



Better Together: Immunotherapy as Future Combination Strategy for Breast Cancer

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Introduction

In our recent review we described the deep change obtained in the management of breast cancer (BC) over the last decade using combinations of different types of treatment [1]. Better knowledge of oncogenesis, biological characteristics of different types of cancer and the introduction of new drugs have identified possible combinations to overcome the multiple mechanisms of resistance put in place by tumor cells to achieve proliferation and progression.

Given the important and increasingly encouraging results obtained with immunological therapies, particularly in the treatment of melanoma [2-6], non-small cell lung carcinoma (NSCLC) [7], and renal cell carcinoma (RCC) [8], we considered necessary to contribute with a brief comment about these new therapeutic approaches and their possible role in the management of BC, particularly as a combination strategy.

The understanding of the so called "immune checkpoints", capable of regulating T-cell suppression, has led to the development of immune checkpoint inhibitors for cancer treatment. Nowadays the better known immune checkpoint inhibitors are represented by the blocking antibody (Ab) against T-Lymphocyte-associated-protein-4 (CTLA-4), the Abs against programmed death-1 (PD-1) and the Ab against programmed death-ligand-1 (PD-L1).

CTLA-4 expression is induced in activated T-cell, it competes with CD28 receptor binding CD80 and CD86 with approximately 100-fold greater affinity than the CD28 receptor, and leads to a reduction of T-cell population therefore; CTLA-4 downregulates T-cell function. As a confirmation of the T-cells function suppression induced by CTLA-4, mice deficient in CTLA-4 die from fatal lymphoproliferation [9,10]. The inhibition of CTLA-4 involves the loss of control on T-cells determining cancer cells death and antitumor immunity. Ipilimumab (Yervoy, Bristol Myers Squibb, Princeton, NJ) was the first anti-CTLA-4 Ab clinically approved after the milestone trials that showed a significant improvement in overall survival (OS) for patients with metastatic melanoma [2,3].

PD-1 is expressed on antigen-experienced memory T-cells in peripheral tissues; when PD-1 is engaged by its ligands, PD-L1 and PD-L2, it inhibits T-cell activation thus resulting in a negative regulation of T-cell activity. PD-1 is also expressed on B-cells, monocytes, dendritic cells and NK cells therefore, blockade of the PD-1 pathway may influence the function of these cell as well [11]. The preclinical rationale that inhibition of PD-1 could lead to antitumor activity mediated by the immune system was confirmed by the phase III clinical trials in different solid tumors such as melanoma, NSCLC and RCC. Nivolumab (Opdivo, Bristol-Myers Squibb, Princeton, NJ)

is an IgG4 fully human anti-PD-1 Ab recently approved for the treatment of metastatic melanoma, NSCLC and RCC [5,7,8], and Pembrolizumab (Keytruda, Merck, Darmstadt, Germany) is an IgG4 engineered humanized anti-PD-1 Ab approved for the treatment of metastatic melanoma and NSCLC [6,12].

PD-L1 is expressed on many cell types, including T-cells, B-cells, monocytes, antigen-presenting cell and epithelial cells. PD-L1 is believed to exert negative signals on T-cells not only by binding PD-1 but also by interacting with B7, expressed on dendritic cells [13]. Atezolizumab (MPDL3280A, Roche) is an IgG4 engineered fully human anti-PD-L1 Ab that doubled OS compared with docetaxel in previously treated patients with PD-L1-positive NSCLC, according to results from the phase II POPLAR study and BIRCH trial [14,15].

In BC the presence of lymphocytic infiltrates as possible prognostic factor was evaluated decades ago. Recently, tumor-infiltrating lymphocytes (TILs) are described as a prognostic factor in Triple Negative Breast Cancer (TNBC). Indeed, Loi et al. demonstrated the association between TILs and better outcomes in this BC subtype evaluating data from the BIG 02-98 trial [16]. In particular, the increment in stromal and intratumoral lymphocytic infiltrations is correlated with the reduction of risk of death. These data were confirmed by Adams et al. that evaluated the correlation between TILs and prognosis in two large phase III clinical trials: ECOG 2197 and ECOG 1199. The authors demonstrated that stromal TILs are an independent prognostic factor for disease free survival (DFS), distant recurrence-free interval (DRFI) and OS [17]. It is important to underline that all these results concern the adjuvant setting and encourage further evaluations to standardize a definition of TILs that could be included in the routine histopathological examination. Confirming the critical role of the immune system in TNBC, an interesting work by Burstein et al. explored TNBC molecular phenotypes using RNA gene expression profiling and described four subtypes of these tumors: Luminal Androgen Receptor (LAR), Mesenchymal (MES), Basal Like Immune-Suppressed (BLIS) and Basal Like Immune-Activated (BLIA). A correlation between these molecular phenotypes and clinical features shows that BLIS subtype has a significantly poorer prognosis in terms of DFS and Disease Specific Survival (DSS) versus all subtypes and conversely the BLIA subtype present the best results in terms of DFS and DSS [18]. These data further support the importance of the immune system in the control of tumor growth and progression. Therefore, Ab anti-CTLA-4, Ab anti-PD1 and Ab anti-PDL1 could represent a new effective treatment option in the management of BC. Different studies have demonstrated a positive correlation between CTLA-4, PD-1 and PD-L1 with increase in TILs and improved outcomes [19-22]. The checkpoint inhibitors Pembrolizumab and Atezolizumab were

evaluated in phase I studies in TNBC [23,24]. In the KEYNOTE-012 trial, approximately 56% of patients treated with Pembrolizumab experienced a treatment-related adverse event (AE), including fatigue, arthralgia, myalgia, and headache. One patient died of disseminated intravascular coagulation, which was attributed to the immunotherapy. The Overall Response Rate (ORR) was 18.5% with 1 complete and 4 partial responses. Seven patients had stable disease. Furthermore the phase I trial with Atezolizumab showed that between the 12 patients assessed for safety endpoints, 1 patient experienced grade 3-4 adrenal insufficiency; between the 9 patients assessed for response to therapy, ORR was 33%. Both treatments show activity in this subtype of BC, therefore further trials are investigating these molecules in successive phases of study.

A possible strategy to obtain improved outcomes in terms of DFS and OS is the combination of these immune checkpoint inhibitors with chemotherapy and/or hormonal-therapies. It is worth noting that many chemotherapeutic agents are immunogenic and this could implement the immunotherapeutic effect of checkpoint inhibitors [25]. This concept is reinforced by the fact that chemotherapy is not only immunogenic but also capable to determine significant responses in terms of DFS and OS, responses that could be improved by the combination with immunotherapies [26]. Furthermore, the association between hormonal-therapies and immunotherapies could be active in luminal BC. In this BC subtype the preliminary results of the KEYNOTE-028 trial were presented at the 2015 San Antonio Breast Cancer Symposium [27] and showed activity of Pembrolizumab also in Estrogen receptor positive (ER+)/HER2 negative BCs. Also Avelumab, another Ab anti-PD-L1, demonstrated to be active in a phase I trial in which responses were observed in all subtypes of BC [28].

Advances in the understanding of the interaction between the immune system and tumor cells have also contributed to the development of other therapeutic strategies based on chimeric antigen receptor (CAR) modified T cells or NK cells. The classic CARs T cells consist of an extracellular antigen recognition domain, a hinge domain, a transmembrane domain and an intracellular domain [29]. The extracellular antigen-binding moiety renders T cells the ability to bind antigens with retained specificity and affinity [30]. The hinge region mediates CAR flexibility [31] and the transmembrane can indeed influence the function of CAR-T cells [32]. The intracellular domain is responsible for signal delivery within CARs. CAR-T cell immunotherapy demonstrated remarkable clinical responses in hematologic malignancies [33] and now it could represent an interesting challenge in solid tumors treatment. At the same time, given their shorter lifespan and potent cytolytic function, mature NK cells provide attractive candidate effector cells to express CARs and provide an excellent source of therapy for patients with cancer [34].

Another important observation is that the escape from host immunosurveillance resulting in cancer growth and progression depends from different alterations in immune response like the enhancement of immunosuppressive cells such as myeloid-derived suppressor cells (MDSCs). Several approaches to obtain suppression of MDSCs have been sought after [35].

The use of CAR-T or CAR-NK cells and suppression of MDSCs may enhance and sensitize the effects of chemotherapies and checkpoints inhibitors in solid tumors and particularly in BC. Nevertheless more data are still needed to better apply CAR-T, CAR-NK cells and MDSCs techniques in treating solid tumors.

All these data represent the background for a new era in BC management. As we have seen in our precedent review, combination of different types of treatment is an important strategy to overcome cancer resistance and proliferation. Therefore, combination of new molecules with "classic" therapies, such as chemotherapy and hormonal-therapy, could become the new standard for BC treatment. Further clinical trials are warranted to confirm this interesting hypothesis.

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