Immunomodulation via Chemotherapy and Targeted Therapy: A New Paradigm in Breast Cancer Therapy?

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Keywords
Breast cancer · Tumor-infiltrating lymphocytes · Prognosis · Immunotherapy

Summary
Cytotoxic chemotherapy in the treatment of tumors has traditionally been thought to be immunosuppressive. Increasing evidence suggests the contrary and has introduced the concept of ‘immunogenic’ chemotherapy or, in other words, the concept that the innate and adaptive immune systems are critical in determining the long-term efficacy of some cytotoxic-based (and radiotherapy-based) regimens. The underlying mechanisms how these therapies can stimulate an antitumor immune response have been demonstrated recently. In this article, we review the background of this new paradigm and how combinations of traditional agents with the new immunotherapeutic therapies may significantly advance our treatment of breast cancer.

Introduction
Traditionally, cytotoxic chemotherapy has been thought to be immunosuppressive. Increasing evidence has proposed the concept of ‘immunogenic’ chemotherapy or, in other words, the concept that the innate and adaptive immune systems are critical in determining the long-term efficacy of some cytotoxic-based (and radiotherapy-based) regimens. Various mechanistic aspects of the way these therapies can stimulate an antitumor immune response have been revealed recently. In this article, we review the data behind this new paradigm and how combinations of traditional cytotoxic agents with the new targeted agents may significantly advance our treatment of solid tumors and, in particular, of breast cancer.

Cancer Immunoediting
In the past decade, cancer immunology has emerged as a fundamental discipline of oncology, and immunotherapy as a reality for cancer patients [1, 2]. Immunity has 2 seemingly paradoxical effects on cancer. On the one hand, immunity prevents the development of nascent tumors, a concept known as cancer immunosurveillance [3, 4]. On the other...
hand, immunity shapes the intrinsic nature of developing tumors through immunological pressure. This combination of host-protective and tumor-sculpting functions of the immune system is termed cancer immunoediting [5]. Immunoediting is a dynamic process composed of 3 phases: elimination, equilibrium, and escape. Elimination refers to the classical concept of cancer immunosurveillance where premalignant and early-stage malignant cells are directly or indirectly removed by immune cells. The concept of cancer immuno surveillance was initially proposed by Burnet and Thomas [6] and was experimentally validated in the late 1990s using gene-engineered mouse models of immunodeficiency [7–9]. Equilibrium refers to a period of ‘tumor dormancy’ after incomplete immunemediated tumor destruction, where equilibrium is reached between immune-mediated killing and survival of tumor cells [10]. Escape refers to the outgrowth of tumors that have survived and have been selected for by immunological pressure. The importance of cancer immunoediting was recently demonstrated in a carcinogen-induced mouse sarcoma model where a mutated antigen was found to be a major tumor rejection antigen that eventually led to the outgrowth of tumors lacking the immunogenic epitope [11]. Thus, despite tumor immunosurveillance, tumors develop and are shaped in a Darwinian way in the presence of a functioning immune system.

**Immunogenic Chemotherapy: Mechanisms of Immune Stimulation**

In addition to being involved in the natural progression of cancer, immunity can affect the activity of various anticancer agents [12]. Accordingly, recent evidence suggests that some chemotherapeutic drugs, such as anthracyclines and oxaliplatin, rely on the induction of anticancer immune responses. In mouse models of cancer, chemotherapy with anthracyclines or oxaliplatin requires the priming of interferon (IFN)-γ-producing CD8+ T cells for optimal treatment response [13]. In cancer patients, high levels of IFN-γ and CD8+ T cells are predictive of a good clinical response to anthracyclines [14]. The immune-stimulating properties of anthracyclines and oxaliplatin were shown to require preapoptotic translocation of calreticulin (CRT) on the tumor cell surface, post-apoptotic release of the chromatin-binding protein high-mobility group B1 (HMGB1), and extracellular release of adenosine triphosphate (ATP). CRT, HMGB1, and ATP act in concert to promote tumor antigen presentation by dendritic cells (DCs) via activation of CD91, Toll-like receptor (TLR)-4, and purinergic P2X7 receptors, respectively [15]. It was recently demonstrated that chemotherapy-induced autophagy is essential for the release of ATP and subsequent anticancer immunity [16]. Accordingly, autophagy-deficient tumor cells are unable to release ATP in response to anthracyclines or oxaliplatin and fail to elicit CD8+ anticancer T cells. This suggests that patients with autophagy-deficient tumor cells might benefit from therapeutic strategies designed to compensate this process in order to trigger immunogenic signaling. Extracellular ATP thus appears as a central activator of anticancer immunity. However, tumors have been shown to overexpress ecto-nucleotidases able to hydrolyze extracellular ATP to adenosine [17]. The expression of these ecto-nucleotidases, such as CD39 and CD73, will potentially have 2 major consequences: decreasing the concentration of extracellular ATP available for DC activation and increasing the concentration of extracellular adenosine, a potent suppressor of anticancer T cell functions. Several groups have now demonstrated the importance of CD73, considered as the rate-limiting enzyme in the production of extracellular adenosine, in the suppression of anticancer immunity [18]. Taken together, these studies suggest that targeted blockade of ecto-nucleotidases such as CD39 and CD73 may provide effective means to enhance antitumor immune responses.

**Immunity in Targeted Therapy**

Immune responses also play a major role in the efficacy of targeted therapies with monoclonal antibodies (mAbs). 9 mAbs directed against 6 cancer-associated proteins (namely human epidermal growth factor receptor 2 (HER2), CD20, EGFR (epidermal growth factor receptor), CD52, CD33, and VEGF (vascular endothelial growth factor)) are currently approved for the treatment of various types of cancer [2]. Studies have shown that mAbs such as trastuzumab (anti-HER2) and rituximab (anti-CD20) rely in part on immune-mediated killing through antibody-dependent cellular cytotoxicity (ADCC) [19]. While innate immune responses appear to be important for tumor antigen-targeted mAb therapies, recent studies in mice and correlative clinical evidence suggest that mAbs such as trastuzumab may also stimulate adaptive antitumor immunity. 2 studies in mice showed that anti-HER2 mAb therapy required adaptive CD8-dependent immunity to mediate its optimal effect [20, 21]. Experimental evidence supports a model whereby trastuzumab activates MyD88-dependent TLR signaling (most likely via the release of HMGB1 following ADCC), stimulates the release of type 1 IFNs and primes adaptive IFN-γ-producing CD8+ T cells. These studies raise the possibility that combination strategies may be used to capitalize on the adaptive tumor-specific immunity generated by anti-HER2 mAbs. Consistent with this notion, Stagg et al. [21] demonstrated that anti-PD-1 and anti-CD137 mAbs can each synergize with anti-HER2 mAb therapy. Similar synergistic activity between anti-CD137 and anti-HER2 mAbs was reported by a different group [22]. Notably, it was also shown that immunosuppressive chemotherapeutic drugs, such as paclitaxel or cyclophosphamide, can dramatically interfere with the tumor-specific immune memory generated by anti-HER2 mAb [20]. Taken together, these studies suggest that first-line chemotherapeutics should be carefully considered to prioritize those that directly or indirectly prime...
or alter tumor-specific immunity, and need careful attention when used in combination with mAb therapies. They also suggest that one of the mechanisms of synergy between pro-immunogenic anthracyclines and trastuzumab may be through increased antitumor immune responses [21]. Recent evidence suggests that targeted therapies with small inhibitors may also benefit from antitumor immune responses. Accordingly, the administration of a BRAF inhibitor in advanced melanoma patients has been reported to increase the level of tumor-infiltrating T cells within 7 days of treatment, with CD8+ T cells increasing significantly more than CD4+ T cells [23]. This increase in CD8+ T cells correlated with a decrease in tumor size. This suggests that the combined use of small-molecule inhibitors, such as BRAF inhibitors, and immunotherapy might be synergistic.

**T Cell Infiltration, Immune Signatures, Prognosis, and Chemotherapy Response**

Overwhelming data reveals the importance of tumor immune infiltrates in the survival of cancer patients, including breast cancer. Increased infiltration of tumors with CD8+ CD45RO+ memory T cells has been associated with a better prognosis in a variety of epithelial cancers [24–26]. Likewise, an increased ratio of CD8+ to CD4+ T cells (T helper 2 (Th2) cells or T regulatory cells (Tregs)) correlates with a good clinical outcome in several types of cancers [27, 28]. In colorectal cancers, T cell infiltration measured by immunohistochemistry has shown superior prognostic power than standard tumor, node, metastasis (TNM) staging [29]. In breast cancer, 2 large series, both in newly diagnosed or early-stage breast cancer, also support a correlation with better clinical outcomes [30, 31].

Tumor lymphocytic infiltration can also be determined by gene expression signatures. In breast cancer, unsupervised expression profiling of cancer-associated stroma revealed a gene signature predictive of good prognosis that was enriched for CD8+ T cell responses [32]. Also, in over 1,500 newly diagnosed breast cancer samples, a metagene of STAT1 signaling was associated with better outcomes in specific breast cancer subtypes: the ‘triple-negative’ (negative for expression of the estrogen receptor, progesterone receptor, and HER2) and HER2/neu-overexpressing subtypes [33, 34]. Other independent groups have also observed this [35, 36]. Together this data supports that immune modulation may be most important for these breast cancer subtypes.

Other solid cancer types have been traditionally considered more responsive to immunotherapies, such as melanoma and renal cell carcinoma. Why is this association not considered in breast cancer? We speculate that perhaps the immune system is more effective at preventing breast cancer metastases rather than influencing growth of the primary tumor. For example, the report that transplant patients who have been treated with immunosuppressive therapies do not have an increased incidence of breast cancer, in contrast to melanoma, suggests that tumor immunosurveillance does not regulate primary breast tumor initiation and growth [37]. This is also reinforced by other reports that used breast cancer murine models, where immune effects on metastases were independent of primary tumor initiation and growth [38, 39]. Furthermore, an increase in circulating myeloid-derived suppressor cells (MDSCs) in human cancer was associated with stage 4 disease [40]. Lately, suppression of an IRF7-driven type I interferon innate pathway, intrinsic to breast cancer cells, was shown to restrict systemic immunosurveillance and result in increased metastases in a breast cancer murine model, further supporting this concept [41].

In addition, it has been shown in breast cancer that high levels of immune infiltrates are associated with certain breast cancer subtypes: A report in 1992 first highlighted this association with rapidly proliferating breast tumors [42]. Why this could be more relevant for breast cancers that are negative for estrogen receptor expression as well as the HER2-overexpressing breast cancer subtypes as opposed to other breast cancer subtypes is unknown; we speculate that it could be due to the poorly differentiated nature and high genomic instability of these subtypes [43]. Furthermore, HER2 is a well-described tumor antigen. However, this association with different breast cancer subtypes may explain why prognosis has been inconsistently associated with breast cancer and immune infiltrates. In the triple-negative subtype, baseline immune infiltrate is strongly associated with prognosis, independent of the adjuvant type of chemotherapy given [43, 44]. Hence, it seems that, for certain patients, immune memory has already been generated. Lately, baseline immune infiltrates at diagnosis have been shown to predict benefit from immunogenic therapies such as higher-dose doxorubicin and trastuzumab. These effects were seen only in the HER2-overexpressing subtype [43, 44]. This raises the interesting possibility that a baseline pre-existing T cell response predicts for a better outcome to immunogenic-based therapies in HER2-overexpressing disease.

**Immune Checkpoint Inhibitors**

**Anti-CTLA-4 mAb**

Several members of the immunoglobulin superfamily of receptors including CTLA-4, PD-1, BTLA (band T lymphocyte attenuator), TIM-3 (T cell immunoglobulin and mucin domain-containing protein 3) and VISTA (V-domain immunoglobulin suppressor of T cell activation) serve as inhibitory immune checkpoints that prevent uncontrolled immune reactions [45]. Much of the recent successes in cancer immunotherapy come from the generation of blocking mAbs targeting these inhibitory receptors. The anti-CTLA-4 mAb ipilimumab was the first to be tested in a phase III clinical trial [46, 47]. In 2011, the FDA approved the use of ipilimumab in patients with metastatic melanoma, either as initial therapy or after relapse. Anti-CTLA-4 mAb therapy enhances the anti-

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**Immunomodulation as Therapy in Breast Cancer**

Breast Care 2012;7:267–272
tumor function of CD8+ T cells, increases the ratio of CD8+ T cells to Foxp3+ Tregs and inhibits the suppressive function of Tregs [1]. CTLA-4 blockade has also been shown to expand a subpopulation of tumor-infiltrating CD4+ T cells that express high levels of ICOS and secrete IFN-γ [48]. These CD4+ ICOS+ T cells might play a role in the therapeutic activity of anti-CTLA-4 mAb therapy, as their frequency correlates with survival in treated melanoma patients. The major drawback to anti-CTLA-4 mAb therapy is the generation of autoimmune toxicities due to off-target effects. It has been reported that up to 23% of patients treated with ipilimumab developed serious grade 3–4 adverse events, reflecting the importance of CTLA-4 in maintaining immune homeostasis. Unfortunately, toxicity has not always been associated with therapeutic benefit. Thus, a major challenge in the use of anti-CTLA-4 mAbs is to define favorable clinical settings that strike an optimum balance between tumor immunity and autoimmunity.

Anti-PD-1 mAb

PD-1 is another inhibitory co-receptor expressed on activated and exhausted T cells. Administration of blocking anti-PD-1 mAbs enhances adaptive antitumor immune responses by preventing T cell exhaustion. PD-1 is expressed by activated CD4+ and CD8+ T cells, B cells, monocytes and natural killer (NK) cells. It has two ligands, PD-L1 and PD-L2, with distinct expression profiles. Expression of PD-L1 has been shown to be associated with poor prognosis in melanoma and hepatocellular carcinoma [49, 50]. Anti-PD-1 and anti-PD-L1 mAbs have been shown to reduce the tumor burden in a number of experimental cancer models. Recently, a phase II non-randomized clinical trial evaluating anti-PD-1 mAb therapy in patients with melanoma, renal cell carcinoma, prostate cancer, non-small cell lung cancer or colorectal cancer reported that 6/16 (37.5%) evaluable patients had objective tumor responses [51]. Taken together, clinical studies with anti-PD-1 and anti-PD-L1 mAbs yield very promising results. Of interest, anti-PD-1 mAbs appear to have safer toxicity profiles than anti-CTLA-4 mAbs [52].

Combining Immune Checkpoint Inhibitors

While inhibition of a single immune checkpoint can prolong the survival of cancer patients, an important question that remains is whether combinatorial checkpoint blockade can by synergistic in promoting antitumor activity. As reported by Curran et al. [53], ‘blockade of single negative costimulatory pathways often leads to enhanced effector T-cell (Teff) infiltration of tumors, but these Teff cells accumulate high levels of the unblocked negative coreceptors that eventually limit their expansion’. The first combination of immune checkpoint inhibitors to be tested was the combination of anti-CTLA-4 and anti-PD-1 mAbs. Curran et al. demonstrated that blockade of CTLA-4 and PD-1 allows CD8+ and CD4+ T cells to survive in the tumor microenvironment, to proliferate and to carry out effector functions. More recently, TIM-3 has been identified as another important inhibitory receptor expressed by exhausted CD8+ T cells. It was shown that the most dysfunctional tumor-infiltrating CD8+ T cells actually co-express PD-1 and TIM-3 [54]. Based on these findings, a direct comparison of the therapeutic activity of anti-CTLA-4, anti-PD-1 and anti-TIM-3 mAbs was made in various mouse models of cancer [55]. It was observed that anti-CTLA-4 mAb was only weakly effective against established tumors, which is consistent with other studies. However, the same regimen of anti-PD-1 mAb or anti-TIM-3 mAb, administered as single agent, significantly delayed established tumor growth. Most importantly, the combination of anti-PD-1 and anti-TIM-3 mAbs had the most potent anticancer effect against well-established experimental and carcinogen-induced tumors. Nevertheless, the extent of cooperative interactions between various immune checkpoints still remains largely unknown. LAG-3 is another recently identified inhibitory receptor that acts to limit effector T cell function and to augment the suppressive activity of Tregs. Woo et al. [56] recently revealed that PD-1 and LAG-3 are extensively co-expressed by tumor-infiltrating T cells and that combined blockade of PD-1 and LAG-3 provokes potent synergistic antitumor immune responses.

Other Regulators of T Cell Function

TNF Receptor Superfamily

Members of the tumor necrosis factor (TNF) receptor superfamily also play an important role as regulators of T cell function [57]. Activation of these costimulatory receptors may further enhance the generation of tumor-reactive T cells in the context of cancer therapy. Costimulatory receptors of the TNF receptor family are composed of OX40 (CD134), 4–1BB (CD137), CD27, CD30, and HVEM (herpes virus entry mediator). When activated, each of these receptors can enhance cytokine production and T cell proliferation in response to T cell receptor (TCR) signaling. OX40 and CD137 activation are particularly effective in allowing activated T cells to survive and proliferate in the late phase of immune responses. The administration of agonistic mAbs against OX40 or CD137 has been shown to enhance tumor immunity and induce regression of established mouse models of cancer [58–60]. The use of agonists to costimulatory receptors or antagonists to inhibitory receptors may rescue or enhance the activity of tumor-reactive T cells.

Blocking Tumor Immunosuppressive Factors

Targeting immunosuppression by soluble mediators is another attractive approach for cancer immunotherapy. A plethora of immunosuppressive factors has been associated with tumorogenesis, including transforming growth factor β (TGF-β), indoleamine 2,3-dioxygenase (IDO), arginase, prostaglandin-E2 (PGE2), and extracellular adenosine [3]. To determine which immunosuppressive factors are minimally required for maintaining tumor tolerance in a given patient population remains a great challenge. Recent studies in mouse models of cancer
and clinical correlative studies suggest that interleukin (IL)-23 may be a key cytokine governing the balance between pro- and antitumorigenic immune responses [61–63].

Enzymes that metabolize l-arginine (such as arginase I), the tryptophan-catabolizing enzyme IDO as well as enzymes that regulate extracellular adenosine levels (such as the ectonucleotidases CD39 and CD73) also significantly contribute to the inhibition of anticancer immune responses [17, 64]. CD73 works at a critical checkpoint in the conversion of immune-activating ATP into immunosuppressive adenosine, making it a potential therapeutic target. Tumors often over-express CD73 as a consequence of tissue hypoxia or, in the case of breast cancer, loss of estrogen receptor expression. Proof-of-principle studies have revealed that anti-CD73 mAb therapy can reduce tumor burden and prevent metastasis in mice [65–69]. While tumor-derived CD73 is a significant contributor to the generation of adenosine, host CD73 also exacerbates tumorigenesis, highlighted by the reduced susceptibility of CD73-deficient mice against a number of transplantable and spontaneous tumors. Given the promising results of anti-CD73 targeted therapy in mice, future studies aimed at translating this approach into the clinic are warranted. Combination of these agents with immunogenic cytotoxic agents could also be a reasonable approach in order to enhance tumor antigen recognition by the host.

Conclusions and Possible Future Directions

As seen, the role of enhancing host antitumor immunity as part of cancer therapy now seems to be a feasible option, and the increased understanding of its interplay in therapies using both traditional cytotoxic and the new targeted agents suggests that combinations of these with directly immunomodulatory agents could lead to a viable new paradigm in breast cancer as well as other cancer types. The key will be the identification of those patients who require specific immune therapies and of the respective best specific therapy. We propose that breast cancer patients (the triple-negative and HER2-overexpressing) depending on whether they have existing lymphocytic infiltrate (TILs) at diagnosis. Those that do (top) will need optimal standard cytotoxic chemotherapy including an anthracycline and radiotherapy with immunogenic capabilities. Levels of T cell-inhibitory ligands present in the tumor may dictate the need for the addition of further immunotherapy. In contrast, for patients without TILs (bottom) cancer therapy upfront must include maximum immunotherapy in combination with standard cytotoxic chemotherapy (± trastuzumab for HER2-positive patients) in order to stimulate the host immune system to see tumor antigen. This could involve immunogenic chemotherapy (provoke tumor antigen recognition), targeted mAbs (ADCC, stimulating adaptive immunity), inhibition of CD73 (inducing ATP), and TLR-3 agonists (stimulating cytokine production to expand antigen-specific CD8+ T cells and generate IFN-γ-producing CD8+ T cells).

patients in combination with classical treatment, which includes cytotoxic chemotherapy, radiotherapy, and trastuzumab (fig. 1). Understanding the ligands that switch off T cells in breast cancer will also be the key to determining the T-cell immunotherapies most interesting for evaluation.

All preclinical data in mouse models, as well as prognostic and predictive data in human breast tumors, are strongly supportive of their role in determining long-term outcomes for these molecular subgroups of breast cancer.

Disclosure Statement

There are no conflicts of interest.

References


