Immune checkpoints
A therapeutic target in triple negative breast cancer

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Early clinical trials investigating monoclonal antibodies targeting the T-cell inhibitory receptor programmed cell death 1 (PD-1) and its ligand PD-L1 have shown efficacy in melanoma, non-small cell lung cancer and renal cell carcinoma. We recently demonstrated PD-L1 expression in 20% of triple negative breast cancers suggesting that targeting the PD-1/PD-L1 immune checkpoint may be an effective treatment modality in patients with this disease.

Cancer Immunotherapy was named *Science* magazine's Breakthrough of the Year in 2013.[1] This recognition was largely driven by the success of ipilimumab, the monoclonal antibody that targets cytotoxic T-lymphocyte-associated antigen 4 (CTLA-4), an inhibitory receptor expressed on activated CD8+ T cells. CTLA-4 attenuates the immune response by countering the activity of CD28, a co-stimulatory receptor.[2] By targeting CTLA-4, ipilimumab effectively takes the "brakes" off T cells, thereby potentiating the antitumor immune response. Ipilimumab received FDA approval in 2011 after a pivotal clinical trial showed improved overall survival (OS) in patients with unresectable stage III/IV melanoma.[3]

CTLA-4 is considered an immune checkpoint and ipilimumab is the first member of a new class of immunotherapeutic agents commonly referred to as "checkpoint blockade." Another immune checkpoint that has received significant attention is programmed cell death 1 (PD-1), a second T-cell receptor that limits the T-cell response within tissues.[4] Monoclonal antibodies targeting PD-1, and its ligand PD-L1, have been investigated in several early phase clinical trials. Blockade of PD-L1 by the monoclonal antibody BMS-936559 was investigated in a Phase I trial evaluating 160 patients with advanced solid tumors showing modest objective response rates (ORR) of 6–17%.[5] Greater responses were seen in a Phase I trial evaluating the anti-PD-1 antibody, BMS-936558 (nivolumab) in advanced non-small cell lung cancer, melanoma, and renal cell carcinoma with ORR of 18%, 28%, and 27%, respectively.[6] Durable responses were seen in 21 of 30 evaluable patients. Pretreatment biopsies were available from 42 patients permitting the evaluation of PD-L1 expression by immunohistochemistry. Interestingly, none of 17 patients with PD-L1 negative tumors had an objective response to treatment. Although the numbers are small, these data suggest that PD-L1 expression may be a tumor biomarker for beneficial response to anti-PD-1 therapy.[6] This hypothesis is supported by data presented at the 2013 American Society of Clinical Oncology Annual Meeting showing that of 101 melanoma patients treated with nivolumab, those with PD-L1 positive tumors exhibited higher ORR and longer progression-free duration and better overall survival (OS).[7] Ongoing clinical trials investigating nivolumab, and other agents targeting PD-1 and PD-L1, are underway to further address the question of whether PD-L1 may serve as a predictive biomarker for this therapeutic avenue.

Our group has recently reported that PD-L1 is expressed in approximately 20% of patients with triple negative breast cancer (TNBC), a subtype of breast cancer that lacks therapeutic targets.[8] Furthermore, we showed that loss of phosphatase and tensin homolog (PTEN), a negative regulator of the phosphatidylinositide 3-kinase (PI3K) pathway, increased PD-L1 cell surface expression, which was associated with decreased proliferation and increased apoptosis of T cells. Cancer cell treatment with therapeutic agents targeting the PI3K pathway, including the AKT inhibitor MK-2206 and rapamycin, also significantly decreased PD-L1 surface expression. PD-L1 mRNA levels were altered after either PTEN knockdown or PI3K inhibition, providing evidence for transcriptional regulation. Drugs targeting the PI3K pathway are currently being investigated in clinical trials across a variety of solid tumor types. It is largely thought that these agents work by targeting tumor cell growth. However, our data suggest that agents targeting PI3K signaling may also function by enhancing adaptive immune responses.

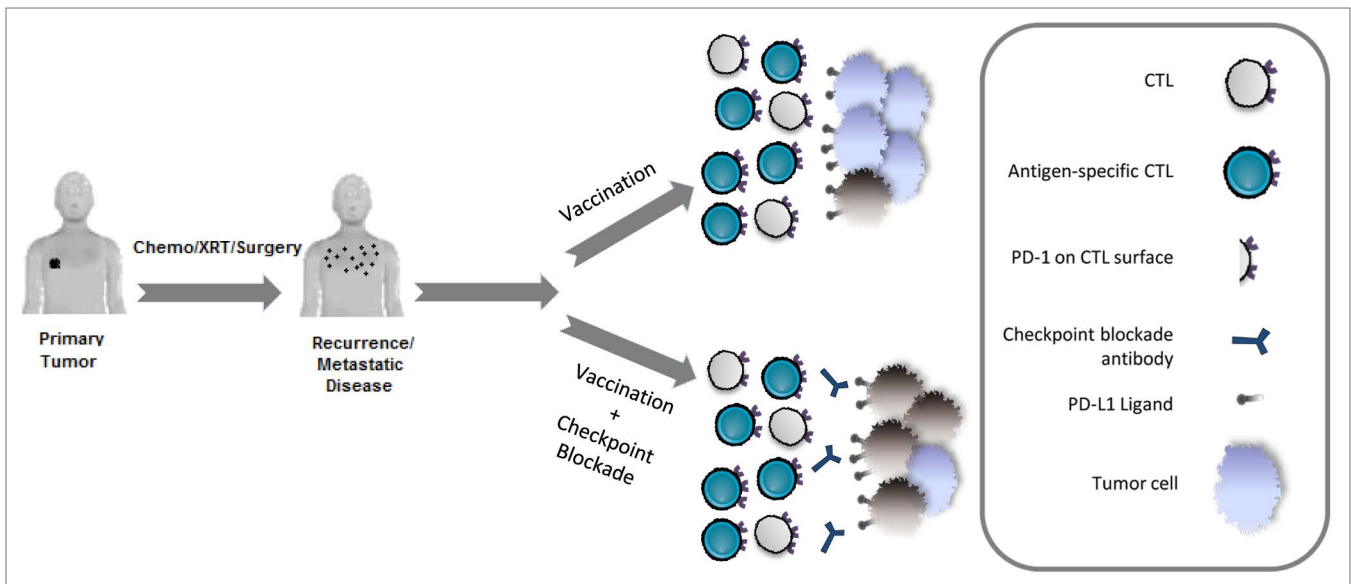Our data provide a rationale for using anti-PD-1 or anti-PD-L1 therapy in

Figure 1. Combination immunotherapy. For patients with metastatic breast cancer, monotherapy consisting of vaccination or administration of monoclonal antibodies targeting immune checkpoints such as programmed cell death 1 (PD-1) or its ligand (PD-L1) may be insufficient to eradicate disease. A combination strategy wherein an antigen-specific cytotoxic T lymphocyte (CTL) response is stimulated by vaccination and an anti-PD-1 or anti-PD-L1 antibody is used to overcome inhibitory pathways, may enhance the antitumor immune response thereby resulting in increased tumor cell death and improved patient outcomes.

TNBC and in fact, this treatment modality is currently under investigation in several ongoing trials. Merck (MK3475; NCT01848834) and Amplimmune (AMP-514; NCT02013804) are evaluating anti-PD-1 antibodies in Phase I trials enrolling patients with advanced malignancies that include TNBC. Bristol-Myers Squibb is conducting a Phase I/II trial investigating nivolumab and the combination of nivolumab plus ipilimumab in TNBC patients as well as patients with advanced gastric cancer, pancreatic cancer, and small cell lung cancer (NCT01928394).

Moving forward, it is likely that combination immunotherapy approaches will lead to improved survival in cancer patients. Our group has long been interested in administering peptide vaccines in the adjuvant setting to prevent disease recurrence in patients that have been rendered disease-free with standard-of-care therapy but that are at high risk for recurrence. Phase I/II studies evaluating nelipepimut-S + GM-CSF (NeuVax) showed 60-mo recurrence-free survival rates of 90% for vaccinated patients vs. 80% for non-vaccinated controls.[9] Based on these encouraging data, a multinational, prospective, randomized, double-blind,

controlled Phase III registration trial is currently enrolling (NCT01479244). It should be emphasized that these trials have enrolled patients in the adjuvant setting when there is minimal residual disease. Earlier studies evaluating peptide vaccines in patients with diffusely metastatic disease have been disappointing with objective response rates of less than 3%.[10] In order for a tumor vaccine to have optimal therapeutic effect in patients with metastatic tumors, it is likely that the immunizing agent will need to be combined with agents such as monoclonal antibodies targeting immune checkpoints (see **Fig. 1**). In this scenario, the vaccine would stimulate antigen-specific T cells and the checkpoint blockade agent would take the "brakes" off, allowing for propagation of that tumor specific immune response. Conversely, in the case of breast cancer which is not thought to be highly immunogenic, stimulation of a T-cell response by vaccination may be required for optimal activity of checkpoint blockade agents.

In summary, our recently reported work showing expression of PD-L1 in approximately 20% of TNBC identifies a potential therapeutic target in this subtype of breast cancer and provides rationale to

evaluate immunotherapy targeting PD-1/PD-L1 in these patients. Such novel therapeutic strategies are desperately needed for patients afflicted with this particularly malevolent disease, exhibiting poorer outcomes, shorter disease-free survival intervals and worse OS than breast cancer patients expressing markers, such as hormone receptors and the oncogene HER2, for which well-established targeted therapeutics are available.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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