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T Cell Coinhibition and Immunotherapy in Human Breast Cancer

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Abstract

Costimulation and coinhibition generated by the B7 family and their receptor CD28 family have key roles in regulating T lymphocyte activation and tolerance. These pathways are very attractive therapeutic targets for human cancers including breast cancer. Gene polymorphisms of B7x (B7-H4/B7S1), PD-1 (CD279), and CTLA-4 (CD152) are associated with increased risk of developing breast cancer although the underlying mechanisms are unclear. In human breast cancer microenvironment, up-regulation of coinhibitory B7/CD28 members B7x, B7-H3 (CD276), and PD-L1 (B7-H1/CD274) on tumor cells as well as PD-1 and PD-L1 on tumor-infiltrating immune cells are emerging as immune evasion pathways. Chemotherapy can affect the expression of these molecules, and therefore may dampen the immune response against breast cancer. Immunotherapy targeting T cell coinhibition as monotherapy or combined with standard therapies are in early stages of clinical development, but hold great promise for treatment of human breast cancer.

Introduction

Despite the development of more effective cytotoxic, hormonal, and anti-HER2 directed therapies for treatment of breast cancer, metastatic disease remains incurable, and one third of women with localized disease will develop metastases and die of the disease (Newman, 2009). Treatment options are particularly limited for patients with “triple negative” breast cancer (TNBC), defined as tumors lacking the expression of the estrogen receptor (ER) and progesterone receptor (PR) and the over-expression of human epidermal growth factor receptor 2 (HER2/neu) protein or HER2/neu gene amplification. TNBC accounts for about 15% of all breast cancers in the United States, and occurs more commonly in younger women and women of black race or Hispanic ethnicity (Brown *et al.*, 2008). TNBC is associated with a higher risk of distant recurrence, earlier time to recurrence, and worse prognosis after recurrence (Liedtke *et al.*, 2008). New therapeutic strategies are therefore needed for breast cancer in general and TNBC in particular. Immunotherapy approaches offer the hope of activating a patient’s immune system to specifically target and eliminate

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cancer cells even once they have metastasized to distal organs. However, this hope is not reflected by the current reality. The slow pace of progress is likely due to incomplete understanding of immune regulation in the context of cancer, but this understanding is now advancing and being translated into improved anti-tumor immunotherapy. In this review we discuss some of the developing T cell-based immunotherapy strategies in breast cancer, the role of T cell coinhibition in breast cancer outcomes and efficacy of immunological intervention, and current progress in coinhibition blockade as T cell immunotherapy.

T Cell-based Immunotherapy for Breast Cancer

Various immunotherapeutic strategies have been tested in preclinical and clinical settings for the treatment of breast cancer. Among these are vaccines, adoptive T cell transfer, cytokine therapy, and others. Perhaps most successful are “passive immunotherapy” antibodies such as the HER-2/neu blocking antibody trastuzumab (Herceptin, Genentech) which was FDA approved in 1998 and became a standard treatment for HER-2/neu-positive tumors, and which may mediate its antitumor effects in part via antibody dependent cellular cytotoxicity (Goldenberg, 1999). While all treatments likely interact with the T cell arm of the immune system, we focus here specifically on T cell-based immunotherapy and ultimately the role of coinhibition of T cells in disease progression and treatment.

One avenue of T cell immunotherapy in breast cancer is the adoptive transfer of lymphocytes into patients. Such approaches include the transfer of immune cells not specific for breast cancer and transfer of tumor-specific T cells. In a number of non-specific transfer studies, breast cancer patients were treated with allogeneic stem cell transplants in addition to high dose chemotherapy with the goal of inducing graft versus tumor (GVT) responses through the induction of graft versus host disease (GVHD) (reviewed in Wright, 2012). Some patients achieved complete or partial remissions associated with GVHD, including, in some cases, long-term survival (Carella *et al.*, 2005; de Souza *et al.*, 2009; Ueno *et al.*, 1998). However, treatment was also associated with relatively high morbidity and mortality due to GVHD (Bishop *et al.*, 2004; de Souza *et al.*, 2009; Ueno *et al.*, 1998). Adoptive transfer of autologous lymphocytes conditioned *in vitro* has been better tolerated by patients. Prior studies have shown that administration of high-dose interleukin-2 in combination with lymphocyte-activated killer cells were ineffective in breast cancer (Sparano *et al.*, 1994). Currently in clinical trials, these approaches enrich the patient’s own T cells for specificity to particular breast cancer associated antigens and stimulate them before returning them to the patient. In the first reported study of such, T cells from a HER-2+ breast cancer patient were isolated and stimulated with HER-2 peptide-loaded dendritic cells *in vitro* and then reinfused to the patient. These T cells successfully eliminated cancer cells disseminated in the bone marrow, but could not penetrate solid metastases (Bernhard *et al.*, 2008). In another study, cytotoxic T lymphocytes (CTLs) isolated from peripheral blood mononuclear cells (PMBC) of four breast cancer patients were stimulated with MUC1 peptides before return to the patients. The therapy was safe but there were no tumor regressions among the two patients that had macroscopic disease. However, in the two patients that were in remission, CTLs isolated after stimulation and reinfusion displayed higher tumor-specific lysis *ex vivo* than those isolated originally before stimulation (Wright *et al.*, 2009). Attempts to improve the efficacy of adoptive T cell treatments through different *in vitro* activation protocols and

combination with chemotherapy or vaccines are in active or planned clinical trials (clinicaltrials.gov: NCT01147016, NCT01219907, and NCT01022138).

In addition to adoptive T cell therapy, breast cancer vaccines aiming to generate effective anti-tumor CTL responses are in development and clinical trials. These vaccines utilize antigens aberrantly expressed in breast cancer including HER-2/neu, MUC1, carcinoembryonic antigen (CEA), and alpha-lactalbumin (Florescu *et al.*, 2011), administered with adjuvant or via viral vectors, tumor cells, dendritic cells, or DNA. Vaccines have demonstrated safety and limited success in small, early trials but there is still no definitive evidence of survival advantage in larger trials. In a phase I trial that included some breast cancer patients, a pox viral vaccine combining CEA with 3 costimulatory molecules (TRICOM) had modest results including stabilization of disease among 40% of patients (Marshall *et al.*, 2005). A pilot study vaccinating breast cancer patients with a similar TRICOM vaccine including both CEA and MUC1 peptides and involving alternating vaccinia-based and fowlpox-based booster vaccines (PANVACVF, Therion Biologics) was associated with both CD8 and CD4 T cell responses and one breast cancer patient had a greater than 20% reduction in size of a liver metastasis (Gulley *et al.*, 2008). A phase II trial showed an overall survival advantage in patients receiving a MUC1 vaccine (Miles and Papazisis, 2003), but no overall survival advantage was found in a larger phase III trial (Ibrahim *et al.*, 2004). Phase I trials for dendritic cells-based HER-2/neu vaccine Lapuleucel-T were safe and showed T cell proliferative and IFN-γ responses, some instances of stable disease, and one partial response (Park *et al.*, 2007; Peethambaram *et al.*, 2009). Increased survival and reduced recurrence were observed in an ongoing phase I/II clinical trial for a HER-2/neu vaccine composed of peptide and GM-CSF [(Peoples *et al.*, 2005), clinicaltrials.gov: NCT00854789].

While many studies seeking to develop effective adoptive T cell therapy and vaccines are underway, clinical success has been limited and the immunotherapy endeavor has overall been somewhat frustrating. This is probably due to a variety of tumor-induced T cell suppressive mechanisms that operate in the context of breast cancer and can undermine even well-designed immunotherapy approaches.

T Cell Coinhibition in Breast Cancer: Immune Evasion Mechanisms

Breast cancers are very ineffective in stimulating effective immune responses despite expressing antigens recognizable by their host's immune system. Tumors escape immune surveillance by several mechanisms including self-tolerance, loss of tumor specific antigens due to epigenetic changes, tumor induced immune suppression, and other mechanisms (Manjili and Payne, 2012). In this section we will discuss breast tumor-induced immune suppression and specifically, the ability of tumors to suppress the T cell response through T cell coinhibition.

Activation of a T cell requires two signals: the presentation of specific antigen on the major histocompatibility complex (MHC) of an antigen presenting cell (APC) (signal 1), together with a costimulatory signal delivered by the APC to the T cell (signal 2). Costimulation is mainly generated by signaling between members of the B7/CD28 families, classically, B7-1

or B7-2 ligands on the APC binding to CD28 on the T cell (Scandiuzzi *et al.*, 2011). Upon initial T cell activation, additional costimulatory as well as coinhibitory signals follow, and the integration of these signals determine the outcome of the T cell response. The first coinhibitory member of the B7/CD28 families discovered was cytotoxic T lymphocyte antigen 4 (CTLA-4/CD152), a homolog of CD28 which is upregulated in activated T cells and strongly binds B7-1 and B7-2, serving to reduce T cell activation through direct inhibitory signaling and opposition of CD28 binding (Krummel and Allison, 1995; Linsley *et al.*, 1991). Over the past number of years, additional members of the B7 family have been identified, including costimulatory B7h (ICOS-L/CD275) which binds ICOS (Yoshinaga *et al.*, 1999), coinhibitory PD-L1 (B7-H1/CD274) (Freeman *et al.*, 2000) and PD-L2 (B7-DC/CD273) which bind PD-1 (Latchman *et al.*, 2001), B7-H3 (CD276) (Chapoval *et al.*, 2001) which can be both costimulatory and coinhibitory, and inhibitory B7x (B7-H4/B7S1) (Prasad *et al.*, 2003; Sica *et al.*, 2003; Zang *et al.*, 2003). In contrast to B7-1 and B7-2, whose expression is limited to lymphoid organs, PD-L1, B7-H3, and B7x can be expressed in non-lymphoid organs as well as on tumor cells in various cancers, where they are thought to contribute to tumor immune evasion. The balance of costimulation and coinhibition is an important checkpoint in T cell function. In breast cancer, the balance of costimulation and coinhibition appears skewed towards coinhibition due to dysregulation of the expression of several B7 and CD28 family members.

CTLA-4 gene polymorphisms in human breast cancer

CTLA-4 expression on T cells is induced by antigenic stimulation while regulatory T cells constitutively express CTLA-4. CTLA-4 gene polymorphisms were found to be associated with breast cancer risk in a comprehensive meta-analysis (Zhang *et al.*, 2011) and in the Chinese Han population (Li *et al.*, 2012). Studies have revealed increased soluble CTLA-4 in plasma and increased FoxP3 and CTLA-4 transcripts in peripheral blood mononuclear cells of breast cancer patients as compared to normal controls (Jaberipour *et al.*, 2010). All of these studies indicate that there is a certain degree of immune dysregulation in breast cancer patients involving CTLA-4, not only in the tumor microenvironment but also possibly extending to the systemic immune system. Still, the extent to which CTLA-4 contributes to tumor immunity in breast cancer is currently uncertain.

PD-L1 expression in human breast cancer

PD-L1 is not expressed in normal breast tissue but is highly expressed in breast cancer tissue samples and breast cancer cell lines. PD-L1 polymorphisms are associated with sporadic breast cancer in the Chinese Han population (Hua *et al.*, 2011), but the significance of this association is not yet known. The expression of PD-L1 was studied in 44 patients with breast cancer and was correlated to clinicopathological parameters (Ghebeh *et al.*, 2006). Tumor expression of PD-L1 is associated with Grade III, estrogen (ER)/progesterone receptor (PR) negative tumors. When PD-L1 expression was evaluated in tumor infiltrating lymphocytes, a higher expression was associated with larger size, Grade III and HER2/neu positive tumors. It was also reported that tumor PD-L1 expression and Foxp3+ regulatory T cell (Treg) infiltration were highly correlated in breast cancer tissue specimens (Ghebeh *et al.*, 2008). Moreover the presence of Foxp3+ Tregs, tumor PD-L1 expression, and PD-1+ tumor infiltrating lymphocytes was correlated with a high histological grade, ER negative

status, and severe lymphocyte infiltration. Taken together, these observations suggest that the expression of PD-L1 by the tumor or PD-1/PD-L1 by the tumor infiltrating lymphocytes may have a role in modulating the immune response. Furthermore, PD-L1 expression is increased in tumors that have a higher proliferation index as measured by Ki-67 and in cell cultures PD-L1 expression was down-regulated in cells in a quiescent state (Ghebeh *et al.*, 2007). These observations suggest that the PD-L1/PD-1 pathway may be more important in certain breast cancer subtypes like triple negative breast cancers which have a higher proliferation rate, higher grade, and have a high lymphocytic response while in low grade tumors these molecules may play a role in the later stages contributing to their invasiveness.

Loss of the tumor suppressor gene phosphatase and tensin homolog (PTEN) in gliomas results in activation of the phosphatidylinositol 3-kinase (PI3k) pathway and increased PD-L1 expression (Parsa *et al.*, 2007), and activation of the PI3k pathway mediated via PI3k mutations or PTEN loss is also known to be common in breast cancer. In breast and prostate cancer cell lines, treatment with PI3/Akt inhibitors resulted in down-regulation of PD-L1 expression and a reversal of their immunoresistant phenotype (Crane *et al.*, 2009). In anaplastic lymphoma kinase (ALK)+ T cell lymphoma, the chimeric tyrosine kinase from the nucleophosmin/ALK translocation results in increased expression of PD-L1 by activating the transcription activator STAT3 (Marzec *et al.*, 2008). Thus, oncogenic pathways may be directly involved in the up-regulation of PD-L1, contributing to immune resistance, and blockade of the PD-1 signal transduction pathway can reverse immune resistance. The effects of chemotherapeutic agents on PD-L1 expression were studied and it was shown that doxorubicin down-regulates PD-L1 expression on the cell surface but up-regulates its expression in the nucleus, and this up-regulation of PDL1 was inhibited by blocking the PI3/AKT pathway (Ghebeh *et al.*, 2010). In contrast, other chemotherapeutic agents like paclitaxel, etoposide, and fluorouracil have been shown to up-regulate PD-L1 expression which contributes to immune resistance (Hasan *et al.*, 2011; Zhang *et al.*, 2008). These observations show that chemotherapy can affect immune resistance and with the advent of neoadjuvant chemotherapy as a standard treatment, this provides us a window of opportunity to study the effects of chemotherapy on these molecules in pre- and post-treatment. Moreover this principle is also important when evaluating monoclonal antibodies against PD-L1 in clinical trials, as these might have differential effects depending on whether these are evaluated in patients who have received anthracycline or taxane based chemotherapy, both of which are used in breast cancer.

B7-H3 expression in human breast cancer

B7- H3 is expressed in breast cancer cell lines and tissue samples but not in normal breast tissue (Arigami *et al.*, 2010). The receptor for this ligand is still unknown. Currently, the majority of evidence in humans suggests a predominantly coinhibitory role for B7-H3, and in cancers its expression has been associated with worse outcomes (Crispen *et al.*, 2008; Zang *et al.*, 2007). B7-H3 expression was evaluated in breast tumors of 82 breast cancer patients using quantitative real-time reverse transcription-polymerase chain reaction and immunohistochemistry. Expression of B7-H3 correlated with increased tumor size, lymphovascular invasion, and sentinel lymph node and overall lymph node metastasis, but was not significantly associated with tumor grade, ER/PR, or Her2 status. These

observations suggest that B7-H3 may play a more important role in tumor metastases, rather than the early phases of the tumor immune response. *In vitro* silencing of B7-H3 by siRNA resulted in increased sensitivity of breast cancer cell lines to paclitaxel (Liu *et al.*, 2011) by interfering with the Jak/Stat3 pathway. Overall the pattern of B7-H3 expression in breast cancer tissue samples appears to be different from PD-L1 and whether these molecules play important roles in different stages of the disease needs to be determined.

B7x gene polymorphisms and protein expression in human breast cancer

B7x [B7-H4/B7-S1] is a protein of the B7 family with a yet-unidentified receptor expressed on activated T cells (Prasad *et al.*, 2003; Sica *et al.*, 2003; Zang *et al.*, 2003). B7x inhibits CD4 and CD8 T cell proliferation and cytokine production (Zang *et al.*, 2003). A study identified three single nucleotide polymorphisms (SNPs) in untranslated regions of the B7x gene which were associated with rates of sporadic breast cancer in a Chinese Han population (Zhang *et al.*, 2009). These SNPs may affect the efficiency of cellular production of B7x rather than the function of the protein itself. Two SNPs were associated with a decreased risk of cancer, while one SNP was linked to a greater incidence of cancer, increased lymph node metastasis, and altered steroid hormone receptor status. It was reported that knockdown of B7x expression in breast cancer cell lines *in vitro* by siRNA leads to increased caspase activity and apoptosis of tumor cells (Salceda *et al.*, 2005). Higher expression of B7x was found in breast and ovarian cancers as compared to their corresponding normal tissues (Qian *et al.*, 2011), indicating that B7x may be more important in tumor biology in these cancers. In contrast to other coinhibitory molecules, B7x can be expressed at low levels in normal breast tissue epithelium (Tringler *et al.*, 2005). In a study of 419 cases of human breast cancer, more than 95% of tissue samples expressed B7x (Tringler *et al.*, 2005). Increased staining intensity correlated with PR negative status and neoadjuvant chemotherapy while the proportion of B7x positive cells was correlated with PR negative and Her2 negative tumors. These observations suggest that B7x expression is highly up-regulated in breast cancer and that B7x may be a useful diagnostic marker and potential therapeutic target.

Blockade of T Cell Coinhibition for Breast Cancer Therapy

After decades of evaluation of various immune therapies, researchers have finally achieved some therapeutic success with blockade of T cell coinhibitory molecules in the past few years. The proof of principle for blockade of coinhibitory therapy in humans came from preclinical mouse models (Leach *et al.*, 1996) and clinical studies in metastatic melanoma showing tumor regression and an improvement in overall survival after treatment with the anti-CTLA-4 antibody (Downey *et al.*, 2007; Hodi, 2007; Hodi *et al.*, 2003; Phan *et al.*, 2003; Robert *et al.*, 2011). CTLA-4 and PD-1 antibodies have produced some impressive results in the few cases of durable remission seen in select patients treated with these antibodies, proving that stimulating the immune system can effectively induce long standing anti-tumor immunity even in advanced cancers. These drugs can also have adverse autoimmune side effects including pneumonitis, vitiligo, colitis, hepatitis, hypophysitis, sarcoidosis, endophthalmitis, diabetes mellitus, and myasthenia gravis. Currently there are no proven biomarkers available to predict response to immune therapy, although in a recent

trial, expression of PD-L1 ligand in tumor specimens shows promise as a biomarker (Topalian *et al.*, 2012). The results of some T cell coinhibitory blockade clinical trials are discussed below.

Blockade of CTLA-4 in human breast cancer

Initial enthusiasm for CTLA-4 antibody based therapy in breast cancer was generated from mouse models in which mice treated with a GM-CSF-expressing vaccine followed by anti-CTLA-4 antibody rejected SM1 cell line-induced mammary carcinoma and were also immune to subsequent re-challenge with the same cell line (Hurwitz *et al.*, 1998). Following this, Tremelimumab (CP-675,206 or ticilimumab; Pfizer) a fully human IgG2 anti-CTLA-4 monoclonal antibody that blocks the binding of CTLA-4 to B7-1 and B7-2 was tested in combination with exemestane, an aromatase inhibitor that blocks the production of estrogen (Vonderheide *et al.*, 2010). In this phase 1 study, 26 patients with advanced hormone receptor positive breast cancer were treated with tremelimumab (3–10 mg/kg) every 28 days or every 90 days along with exemestane 25 mg orally daily. Since other studies suggested that there was an increase in diarrhea with the 28 day dosing schedule, this was discontinued later in the trial. Dose limiting toxicities were liver dysfunction and diarrhea and the maximum tolerated dose was 6 mg/kg every 90 days. The overall response rate (ORR) was stable disease in 11 patients (42%). Treatment was associated with increase in the ratio of ICOS+ CD4 and CD8 T cells when compared with Foxp3+ Treg cells, suggesting enhanced cellular immune function. In another phase II trial investigating Ipilimumab (MDX-010, Yervoy; Bristol-Myers Squibb) at 3 mg/kg in subjects with metastatic breast cancer (clinicaltrials.gov: NCT0083278), enrollment was stopped early as there were no objective responses as defined by the RECIST criteria in 31 subjects who were treated. Trials which combine ipilimumab with cryoablation to prevent recurrence before mastectomy are in progress (clinical-trials. gov: NCT01502592). These clinical trials show that CTLA-4 inhibition alone is not an effective approach in breast cancer.

Blockade of PD-1/PD-L1 pathway

In a phase 1 study, the anti-PD-1 antibody (BMS-936558, Bristol-Myers Squibb) was evaluated in 296 patients with advanced refractory solid tumors — non-small cell lung, renal, melanoma, colon, and castration resistant prostate cancer — and was studied at different doses of 1.0, 3.0, or 10.0 mg per kilogram of body weight (Topalian *et al.*, 2012). The maximum tolerated dose (MTD) was not reached and the common side effects were skin rash, diarrhea, and pruritus. Grade 3 or 4 adverse events included diarrhea, pneumonitis, and liver dysfunction. The ORR was 18% in patients with non-small cell lung cancer, 28% in melanoma, and 27% in patients with renal cell cancer with some durable responses. Of note, 36% of patients whose tumors expressed PD-L1 (defined as >5%) demonstrated an objective response while none of the patients whose tumors did not express PD-L1 demonstrated a response. In another phase 1 study the anti-PD-L1 antibody (BMS-936559, Bristol-Myers Squibb) was evaluated to assess the safety and efficacy in advanced cancers (Brahmer *et al.*, 2012). A total of 207 patients were treated at increasing dose levels from 0.3 to 10 mg/kg every 14 days in 6-week cycles for up to 16 cycles. The ORR was 6 to 17% in various tumors with median response duration equal to or greater than 24 weeks, but none of the four breast cancer patients treated had a response. With respect to

breast cancer patients, the absence of response noted may be due to the small number of patients treated in this study (n=4) or they might have had PD-L1 negative breast cancer as we know not all breast cancers express PD-L1. Another PD -L1 antibody (MPDL3280A) is currently being evaluated in advanced solid cancers along with bevacizumab (clinicaltrials.gov: NCT01633970).

Blockade of the B7-H3 pathway

A phase 1 trial of anti B7-H3 antibody (MGA 271) is underway in the treatment of refractory solid tumors (clinicaltrials.gov: NCT01391143) (Seliger and Quandt, 2012).

Concluding Remarks

The past few years have witnessed a new era in the understanding of tumor immunity with the discovery of the B7 family of coinhibitory molecules and their functions. But we are still in the early stages of understanding B7 coinhibitory molecule expression and co-expression in various tumors, mechanisms of signal transduction, and the interaction of these pathways with other oncogenic pathways contributing to tumor immunity. Even though breast cancer therapies have improved in the past decade, treatment of certain breast cancer subtypes like TNBC remains challenging and currently the cure rates for metastatic disease are dismal. T cell immunotherapy has shown some success in metastatic disease treatment, but progress is likely hindered by cancer-induced immunosuppression including T cell coinhibition. While still in development, coinhibition blockade, likely in combination with other T cell immunotherapies as well as chemotherapies, may prove to be an effective approach in breast cancer therapy.

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References

Arigami T, Narita N, Mizuno R, Nguyen L, Ye X, Chung A, Giuliano AE, Hoon DS. B7-H3 ligand expression by primary breast cancer and associated with regional nodal metastasis. Ann Surg. 2010; 252(6):1044–1051. [PubMed: 21107115]

Bernhard H, Neudorfer J, Gebhard K, Conrad H, Hermann C, Nahrig J, Fend F, Weber W, Busch DH, Peschel C. Adoptive transfer of autologous, HER2-specific, cytotoxic T lymphocytes for the treatment of HER2-overexpressing breast cancer. Cancer Immunol Immunother. 2008; 57(2):271–280. [PubMed: 17646988]

Bishop MR, Fowler DH, Marchigiani D, Castro K, Kasten-Sportes C, Steinberg SM, Gea-Banacloche JC, Dean R, Chow CK, Carter C, Read EJ, Leitman S, Gress R. Allogeneic lymphocytes induce tumor regression of advanced metastatic breast cancer. J Clin Oncol. 2004; 22(19):3886–3892. [PubMed: 15314059]

Brahmer JR, Tykodi SS, Chow LQ, Hwu WJ, Topalian SL, Hwu P, Drake CG, Camacho LH, Kauh J, Odunsi K, Pitot HC, Hamid O, Bhatia S, Martins R, Eaton K, Chen S, Salay TM, Alaparthy S, Grosso JF, Korman AJ, et al. Safety and activity of anti-PD-L1 antibody in patients with advanced cancer. N Engl J Med. 2012; 366(26):2455–2465. [PubMed: 22658128]

Brown M, Tsodikov A, Bauer KR, Parise CA, Caggiano V. The role of human epidermal growth factor receptor 2 in the survival of women with estrogen and progesterone receptor-negative,

invasive breast cancer: The California Cancer Registry, 1999–2004. Cancer. 2008; 112(4):737–747. [PubMed: 18189290]

Carella AM, Beltrami G, Corsetti MT, Nati S, Musto P, Scalzulli P, Gonella R, Ballestrero A, Patrone F. Reduced intensity conditioning for allograft after cytoreductive autograft in metastatic breast cancer. Lancet. 2005; 366(9482):318–320. [PubMed: 16039336]

Chapoval AI, Ni J, Lau JS, Wilcox RA, Flies DB, Liu D, Dong H, Sica GL, Zhu G, Tamada K, Chen L. B7-H3: a costimulatory molecule for T cell activation and IFN-gamma production. Nat Immunol. 2001; 2(3):269–274. [PubMed: 11224528]

Crane CA, Panner A, Murray JC, Wilson SP, Xu H, Chen L, Simko JP, Waldman FM, Pieper RO, Parsa AT. PI(3) kinase is associated with a mechanism of immunoresistance in breast and prostate cancer. Oncogene. 2009; 28(2):306–312. [PubMed: 18850006]

Crispen PL, Sheinin Y, Roth TJ, Lohse CM, Kuntz SM, Frigola X, Thompson RH, Boorjian SA, Dong H, Leibovich BC, Blute ML, Kwon ED. Tumor cell and tumor vasculature expression of B7-H3 predict survival in clear cell renal cell carcinoma. Clin Cancer Res. 2008; 14(16):5150–5157. [PubMed: 18694993]

De Souza JA, Davis ML, Rondon G, Cheng YC, Jones RB, Champlin RE, Ueno NT. Prolonged disease control by nonmyeloablative allogeneic transplantation for metastatic breast cancer. Bone Marrow Transplant. 2009; 44(2):81–87. [PubMed: 19448681]

Downey SG, Klapper JA, Smith FO, Yang JC, Sherry RM, Royal RE, Kammula US, Hughes MS, Allen TE, Levy CL, Yellin M, Nichol G, White DE, Steinberg SM, Rosenberg SA. Prognostic factors related to clinical response in patients with metastatic melanoma treated by CTL-associated antigen-4 blockade. Clin Cancer Res. 2007; 13(22 Pt 1):6681–6688. [PubMed: 17982122]

Florescu A, Amir E, Bouganim N, Clemons M. Immune therapy for breast cancer in 2010-hype or hope? Curr Oncol. 2011; 18(1):e9–e18. [PubMed: 21331271]

Freeman GJ, Long AJ, Iwai Y, Bourque K, Chernova T, Nishimura H, Fitz LJ, Malenkovich N, Okazaki T, Byrne MC, Horton HF, Fouser L, Carter L, Ling V, Bowman MR, Carreno BM, Collins M, Wood CR, Honjo T. Engagement of the PD-1 immunoinhibitory receptor by a novel B7 family member leads to negative regulation of lymphocyte activation. J Exp Med. 2000; 192(7):1027–1034. [PubMed: 11015443]

Ghebeh H, Barhoush E, Tulbah A, Elkum N, Al-Tweigeri T, Dermime S. FOXP3+ Tregs and B7-H1+/PD-1+ T lymphocytes coinfiltrate the tumor tissues of high-risk breast cancer patients: Implication for immunotherapy. BMC Cancer. 2008; 8:57. [PubMed: 18294387]

Ghebeh H, Lehe C, Barhoush E, Al-Romaih K, Tulbah A, Al-Alwan M, Hendrayani SF, Manogaran P, Alaiya A, Al-Tweigeri T, Aboussekhra A, Dermime S. Doxorubicin downregulates cell surface B7-H1 expression and upregulates its nuclear expression in breast cancer cells: role of B7-H1 as an anti-apoptotic molecule. Breast Cancer Res. 2010; 12(4):R48. [PubMed: 20626886]

Ghebeh H, Mohammed S, Al-Omair A, Qattan A, Lehe C, Al- Qudaihi G, Elkum N, Alshabanah M, Bin Amer S, Tulbah A, Ajarim D, Al-Tweigeri T, Dermime S. The B7-H1 (PD-L1) T lymphocyteinhibitory molecule is expressed in breast cancer patients with infiltrating ductal carcinoma: correlation with important high-risk prognostic factors. Neoplasia. 2006; 8(3):190–198. [PubMed: 16611412]

Ghebeh H, Tulbah A, Mohammed S, Elkum N, Bin Amer SM, Al-Tweigeri T, Dermime S. Expression of B7-H1 in breast cancer patients is strongly associated with high proliferative Ki-67-expressing tumor cells. Int J Cancer. 2007; 121(4):751–758. [PubMed: 17415709]

Goldenberg MM. Trastuzumab, a recombinant DNA-derived humanized monoclonal antibody, a novel agent for the treatment of metastatic breast cancer. Clin Ther. 1999; 21(2):309–318. [PubMed: 10211534]

Gulley JL, Arlen PM, Tsang KY, Yokokawa J, Palena C, Poole DJ, Remondo C, Cereda V, Jones JL, Pazdur MP, Higgins JP, Hodge JW, Steinberg SM, Kotz H, Dahut WL, Schlom J. Pilot study of vaccination with recombinant CEA-MUC-1-TRICOM poxviral-based vaccines in patients with metastatic carcinoma. Clin Cancer Res. 2008; 14(10):3060–3069. [PubMed: 18483372]

Hasan A, Ghebeh H, Lehe C, Ahmad R, Dermime S. Therapeutic targeting of B7-H1 in breast cancer. Expert Opin Ther Targets. 2011; 15(10):1211–1225. [PubMed: 21870995]

Hodi FS. Cytotoxic T-lymphocyte-associated antigen-4. Clin Cancer Res. 2007; 13(18 Pt 1):5238–5242. [PubMed: 17875750]

Hodi FS, Mihm MC, Soiffer RJ, Haluska FG, Butler M, Seiden MV, Davis T, Henry-Spires R, Macrae S, Willman A, Padera R, Jaklitsch MT, Shankar S, Chen TC, Korman A, Allison JP, Dranoff G. Biologic activity of cytotoxic T lymphocyte-associated antigen 4 antibody blockade in previously vaccinated metastatic melanoma and ovarian carcinoma patients. Proc Natl Acad Sci U S A. 2003; 100(8):4712–4717. [PubMed: 12682289]

Hua Z, Li D, Xiang G, Xu F, Jie G, Fu Z, Jie Z, Da P. PD-1 polymorphisms are associated with sporadic breast cancer in Chinese Han population of Northeast China. Breast Cancer Res Treat. 2011; 129(1):195–201. [PubMed: 21487727]

Hurwitz AA, Yu TF, Leach DR, Allison JP. CTLA-4 blockade synergizes with tumor-derived granulocyte-macrophage colony-stimulating factor for treatment of an experimental mammary carcinoma. Proc Natl Acad Sci U S A. 1998; 95(17):10067–10071. [PubMed: 9707601]

Ibrahim NK, Murray J, Parker J, Finke L, Miles D. Group OBOTTS. Humoral immune responses to naturally occurring STn in metastatic breast cancer patients treated with STn-klh vaccine [abstract 2547]. J Clin Oncol (2004 ASCO Annual Meeting Proceedings). 2004; 22(14S):2547.

Jaberipour M, Habibagahi M, Hosseini A, Habibabad SR, Talei A, Ghaderi A. Increased CTLA-4 and FOXP3 transcripts in peripheral blood mononuclear cells of patients with breast cancer. Pathol Oncol Res. 2010; 16(4):547–551. [PubMed: 20306312]

Krummel MF, Allison JP. CD28 and CTLA-4 have opposing effects on the response of T cells to stimulation. J Exp Med. 1995; 182(2):459–465. [PubMed: 7543139]

Latchman Y, Wood CR, Chernova T, Chaudhary D, Borde M, Chernova I, Iwai Y, Long AJ, Brown JA, Nunes R, Greenfield EA, Bourque K, Boussiotis VA, Carter LL, Carreno BM, Malenkovich N, Nishimura H, Okazaki T, Honjo T, Sharpe AH, et al. PD-L2 is a second ligand for PD-1 and inhibits T cell activation. Nat Immunol. 2001; 2(3):261–268. [PubMed: 11224527]

Leach DR, Krummel MF, Allison JP. Enhancement of antitumor immunity by CTLA-4 blockade. Science. 1996; 271(5256):1734–1736. [PubMed: 8596936]

Li D, Zhang Q, Xu F, Fu Z, Yuan W, Pang D. Association of CTLA-4 gene polymorphisms with sporadic breast cancer risk and clinical features in Han women of northeast China. Mol Cell Biochem. 2012; 364(1–2):283–290. [PubMed: 22249287]

Liedtke C, Mazouni C, Hess KR, André F, Tordai A, Mejia JA, Symmans WF, Gonzalez-Angulo AM, Hennessy B, Green M, Cristofanilli M, Hortobagyi GN, Pusztai L. Response to neoadjuvant therapy and long-term survival in patients with triple-negative breast cancer. J Clin Oncol. 2008; 26(8):1275–1281. [PubMed: 18250347]

Linsley PS, Brady W, Urnes M, Grosmaire LS, Damle NK, Ledbetter JA. CTLA-4 is a second receptor for the B cell activation antigen B7. J Exp Med. 1991; 174(3):561–569. [PubMed: 1714933]

Liu H, Tekle C, Chen YW, Kristian A, Zhao Y, Zhou M, Liu Z, Ding Y, Wang B, Maelandsmo GM, Nesland JM, Fodstad O, Tan M. B7-H3 silencing increases paclitaxel sensitivity by abrogating Jak2/Stat3 phosphorylation. Mol Cancer Ther. 2011; 10(6):960–971. [PubMed: 21518725]

Manjili MH, Payne KK. Cancer immunotherapy: Re-programming cells of the innate and adaptive immune systems. Oncoimmunology. 2012; 1(2):201–204. [PubMed: 22720242]

Marshall JL, Gulley JL, Arlen PM, Beetham PK, Tsang KY, Slack R, Hodge JW, Doren S, Grosenbach DW, Hwang J, Fox E, Odogwu L, Park S, Panicali D, Schlom J. Phase I study of sequential vaccinations with fowlpox-CEA(6D)-TRICOM alone and sequentially with vaccinia-CEA(6D)-TRICOM, with and without granulocytemacrophage colony-stimulating factor, in patients with carcinoembryonic antigen-expressing carcinomas. J Clin Oncol. 2005; 23(4):720–731. [PubMed: 15613691]

Marzec M, Zhang Q, Goradia A, Raghunath PN, Liu X, Paessler M, Wang HY, Wysocka M, Cheng M, Ruggeri BA, Wasik MA. Oncogenic kinase NPM/ALK induces through STAT3 expression of immunosuppressive protein CD274 (PD-L1, B7-H1). Proc Natl Acad Sci U S A. 2008; 105(52): 20852–20857. [PubMed: 19088198]

Miles D, Papazisis K. Rationale for the clinical development of STn-KLH (Theratope) and anti-MUC-1 vaccines in breast cancer. Clin Breast Cancer. 2003; 3(Suppl 4):S134–S138. [PubMed: 12620150]

Newman LA. Epidemiology of locally advanced breast cancer. Semin Radiat Oncol. 2009; 19(4):195–203. [PubMed: 19732683]

Park JW, Melisko ME, Esserman LJ, Jones LA, Wollan JB, Sims R. Treatment with autologous antigen-presenting cells activated with the HER-2 based antigen Lapuleucel-T: results of a phase I study in immunologic and clinical activity in HER-2 overexpressing breast cancer. J Clin Oncol. 2007; 25(24):3680–3687. [PubMed: 17704416]

Parsa AT, Waldron JS, Panner A, Crane CA, Parney IF, Barry JJ, Cachola KE, Murray JC, Tihan T, Jensen MC, Mischel PS, Stokoe D, Pieper RO. Loss of tumor suppressor PTEN function increases B7-H1 expression and immunoresistance in glioma. Nat Med. 2007; 13(1):84–88. [PubMed: 17159987]

Peethambaram PP, Melisko ME, Rinn KJ, Alberts SR, Provost NM, Jones LA, Sims RB, Lin LR, Frohlich MW, Park JW. A phase I trial of immunotherapy with lapuleucel-T (APC8024) in patients with refractory metastatic tumors that express HER-2/neu. Clin Cancer Res. 2009; 15(18): 5937–5944. [PubMed: 19723649]

Peoples GE, Gurney JM, Hueman MT, Woll MM, Ryan GB, Storrer CE, Fisher C, Shriver CD, Ioannides CG, Ponniah S. Clinical trial results of a HER2/neu (E75) vaccine to prevent recurrence in high-risk breast cancer patients. J Clin Oncol. 2005; 23(30):7536–7545. [PubMed: 16157940]

Phan GQ, Yang JC, Sherry RM, Hwu P, Topalian SL, Schwartzentruber DJ, Restifo NP, Haworth LR, Seipp CA, Freezer LJ, Morton KE, Mavroukakis SA, Duray PH, Steinberg SM, Allison JP, Davis TA, Rosenberg SA. Cancer regression and autoimmunity induced by cytotoxic T lymphocyte-associated antigen 4 blockade in patients with metastatic melanoma. Proc Natl Acad Sci U S A. 2003; 100(14):8372–8377. [PubMed: 12826605]

Prasad DV, Richards S, Mai XM, Dong C. B7S1, a novel B7 family member that negatively regulates T cell activation. Immunity. 2003; 18(6):863–873. [PubMed: 12818166]

Qian Y, Shen L, Cheng L, Wu Z, Yao H. B7-H4 expression in various tumors determined using a novel developed monoclonal antibody. Clin Exp Med. 2011; 11(3):163–170. [PubMed: 21190056]

Robert C, Thomas L, Bondarenko I, O'Day S, M DJ, Garbe C, Lebbe C, Baurain JF, Testori A, Grob JJ, Davidson N, Richards J, Maio M, Hauschild A, Miller WH Jr, Gascon P, Lotem M, Harmankaya K, Ibrahim R, Francis S, et al. Ipilimumab plus dacarbazine for previously untreated metastatic melanoma. N Engl J Med. 2011; 364(26):2517–2526. [PubMed: 21639810]

Salceda S, Tang T, Kmet M, Munteanu A, Ghosh M, Macina R, Liu W, Pilkington G, Papkoff J. The immunomodulatory protein B7-H4 is overexpressed in breast and ovarian cancers and promotes epithelial cell transformation. Exp Cell Res. 2005; 306(1):128–141. [PubMed: 15878339]

Scandiuzzi L, Ghosh K, Zang X. T cell costimulation and coinhibition: genetics and disease. Discov Med. 2011; 12(63):119–128. [PubMed: 21878189]

Seliger B, Quandt D. The expression, function, and clinical relevance of B7 family members in cancer. Cancer Immunol Immunother. 2012; 61(8):1327–1341. [PubMed: 22695874]

Sica GL, Choi IH, Zhu G, Tamada K, Wang SD, Tamura H, Chapoval AI, Flies DB, Bajorath J, Chen L. B7-H4, a molecule of the B7 family, negatively regulates T cell immunity. Immunity. 2003; 18(6):849–861. [PubMed: 12818165]

Sparano JA, Fisher RI, Weiss GR, Margolin K, Aronson FR, Hawkins MJ, Atkins MB, Dutcher JP, Gaynor ER, Boldt DH, et al. Phase II trials of high-dose interleukin-2 and lymphokine-activated killer cells in advanced breast carcinoma and carcinoma of the lung, ovary, and pancreas and other tumors. J Immunother Emphasis Tumor Immunol. 1994; 16(3):216–223. [PubMed: 7834121]

Topalian SL, Hodi FS, Brahmer JR, Gettinger SN, Smith DC, Mcdermott DF, Powderly JD, Carvajal RD, Sosman JA, Atkins MB, Leming PD, Spigel DR, Antonia SJ, Horn L, Drake CG, Pardoll DM, Chen L, Sharfman WH, Anders RA, Taube JM, et al. Safety, activity, and immune correlates of anti-PD-1 antibody in cancer. N Engl J Med. 2012; 366(26):2443–2454. [PubMed: 22658127]

Tringler B, Zhuo S, Pilkington G, Torkko KC, Singh M, Lucia MS, Heinz DE, Papkoff J, Shroyer KR. B7-H4 is highly expressed in ductal and lobular breast cancer. Clin Cancer Res. 2005; 11(5):1842–1848. [PubMed: 15756008]

Ueno NT, Rondon G, Mirza NQ, Geisler DK, Anderlini P, Giralt SA, Andersson BS, Claxton DF, Gajewski JL, Khouri IF, Korbling M, Mehra RC, Przepiorka D, Rahman Z, Samuels BI, Van Besien K, Hortobagyi GN, Champlin RE. Allogeneic peripheral-blood progenitor- cell

transplantation for poor-risk patients with metastatic breast cancer. J Clin Oncol. 1998; 16(3):986–993. [PubMed: 9508181]

Vonderheide RH, Lorusso PM, Khalil M, Gartner EM, Khaira D, Soulieres D, Dorazio P, Trosko JA, Ruter J, Mariani GL, Usari T, Domchek SM. Tremelimumab in combination with exemestane in patients with advanced breast cancer and treatment-associated modulation of inducible costimulator expression on patient T cells. Clin Cancer Res. 2010; 16(13):3485–3494. [PubMed: 20479064]

Wright SE. Immunotherapy of breast cancer. Expert Opin Biol Ther. 2012; 12(4):479–490. [PubMed: 22413825]

Wright SE, Rewers-Felkins KA, Quinlin IS, Phillips CA, Townsend M, Philip R, Zorsky P, Klug P, Dai L, Hussain M, Thomas AA, Sundaramurthy C. Tumor burden influences cytotoxic T cell development in metastatic breast cancer patients — a phase I/II study. Immunol Invest. 2009; 38(8):820–838. [PubMed: 19860591]

Yoshinaga SK, Whoriskey JS, Khare SD, Sarmiento U, Guo J, Horan T, Shih G, Zhang M, Coccia MA, Kohno T, Tafuri-Bladt A, Brankow D, Campbell P, Chang D, Chiu L, Dai T, Duncan G, Elliott GS, Hui A, McCabe SM, et al. T-cell co-stimulation through B7RP-1 and ICOS. Nature. 1999; 402(6763):827–832. [PubMed: 10617205]

Zang X, Loke P, Kim J, Murphy K, Waitz R, Allison JP. B7x: a widely expressed B7 family member that inhibits T cell activation. Proc Natl Acad Sci U S A. 2003; 100(18):10388–10392. [PubMed: 12920180]

Zang X, Thompson RH, Al-Ahmadie HA, Serio AM, Reuter VE, Eastham JA, Scardino PT, Sharma P, Allison JP. B7-H3 and B7x are highly expressed in human prostate cancer and associated with disease spread and poor outcome. Proc Natl Acad Sci U S A. 2007; 104(49):19458–19463. [PubMed: 18042703]

Zhang B, Beeghly-Fadiel A, Long J, Zheng W. Genetic variants associated with breast-cancer risk: comprehensive research synopsis, meta-analysis, and epidemiological evidence. Lancet Oncol. 2011; 12(5):477–488. [PubMed: 21514219]

Zhang J, Zhang M, Jiang W, Wang L, Fu Z, Li D, Pang D. B7-H4 gene polymorphisms are associated with sporadic breast cancer in a Chinese Han population. BMC Cancer. 2009; 9:394. [PubMed: 19903360]

Zhang P, Su DM, Liang M, Fu J. Chemopreventive agents induce programmed death-1-ligand 1 (PD-L1) surface expression in breast cancer cells and promote PD-L1-mediated T cell apoptosis. Mol Immunol. 2008; 45(5):1470–1476. [PubMed: 17920123]