of atezolizumab combination with nab-paclitaxel as a front-line therapy in a larger population of patients with metastatic TNBC (NCT02425891).

## 3. CDK4/6 inhibitors

Abemaciclib is a CDK4/6 inhibitor that showed promising outcomes in the clinical setting for patients with breast cancer and non-small cell lung cancer (NSCLC). Dempsey et al. reported that abemaciclib pretreatment synergized the antitumor effects of PD-L1 immunotherapies in a preclinical model of NSCLC in immuno-competent mice bearing CT26 tumors. The combination produced a superior anti-tumor effect with about 55% complete tumor regression compared to 0% in groups treated with monotherapies. Interestingly, the mice co-treated with abemaciclib/PD-L1 showed the development of anti-tumor-immune memory as they maintained complete tumor regression after the treatment has stopped and resisted CT-26 cells re-challenge (Dempsey et al., 2017). Data generated from this study encourages the initiation of similar studies on breast cancer models and the extension to the clinical setting after careful consideration of the probable toxic reactions.

## 4. Stimulator of interferon genes (STING) agonists

The stimulator of IFN genes (STING) is an adaptor molecule that plays a key role in cytosolic DNA sensing. STING signaling in dendritic cells (DCs) is usually activated by tumor-derived DNA, leading to increased type I interferon (INF) production and activation of DCs. Activated DCs lead to subsequent activation and cross-priming of tumor cytotoxic CD8 + T cells which attack and eliminate the tumor cells (Demaria et al., 2015). Local injection of ADU-S100, a synthetic cyclic dinucleotide STING agonist, into the tumor site exhibited significant antitumor activity in various *in vivo* models including 4T1 TNBC model (Corrales et al., 2015). A recent study by Foote et al., indicated that treatment with ADU-S100 was successful in delaying tumor progression in immune tolerant nue/N transgenic mouse model of HER2 + breast cancer (Foote et al., 2017). Nevertheless, ADU-S100 monotherapy failed to induce activation and expansion of CD8 + tumor antigen specific T cells. The triple combination of PD-1 antibody and OX-40 activator synergized the effect of ADU-S100 resulting in a successful priming of CD8 + T cells, overcoming immune tolerance and led to tumor clearance in 40% of mice compared to 10% in ADU-S100-only group (Foote et al., 2017). In a separate study, combined treatment by STING agonist and PD-L1 antibody elicited successful antitumor effects in squamous cell carcinoma preclinical model (Gadkaree et al., 2017).

## 5. Indoleamine 2,3-deoxygenase (IDO) inhibitors

Myeloid-derived suppressor cells (MSDCs) in the tumor microenvironment together with the tumor cells, produce metabolic enzymes such as indoleamine 2,3-deoxygenase (IDO) and arginase (Godin-Ethier et al., 2011; Munder, 2009). These enzymes consume the amino acids essential for the proper functioning of T-cells from the tumor microenvironment and therefore plays an important role in the tumor immune escape. The up-regulated levels of the immunosuppressive enzyme IDO1 in a wide-range of neoplasms is correlated with poor prognosis (Godin-Ethier et al., 2011). Inhibition of IDO is thus suggested to augment anti-tumor immunity and to enhance the responsiveness to immune checkpoint inhibitors. Preliminary data from phase I/II study of combined treatment of IDO1 inhibitor epacadostat together with pembrolizumab in patients with advanced stage tumors showed a promising clinical activity. The ORR in evaluable patients with melanoma was 57% (4 out of 7). The ORR was 40% (2 out of 5) in evaluable patients with renal cell carcinoma(RCC) (Gangadhar et al., 2015).

## 6. Epigenetic modifiers

Epigenetic silencing of immune recognition and antigen processing genes is one of the mechanisms of tumor immune escape (Choudhary et al., 2009; Hellebrekers et al., 2006). Epigenetic reprogramming *via* small molecule inhibitors of histone-deacetylases(HDAC) has shown significant anti-tumor effects in a variety of cancer types including breast cancer (Bai et al., 2011; Trapani et al., 2017). HDAC inhibitors can also prime the anti-tumor immune response (Terranova-Barberio

et al., 2016). The HDAC-6 inhibitor ricolinostat modulates the different subsets of immune cells. Sodre et al. (2017) indicated that ricolinostat opposes the function of immune suppressor T-regulatory cells (Tregs) and MDSCs. On the contrary, it boosts the accumulation of anti-tumor CD8+ effector T-cells. These studies were done *ex vivo* on peripheral blood monocytes isolated from melanoma patients or TILs harvested from melanoma biopsy specimens. Etinostat, a selective HDAC-1 inhibitor, was shown to increase T-cell-mediated cytotoxicity *in vitro* in prostate (LNCaP) and breast cancer (MDA-MB231) cells by reversal of the cancer immune escape phenotype (Gameiro et al., 2016). Entinostat was also reported to augment the efficacy of PD-1 immune checkpoint antibody via opposing the function of MDSCs in BALB/c and C57BL/6 mice bearing lung (LLC) and renal (RENCA) neoplasms (Orillion et al., 2017).

## 7. PEGylated recombinant hyaluronidase (PEGPH20)

The tumor microenvironment in many solid tumors, including breast cancer is characterized by the increased buildup of hyaluronan (HA). Such accumulation of HA was correlated with the resistance to therapy and poor prognosis. The increased HA in the tumor microenvironment hinders the delivery of monoclonal antibodies as well as immune cell infiltration to the tumor site (Singha et al., 2015). Rosengren et al., showed that HA depletion by PEGylated recombinant hyaluronidase (PEGPH20) enhanced the delivery of PD-1 and PD-L1 antibodies in a preclinical model of pancreatic cancer (Rosengren et al., 2016). Clift el al., indicated that PEGPH20 augments intratumoral accumulation of CD8+ TILs leading to enhanced antitumor activity of anti-PD-L1 antibody in BALB/c mice bearing breast tumors (Clift et al., 2017). A phase I trial investigating the effect of combined treatment with PEGPH20 and immune checkpoint inhibitors in patients with advanced gastric adenocarcinoma and NSCLC is currently ongoing (NCT02563548).

## 8. Targeting T-cell immunoglobulin mucin-3 (TIM-3)

Tim-3 is another T-cell inhibitory receptor that is commonly upregulated on exhausted/dysfunctional CD8+ T cells in patients with HIV or HCV viral infections (Golden-Mason et al., 2009; Jones et al., 2008). Sakuishi et al., reported that Tim-3 is co-expressed with PD-1 on TILs from BALB/c mice bearing breast and colon tumors. In tumor tissues CD8+ TILs that are positive for Tim-1 and PD-1 were predominant and exhibited a significant deficit in T cell effector functioning. Interestingly co-treatment with antibodies against Tim-3/PD-L1 produced a huge leap in the anti-tumor activity with complete tumor regression in 50% of the treated tumor-bearing mice compared to little or no effect in the monotherapy groups (Sakuishi et al., 2010). The results of this preclinical study are encouraging and should be exploited to the clinical setting after careful testing of any possible toxic reactions.

## 9. Targeting cytotoxic T lymphocyte associated protein-4 (CTLA-4)

CTLA-4 inhibits T-cell activation by binding and neutralizing co-stimulatory molecules such as CD80 and CD86 (Mao et al., 2010). The CTLA-4 antibody, Ipilimumab, was approved by the Food and Drug Administration (FDA) for advanced melanoma patients to enhance the overall survival. The ORR in these patients on Ipilimumab monotherapy was 11% (Intlekofer and Thompson, 2013). Data from a phase I clinical study (NCT01927419) indicated that combined treatment of advanced melanoma patients with lpilimumab together with nivolumab (PD-1 antibody) in the first line stetting significantly boosted the ORR to reach 61% (Postow et al., 2015). Preliminary data from the phase II CheckMate-069 study (NCT01927419) confirmed the benefit of the same combination in improving the ORR in advanced melanoma patients compared to the monotherapy and revealed a significant increase in the 2-year overall survival (63.8 combo vs 53.6% lpilimumab). However, the incidence of grade 3–4 adverse effects was significantly higher (59% combo vs 20% lpilimumab) in the combined treatment arm. Grade 3–4 adverse events included colitis and diarrhea (Hodi et al., 2016).

## 10. Tyrosine kinase inhibitors

The AXL receptor tyrosine kinase (AXL) signaling is crucial for epithelial-to-mesenchymal transition and stemness (Antony and Huang, 2017) in addition to playing a role in immune modulation in the tumor microenvironment (Aguilera et al., 2016). Wnuk et al., reported that BGB324 a selective inhibitor of tyrosine kinase AXL reduced intratumoral and tumor microenvironment immune suppression and synergized the antitumor effects of immune checkpoint inhibitors (anti-CTLA4/PD-1). This study was done on preclinical murine models of lung and mammary adenocarcinoma (Wnuk-Lipinska et al., 2017).

Jiang et al. (2016) indicated that the inhibition of focal adhesion kinase (FAK) by VS-4718 in C57BL/6 and FVB/N mice carrying pancreatic tumors, enhanced their response to PD-1 checkpoint inhibitor antibody via opposing the immune suppressive tumor microenvironment. The same kinase was reported to be up-regulated in lung and breast cancer cells where it is implicated in cancer invasion and metastasis (Woo et al., 2017). Therefore, co-treatment with FAK-inhibitor and immune checkpoint inhibitors is expected to display positive outcomes in these tumor types as well.

The vascular endothelial growth factor(VEGF) that serves as a key player in tumor angiogenesis (Pachmayr et al., 2017) has also shown immune-modulatory effects that augment the process of tumor immune evasion. Alfaro et al., reported that VEGF is an immune suppressor as it hinders the maturation of DCs (Alfaro et al., 2009). Arihara et al., showed that VEGF also enhances tumor infiltration with MDSCs (Arihara et al., 2013). Therefore, concurrent inhibition of VEGF receptor signaling is another promising strategy to enhance the antitumor immunity. Combined administration of axitinib (VEGFR1/2/3 inhibitor) and CTLA-4 antibody resulted in an enhanced anti-tumor activity and increased survival of mice-bearing intracranial melanoma metastasis. The mechanism was reported to be through opposing the immune suppressor function of MDSCs and enhancing antigen-presentation by DCs in the tumor microenvironment (Du Four et al., 2016).

## 11. Dual targeting of TGF beta and PDL-1

The transforming growth factor (TGF)-β is an immunosuppressive cytokine that facilitates tumor immune escape by hindering the maturation and function of T-cells and natural killer (NK) cells and hence promoting tumorigenesis (Wrzesinski et al., 2007). The fusion protein M7824 is an innovative immunotherapeutic agent that blocks both PD-L1 and TGF-β signals. Preclinical studies indicated that M7824 exhibited potent antitumor activity in mice bearing breast and colorectal carcinomas. It is noteworthy that M7824 also inhibited spontaneous metastases (Knudson et al., 2017; Lan et al., 2017). The antitumor effects were explained by the activation of CD8+ T cells and NK cells as well as increasing the expression of the major histo-compatibility complex (MHC)-I, MHC-II and PD-L1 by the tumor cells (Knudson et al., 2017). Moreover, M7824 augmented the ratio of M1/M2 macrophages and suppressed accumulation of MDSCs into the tumor (Lan et al., 2017). Toxicity studies in cynomolgus monkeys indicated that the fusion protein is well tolerated with negligible hematologic effects (Lan et al., 2017). The preliminary data from a phase-I clinical trial (NCT02517398) testing the fusion protein in patients with advanced cervical and pancreatic tumors indicated some early clues for clinical efficacy along with good tolerability profile in patients with heavy pretreatments. No grade 4–5 drug-related adverse effects were reported. However, grade 3 adverse effects included colitis with secondary anemia that was dose-limiting in one patient (Gulley et al., 2017).

**Table 1**
Summary of the strategies to enhance tumor response to immunotherapy.

| Immuno-modulator | Immune checkpoint antibody | Clinical/preclinical | Cancer type | Reference |
|---|---|---|---|---|
| Nab-paclitaxel | Anti-PD-L1 (Atezolizumab) | Clinical (Phase Ib& III) | TNBC | (Adams et al., 2016), (NCT02425891) |
| Abemaciclib | Anti-PD-L1 | Preclinical | NSCLC | (Dempsey et al., 2017) |
| Ribociclib/Alpelisib | Anti-PD-1/anti-CTLA-4 | Preclinical | TNBC | (Teo et al., 2017) |
| Palbociclib/Alpelisib | Anti-PD-1/anti-CTLA-4 | Preclinical | TNBC | (Teo et al., 2017) |
| STING agonist | Anti-PD-1 | Preclinical | TNBC | (Foote et al., 2017) |
| STING agonist | Anti-PD-L1 | Preclinical | Squamous cell carcinoma | (Gadkaree et al., 2017) |
| Epacadostat | Anti-PD-1 (Pembrolizumab) | Clinical (Phase I/II) | Melanoma and RCC | (Gangadhar et al., 2015) |
| Entinostat | Anti-PD-1 | Preclinical | Lung cancer and RCC | (Sodre et al., 2017) |
| PEGPH20 | Anti-PD-L1 | Preclinical | TNBC | (Clift et al., 2017) |
| PEGPH20 | Anti-PD-1 (Pembrolizumab) | Clinical (Phase Ib) | Gastric cancer | (NCT02563548) |
| Anti-Tim-3 | Anti-PD-1 | Preclinical | Lung cancer | (Sakuishi et al., 2010) |
| Anti-CTLA-4 | Anti-PD-1 (Nivolumab) | Clinical (phase I) | Advanced melanoma | (Postow et al., 2015) |
| Anti-CTLA-4 | Anti-PD-1 (Nivolumab) | Clinical (Phase II) | Advanced melanoma | (Hodi et al., 2016) |
| Cisplatin | Anti-CTLA-4/Anti-PD-1 | Preclinical | BRCA1 mutated TNBC | (Nolan et al., 2017) |
| BGB324-AXL tyrosine kinase inhibitor | Anti-CTLA4 | Preclinical | Lung and mammary carcinoma | (Wnuk-Lipinska et al., 2017) |
| BGB324-AXL tyrosine kinase inhibitor | Anti- PD-1 | Preclinical | Lung and mammary carcinoma | (Wnuk-Lipinska et al., 2017) |
| VS-4718-FAK inhibitor | Anti-PD-1 | Preclinical | Pancreatic cancer | (Jiang et al., 2016) |
| Axitinib-VEGFR1/2/3 inhibitor | Anti-CTLA-4 | Preclinical | Intracranial melanoma metastasis | (Du Four et al., 2016) |
| M7824 fusion protein | Anti-PD-L1 + Anti-TGF-β | Preclinical | Breast and colorectal carcinoma | (Knudson et al., 2017; Lan et al., 2017) |
| M7824 fusion protein | Anti-PD-L1 + Anti-TGF-β | Clinical (Phase I) | Cervical and pancreatic cancers | (Gulley et al., 2017), (NCT02517398) |
| HD-IR | Anti-PD-L1 | Preclinical | Mammary and colon tumors | (Deng et al., 2014) |
| Palliative radiotherapy | Anti-PD-L1 (Nivolumab) | Clinical (case report) | Lung cancer | (Yuan et al., 2017) |
| hypofractionated γ-irradiation | Anti-PD-1 | Preclinical | Melanoma | (Hettich et al., 2016) |
| stereotactic radiotherapy | Anti-PD-L1 (Nivolumab) | Clinical (Phase I) | Melanoma brain metastasis | (NCT02716948) |
| stereotactic radiotherapy | Anti-PD-1 (Pembrolizumab) | Clinical (Phase I) | NSCLC and melanoma Brain metastasis | (NCT02858869) |

PD–1 = programmed death-1 receptor; PD-L1 = programmed death-1 ligand; TNBC = triple negative breast cancer; NSCLC = non-small cell lung cancer; RCC = renal cell carcinoma; FAK = focal adhesion kinase; HD-IR = high dose ionizing radiation; STING = stimulator of interferon genes; PEGPH20 = PEGylated recombinant hyaluronidase; TGF-β = transforming growth factor beta; CTLA–4 = cytotoxic T-lymphocyte associated protein-4; Tim–3 = T cell immunoglobulin mucin-3.

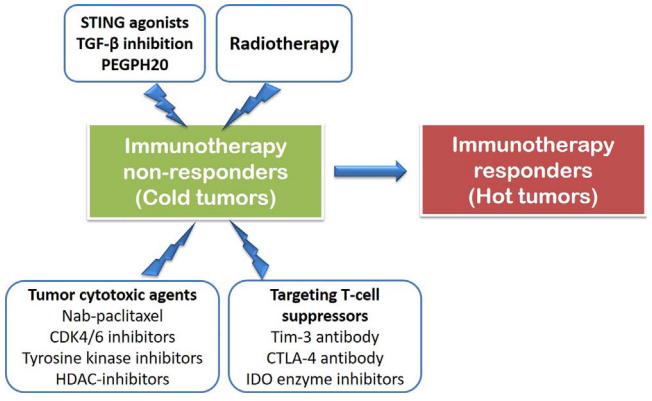


**Fig. 1.** Diagram depicting the implemented immunotherapeutic strategies to convert non-responders triple negative breast cancer to responders. STING, stimulator of interferon genes; TGF-β, transforming growth factor beta; PEGPH20, PEGylated recombinant hyaluronidase; HDAC, Histone deacetylases; TIM-3, T-cell immunoglobulin and mucin-domain containing-3; CTLA-4, cytotoxic T lymphocyte associated protein-4; IDO-indoleamine dehydrogenase.

## 12. Radiotherapy

The exposure to high-dose of ionizing irradiation (HD-IR) leads to tumor cell death and DNA damage and this leads to induction of immune reactions in the tumor microenvironment to inhibit tumor growth. However, there is a chance for local or distant tumor recurrence following IR due to the development of resistance (Deng et al., 2014). The radiotherapy-mediated immunogenic modulation (Gameiro et al., 2014) can be harnessed to enhance the tumor sensitivity to immunotherapies. Deng et al., reported that co-administration of anti–PD-L1 and high dose IR exhibited a synergistic antitumor effect through stimulation of the tumor suppressor cytotoxic CD8+ T cell and induction of MSDCs apoptosis (Deng et al., 2014). In this study BALB/c mice carrying TUBO mammary tumors and C57BL/6 carrying MC38 colon tumor xenografts received local one 12-Gy dose of HD-IR on day 14 after tumor cells inoculation. The administration of PDL-1 antibody (200 μg i.p.) was initiated on the same day of IR dose and continued to be administered every 3 days for a total of four times. A case report study of a patient with PDL-1 negative metastatic squamous cell lung carcinoma indicated that palliative thoracic radiotherapy (30 Gy in 10 fractions) was effective in controlling tumor burden despite the recurrence after nivolumab (PD-1 antibody) (Yuan et al., 2017). Hettich et al., reported that hypofractionated γ-irradiation concomitant with anti-PD-1 checkpoint significantly boosted tumor TILs accumulation with subsequent elimination of bulky neoplasms induced in C57BL/6 mice (Hettich et al., 2016). In this study, the tumors were locally treated with hypofractionated γ-radiation (2 × 12 Gy on successive days) and PD-1 antibody (200 μg i.p.) started on the day of the second radiation fraction and continued as weekly doses. The potential merit of double combination of radiotherapy together with immune checkpoint inhibitors in NSCLC (Campbell and Decker, 2017; Verheyen et al., 1986) and melanoma (Ahmed et al., 2017) has been recently reviewed. Data from retrospective clinical studies supported the potential merit of radiotherapy to augment the outcomes of immunotherapy even in the hard to treat settings such as melanoma intracranial metastasis that has very narrow overall survival (Ahmed et al., 2017). Prospective studies are currently ongoing to evaluate the effects of immune checkpoint inhibitors (nivolumab or pembrolizumab) combined with stereotactic radiotherapy in patients with melanoma (NCT02716948) or NSCLC (NCT02858869) metastasized to the brain.

## 13. Conclusion and future recommendations

Although breast cancer has been initially labeled as a non-immunogenic tumor type, certain subsets of breast cancer that carry the worst prognostic features demonstrated an immunogenic tumor microenvironment. Clinical trials of immune checkpoint inhibitors in breast cancer patients have demonstrated promising results and brought a light of hope for patients with metastatic advanced stage TNBC which is very hard to treat with the conventional protocols. This review highlighted a plenty of promising strategies that can be

exploited to extend the benefit of immunotherapy with immune checkpoint inhibitors in patients who are considered non-responders and to transform them into responders (Table 1 and Fig. 1). Further studies about the toxicity profiles of the investigated combinations are needed to confirm adequate safety and tolerability.

## Conflict of interest

The authors declare that there is no conflict of interests.

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