Immune checkpoint inhibition in triple negative breast cancer: targeting achilles' heel?

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Triple negative breast cancers pose an important challenge both for patients and their clinicians due to their aggressive disease course, poor long-term survival and lack of effective systemic treatment options. Recent scientific advances show that the adaptive immune system harbors the intrinsic capacity to eradicate cancer, generally through mechanisms that involve cytotoxic T-cells. Immune checkpoint inhibition boosts the host-anti-tumor response in many solid tumors, including breast cancer. However, cancer cells acquire ways to evade immunosurveillance and intra-tumoral T-cells are often functionally impaired, resulting in overt clinical cancer. Interestingly, the efficacy of immune checkpoint inhibition appears to correlate with tumor immunogenicity and the tumor mutational burden. Triple negative breast cancer has the highest tumor mutational burden of all breast cancer subtypes and therefore is believed to be the most immunogenic subtype. For this reason, clinical trials to date mainly focus on this specific subtype. Here, we review the accumulating evidence for immune checkpoint blockade in triple negative breast cancer.

INTRODUCTION

Triple negative breast cancers (TNBCs) account for 12-17% of all types of breast cancer and lack (by definition) the expression of estrogen/progesterone receptors and HER2 overexpression and/or amplification. This results in an aggressive disease entity that is resistant to both hormonal and HER2-targeted therapies.[1] In the absence of targeted options and specific treatment guidelines, the current therapy in the advanced TNBC setting consists of standard chemotherapy regimens, associated with poor response rates and short progression-free (PFS) and overall survival (OS).[2]

It is well known that the adaptive immune system has the ability to eradicate malignant cells through mechanisms involving T helper 1-, Natural Killer- and cytotoxic T cells.[3] In various tumors, including TNBC, a high percentage of tumor infiltrating lymphocytes (TILs) is associated with improved OS.[4,5] Furthermore, compared to paired early TNBC samples, the amount of TILs in advanced TNBC decreases, possibly under the influence of earlier cytostatic treatments but also as tumors evade immunosurveillance.[6] Cancer cells often evade immunosurveillance by various mechanisms resulting in T-cell exclusion and exhaustion e.g. by attracting immunosuppressive cells or hijacking immune checkpoints which prevent excessive T-cell activation in physiological conditions. To date, the cytotoxic T lymphocyte-associated antigen 4 (CTLA-4), programmed death-1 (PD-1) and its ligand PD-L1 are the best characterized immune checkpoints and therapeutic administration of antibodies against these proteins (*Table 1*) alleviate the immune system from its cancer-induced restraint.[7]

In a wide array of solid tumors, immune checkpoint blockade (ICB) has emerged as a valuable alternative option to the classical cytotoxic drugs.[7] However, response rates often vary depending on tumor type and (immunosuppressive) characteristics of the tumor micro-environment, and fail-safe predictors for clinical response are currently lacking. Recent evidence hints towards a prediction of response to ICB based on cancer cell mutational burden and the expression of neoantigens,

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TABLE 1. Immune checkpoint inhibitors

product name	target
pembrolizumab	PD-1
nivolumab	PD-1
atezolizumab	PD-L1
avelumab	PD-L1
durvalumab	PD-L1
ipilimumab	CTLA-4
tremelimumab	CTLA-4

PD-1 programmed death-1; PD-L1 PD-1 ligand; CTLA4 cytotoxic T lymphocyte antigen 4

which may be recognized by T-cells.[8] Mismatch repair (MMR) deficiency is one of the mechanisms leading to a high tumor mutational burden. The presence of MMR deficiency predicts the response of solid tumors to the anti-PD-1 antibody pembrolizumab which led the FDA to an accelerated approval for pembrolizumab in all MMR deficient solid tumors.[9,10] MMR deficiency is however very rare in breast cancer, making it difficult to predict responses to ICB, despite anecdotal evidence in case reports.[11]

Although conventionally breast cancer is not considered as a highly immunogenic cancer type, TNBC has the highest tumor mutational burden, highest infiltration rate of T cells and the highest PD-L1 expression of all breast cancer subtypes. As such, TNBC is regarded as being immunogenic.[12] In this review we highlight the existing evidence of ICB in the treatment of metastatic and early TNBC. We will also discuss possible methods to improve the response of ICB in TNBC.

ANTI-PD-1(LIGAND) ANTIBODIES IN TNBC

METASTATIC TNBC: ANTI PD-(L)1 ANTIBODIES AS SINGLE AGENT THERAPY

The vast majority of immunotherapy trials in breast cancer focused on TNBC in the metastatic setting. The Keynote-012 and Keynote-086 studies both investigated the effect of pembrolizumab on metastatic TNBC.[13,14] Keynote-086 trial included 2 cohorts: cohort A involving 170 TNBC patients that received one or more prior therapies and a second cohort consisting of 52 treatment-naive patients (B). Overall response rates (ORR) where modest at best in pretreated patients (4.7% in Keynote-086 cohort A and 18.5% in Keynote-012). Interestingly, the ORR in Keynote-086 cohort B was significantly higher than what was reported for cohort A (23% vs. 4.7% respectively).[14,15] This led to the postulation that the sooner ICBs are given, the more likely patients will respond. Similar findings were also noted in phase Ia trials with atezolizumab, where atezolizumab in first-line had an ORR of 26% in comparison to 11% in previously treated patients.16,17 Patients who had a complete (CR) or partial response (PR) to atezolizumab in monotherapy experienced a long median duration of response (21.1 months) and an extended OS.[16,17] A further sub-analysis of the patients who responded well to nivolumab or atezolizumab revealed that responding patients were enriched in TILs and PD-L1 expression. However, none of these biomarkers proved to be a decent discriminator between responders and non-responders in TNBC.

METASTATIC TNBC: ANTI PD-(L)1 ANTIBODIES IN COMBINATION WITH CHEMOTHERAPY

One way to boost the modest responses with ICB monotherapy is combining PD-1/PD-L1 blockade with chemotherapy. The underlying rationale is that chemotherapy by itself also has the potential to induce immunogenic cell death, thereby increasing antigen presentation to immune effector cells to elicit a strong host-anti-tumor response.[18] Two recent pilot studies investigated the role of chemotherapy with PD-1 or PD-L1 blockade. *Adams et al*. investigated Nab-paclitaxel + atezolizumab, whereas *Tolaney and coworkers* investigated the safety and efficacy of eribulin + pembrolizumab in metastatic TNBC.[19-21] Both groups demonstrated a high ORR (respectively 42% with Nab-Paclitaxel + atezolizumab and 33.3% with eribulin + pembrolizumab), which seems higher than the response rates seen with ICB monotherapy (in indirect comparison). However, this high ORR comes at a cost. Indeed, up to 66% of patients treated with the combination regimen experienced treatment emergent adverse effects (both immune- and cytotoxic related). Taken together these findings ask for caution not to increase the ORR at the cost of serious adverse events and reduction in quality of life without sound evidence of improved long-term outcome. Another important caveat is that bone marrow toxicity (especially lymphopenia) induced by cytotoxic agents may actually decrease the efficacy of immunotherapy. It is evident that further research is needed to shed light on these concerns. Albeit a recent press release from IMpassion130, a large scale phase III clinical trial investigating the addition of atezolizumab to Nab-paclitaxel, described encouraging

TABLE 2. Immune checkpoint inhibitors in the neoadjuvant setting

study	ICB product	associated chemotherapy	N	pCR in TNBC
I-SPY 2[25]	pembrolizumab	Paclitaxel→ AC	69	60%
Keynote-173[24]	pembrolizumab	A: nab-paclitaxel→ AC	A: 10	A: 60%
		B: nab-paclitaxel + carbo→ AC	B: 10	B: 90%
Pusztai[23]	MEDI4736	nab-paclitaxel→ dose dense AC	7	71.4%

AC: Doxorubicin/cyclophosphamide; pCR: pathological complete response

results regarding the primary efficacy analysis and safety profile of this combination treatment.[22] Results from other phase III studies (e.g. the Keynote-355) are also expected within short term.

USE OF ANTI PD-(L)1 ANTIBODIES IN EARLY TNBC

Neoadjuvant therapy serves to downsize (and hopefully downstage) the primary tumour. The appearance of pathological complete remission (pCR) after neoadjuvant treatment is strongly associated with a better long-term outcome in TNBC. As was discussed above, the earlier in the disease course the immune system is activated the higher the chance that this will result in a strong and durable anti-tumour response. Exploratory (single arm phase I-II) clinical trials in the neoadjuvant setting have been partially presented and are summarized in *Table 2*. In summary, the pCR rate is high in the ICB arms with increases up to three-fold compared to an estimation model based on historical controls.[23-25] Of interest, the increase in pCR appears to be independent of the agents associated to ICB. Although encouraging, these results come (unfortunately again) at the cost of important immune related adverse events, possibly due to a more robust immune response in early stage TNBC. To provide answers to the question whether pCR predicts the same long-term outcome as it does in neoadjuvant treatments without ICB, randomized phase III trials are needed. Results from the IMpassion031 and Keynote-522 trials, investigating the effect of neoadjuvant therapy with or without addition of atezolizumab or pembrolizumab, are expected within the next 1-2 years.[26,27]

As is apparent from the above, clinical trials with immunotherapy mainly focus on the neoadjuvant setting in early TNBC. In the adjuvant setting the phase III IMpassion030 trial is currently recruiting patients to evaluate the efficacy and safety of adjuvant chemotherapy with or without atezolizumab.[28]

ANTI CTLA4 ANTIBODIES IN TNBC

To date, anti-CTLA4 immunotherapy has not been tested in TNBC, but results from small exploratory studies in ER-positive breast cancer are available.[29] Despite favorable immunologic changes at the pathology level (e.g. increase in CD8+ effector/FOXP3+ regulatory T cell ratio), results of treatment with anti-CTLA4 checkpoint blockade are disappointing with no objective responses whatsoever, despite dose escalation. Importantly, treatment with anti CTLA4 antibodies is also less tolerated then other ICBs, illustrating the important role of CTLA4 in maintaining immune homeostasis.

BOOSTING THE RESPONSE TO IMMUNOTHERAPY

RADIOTHERAPY MAY BOOST THE IMMUNE SYSTEM FROM A DISTANCE

Radiotherapy may increase immunogenic cancer cell death, re-introduce oxygen in the hypoxic tumor area (important for T cell activation), abolish immunosuppressive MDSCs and augment dendritic cells who pick up antigens and present them to T helper cells in the secondary lymphoid organs to prime cytotoxic T cells.[30] When combined with ICBs, these primed CD8+ T cells may experience an additional boost to their effector phenotype. Although sporadic (pre)clinical evidence of a synergistic effect of radiotherapy with ICB is present in various tumor types like non-small cell lung cancer and melanoma, randomized clinical trials in TNBC are currently lacking.[31] The TONIC trial aspires to shed further light on the potential of induction radiation to make the tumor microenvironment more vulnerable to PD-1 blocking agents and definite results are expected soon.[32]

PARP INHIBITORS MAY BOOST THE IMMUNE SYSTEM FROM WITHIN

Since PARP inhibitors accumulate DNA damage and thus have the potential to alter immunogenicity in the

tumor microenvironment by increasing neoantigen expression, there is a clear rationale for combining PARP inhibitors with ICB's. Remarkably, *Jiao and colleagues* noted an increase in PD-L1 expression upon PARP inhibitor administration in a TNBC xenograft model.[33] These findings provide potentially valuable new insights for future treatments, but need further confirmation.

A GLANCE AT THE FUTURE – (CANCER) METABOLISM?

The activation status of effector T-cells in the tumor microenvironment is dependent of the net balance of pro- and anti-immunogenic factors. In other words, ICB alone may not be effective enough to fully generate a robust host-anti-tumor effect as other factors in the tumor microenvironment (immunosuppressive cell types, nutrient deprivation, toxic metabolites, …) render T cells unfit to challenge malignant cells. Fundamental research into these pathways recently provided new targets such as indoleamine 2,3-dioxygenase and arginase, and clinical trials are cautiously starting up. Importantly, although best characterized, PD-1 and CTLA-4 are only 2 players in the diverse repertoire of inhibitory T cell receptors. Another example of these receptors is the lymphocyte-activation gene 3 (LAG-3) which is a marker of T-cell exhaustion. Approaches combining an anti-LAG-3 antibody with anti PD-L1 in metastatic solid tumors including TNBCs or cytotoxic treatment in hormone receptor positive metastatic breast cancer are ongoing.[34,35]

Of particular interest is the recent evidence in a TNBC mouse model that suggests that not only the expression of PD-L1 is important for its suppressive function, but also its glycosylation status.[36] As glucose levels regulate the extent of glycosylation of several proteins, it is tempting to postulate that cancer cells (and possibly also other constituents of the tumor microenvironment), deliberately take up excessive amounts of glucose and other nutrients to boost post-translational modification of key molecules involved in immune suppression.[37] Metabolic targeting in the TME is a promising research field with a large potential of generating conceptually novel therapeutic approaches.

CONCLUSION

Cancer immunotherapy represents a powerful weapon against cancer. ICB is a promising investigational treatment option for (metastatic) TNBC. More research is needed to optimize the optimal timing of ICB administration, find optimal combination approaches to boost tumor immunogenicity/ICB response and limit treatment toxicity. The predictive effect of PD-L1 expression and tumor-infiltrating lymphocytes seems modest. MMR-deficiency is rare in TNBC and its absence should not exclude consideration of ICB treatment in patients with TNBC.

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