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Current Status of Autologous Breast Tumor Cell-based Vaccines

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Summary

Approximately 9 of 10 breast cancer-related deaths are attributable to metastasis. Yet, less than 4% of breast cancer patients are initially diagnosed with metastatic cancer. Therefore, the majority of breast cancer-related deaths are due to recurrence and progression of nonmetastatic disease. There is tremendous clinical opportunity for novel adjuvant strategies, such as immunotherapies, that have the potential to prevent progressive recurrences. In particular, autologous tumor cell-based vaccines can train a patient's immune system to recognize and eliminate occult disease. Autologous tumor cell-based vaccines have several advantages including safety, multivalency and patient specificity. Furthermore, because lumpectomy or mastectomy is indicated for the vast majority of breast cancer patients, resected tumors offer a readily available, patient-specific source of tumor antigen. Disadvantages of autologous tumor cell-based vaccines include poor immunogenicity and production inconsistencies. This review summarizes recent progress in the development of autologous breast tumor vaccines and offers insight for overcoming existing limitations.

Current Adjuvant Therapies for Breast Cancer

In the United States, approximately 235,030 new cases of invasive breast cancer and 40,430 breast cancer-related deaths are expected in 2014 [1]. About 90% of breast cancer-related deaths are due to metastases and not the primary tumor. Considering that less than4% of new breast cancer patients are diagnosed with Stage IV metastatic cancer [2], the vast majority of the 36,000+ metastasis-related deaths are due to the recurrence and progression of non-metastatic disease.

In an effort to combat tumor recurrence, approximately 80% of patients receive adjuvant therapy such as chemotherapy [3], hormonal and/or radiotherapy following tumor resection. Chemotherapy typically includes a cocktail of drugs, such as anthracyclines or taxanes, and is indicated for patients with tumors larger than 2 cm. With adjuvant chemotherapy, patients younger than 50 experience a 10% increase in 15-year survival rate, while patients older than 50 experience only a 3% increase in survival over the same period [4]. Hormonal therapy, which includes tamoxifen or aromatase inhibitors, is indicated for patients with

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hormone receptor-positive breast cancer. In patients with estrogen receptor (ER)-positive disease, tamoxifen was found to increase the 15-year survival rate by 9.2%. Adjuvant radiotherapy is indicated for breast cancer patients with chest wall involvement or patients undergoing breast conserving surgery. A recent meta-analysis showed that radiotherapy provides a 5% decrease in 15-year breast cancer mortality risk [5].

Improvements in survival rates of breast cancer patients due to adjuvant therapies are somewhat offset by significant toxicities. Chemotherapy is associated with nausea, hair loss, mouth sores, low blood cell counts, neuropathy and secondary cancers while hormonal treatments induce fatigue, hot flashes, mood swings and blood clots. Overtreatment of breast cancer is both common and lethal. A particularly troubling recent estimate suggests that 1 to 3 deaths occur due to overtreatment for every breast cancer death avoided [6].

Even with current adjuvant therapies, 7%, 11% and 13% of Stage I, II and III patients, respectively will experience a tumor recurrence within 5 years. After 10 years, the overall breast cancer recurrence rate is about 20% [7]. These data, taken together with potentially lethal toxicities associated with current adjuvant therapies, support the need for more effective interventions to limit breast cancer recurrence and progression.

A New Era of Immunotherapy

The recent approval of the first active specific immunotherapy (ASI), Provenge® (sipuleucel-T), has energized the development of additional cancer immunotherapies and paved the way for the study of ASIs in earlier stage patients who are less immunocompromised. One such population that would benefit from ASIs in an adjuvant setting is early stage breast cancer patients. Approximately 96% of breast cancer patients are diagnosed between stages I-III. The vast majority of these patients undergo tumor resection and will be classified as having minimal residual disease. Adjuvant ASI could help stimulate tumor-specific T lymphocytes with the potential to eradicate occult lesions or micrometastases [8]. A durable memory T cell response may also protect these patients from recurrence and secondary cancers. Furthermore, ASIs are not expected to induce severe toxicities associated with current adjuvant therapies.

ASI can be accomplished using a multitude of vaccine formulations classified by the nature of the antigen and how it is delivered. Regarding antigen nature, antigens can be presented to the immune system as whole cells [9], tumor-specific or tumor-associated peptides [10,11], carbohydrates [12] or cell lysates [13]. Regarding delivery systems, antigens can be delivered via viral vectors [14], non-viral vectors [15], inactivated tumor cells [16,17], protein or peptide-pulsed antigen presenting cells [18,19], or loaded in targeted and/or controlled-release technologies [20]. In addition, each of the aforementioned ASIs can be supplemented with cytokines or other immunomodulators [9,19,21].

This review will focus on the development of autologous tumor cell-based vaccines for use against breast cancer. We will discuss advantages and disadvantages of autologous cancer vaccines, review recent clinical and preclinical data, and offer suggestions for future exploration to achieve successful clinical application. This review will not cover dendritic

cell-based immunotherapies which utilize autologous tumor cell lysates. This topic has been reviewed elsewhere [22].

Advantages of Autologous Tumor Cell-based Vaccines

Autologous tumor cell-based vaccines (ATCVs) use cells extracted from a patient's own tumor as the source of antigen. ATCVs have a number of advantages including multivalency and patient specificity. Most non-cell-based cancer vaccines are comprised of a single tumor-associated antigen (TAA) or epitope, such as human epithelial growth factor receptor-2 (HER-2) or carcinoembryonic antigen (CEA). As a result, these vaccines are susceptible to tumor escape through epitope downregulation and/or tumor heterogeneity. Antigen cascade or epitope spreading may facilitate a broader immune response capable of eliminating tumor cells which lack a particular epitope. Nevertheless, the use of a single epitope as the basis of a cancer vaccine is risky.

ATCVs contain many potential antigens, both known and unknown. Vaccines comprised of multiple epitopes potentiate a polyclonal response capable of responding to a more diverse population of tumor cells [9,21]. In addition, because all of the potential antigens are derived from a patient's own tumor, ATCVs are patient-specific. In short, ATCVs expose a patient to her complete and individualized antigen repertoire. This is particularly important as each case of breast cancer can contain up to an estimated 105 mutant genes [23]. Therefore, it is likely that no two tumors are completely alike.

An additional benefit of ATCVs is their potential for use against triple-negative breast cancer (TNBC), i.e. breast cancer that lacks expression of ER, progesterone receptor (PgR) and HER-2. Patients diagnosed with TNBC typically have: 1) larger, higher grade tumors at diagnosis; 2) fewer treatment options due to incompatibility of endocrine or HER2–directed therapies; 3) lower 5-yr survival rates, i.e. 77% for TNBC vs. 93% for other breast cancer types; and 4) higher rates of distant recurrence in the first 5 years after diagnosis. Vaccine efficacy is not expected to depend upon expression levels of hormone receptors which are not useful targets for immunotherapy. It was shown that there is no correlation between anti-tumor lymphocytic immunity and ER or PgR status [24]. HER-2 is a potential target for breast cancer immunotherapy [25]. However, other TAAs contained within ATCVs can be expected to compensate for the absence of HER-2. Therefore, ATCVs capable of eliciting patient-specific adaptive immune responses may provide a much needed treatment option for this patient population.

It should be mentioned that several studies have explored the use of allogeneic tumor cells as vaccine antigens. These were recently reviewed elsewhere [26]. Allogeneic tumor cell-based vaccines utilize cell lines that have been harvested from another individual of the same species. Since these vaccines can be mass produced in a laboratory and stored for later use, they are more economical, reproducible and easily characterized as compared to autologous vaccines. However, while allogeneic cell lines may share one or two tumor associate antigens with a patient's tumor, they do not replicate the tumor's antigen complexity which may induce undesirable selective pressure and promote tumor escape [26].

Disadvantages of Autologous Tumor Cell-based Vaccines

Recent preclinical and clinical studies have uncovered two major disadvantages of ATCVs. First, the manufacture of high quality ATCVs is cumbersome and highly variable [9]. In clinical studies involving ATCVs, the rate at which vaccines can be generated successfully from resected tumors is typically 70% to 90%. ATCVs cannot be generated due to the lack of viable resected tumor tissue or problems in the culture, expansion and/or ex vivo manipulation of tumor cells for 10 to 30% of patients. Even with a sufficient supply of tumor tissue, methods to prepare ATCVs are highly variable which may lead to differences in immunogenicity and difficulty when comparing study results. For example, tumors have been digested enzymatically with cocktails containing enzymes such as collagenase and DNAse [27] and/or mechanically disrupted using a variety of tools such as a stomachers, homogenizers, Medimachine or similar apparati [28]. Once in a single-cell suspension, tumor cells can be inactivated by irradiation [29,30], freeze-thaw cycling [28], exposure to chemotherapeutics [27] or high hydrostatic pressure [31]. Additionally, while some protocols mention separating tumor cells from other cells in the suspension, others do not [27,29,30,32]. It is not clear how each of these different preparations affect the yield and/or immunogenicity of ATCVs as the literature lacks a comparative study.

The second major disadvantage is the inherent poor immunogenicity of the tumor cell themselves. TAAs are either mutated self-antigens or overexpressed, non-mutated self-antigens against which the immune system has developed tolerance. Thus, TAAs, unlike foreign bacterial or viral proteins, do not trigger robust immune responses. In addition, many tumors downregulate their expression of major histocompatibility complex (MHC) or co-stimulatory molecules which are both needed to generate a robust immune response [33]. Delivery systems and adjuvants engineered to increase immunogenicity of ATCVs are under exploration by our lab and others.

ATCV Clinical Trials in Breast Cancer

Only three completed and two ongoing clinical trials have explored ATCVs in breast cancer (Table 1). The relative lack ATCV-based trials in breast cancer patients may be due to the wide variety of clinical studies available to this population. There are currently >1600 open clinical studies for breast cancer, more than any other cancer, listed on clinicaltrials.gov.

Due to the poor immunogenicity of tumor cells alone, all ATCV-based trials for breast cancer to date combine autologous breast tumor cells with additional antigens, cytokines or immunomodulators. In one completed study, a vaccine comprised of autologous breast tumor cells infected with Newcastle disease virus (NDV) was evaluated in 121 patients with primary breast cancer, metastatic breast cancer or metastatic ovarian cancer [34]. The intent of the NDV infection was to induce expression of immunostimulatory interferon-β. Primary breast cancer patients were given at least two vaccinations following surgery. Vaccine quality was divided into high and low quality based on the number and viability of tumor cells used in the vaccine. After 4 years, 96% of primary breast cancer patients receiving a high quality vaccine survived compared to 68% of patients receiving a low quality vaccine. Metastatic breast cancer patients receiving a high quality vaccine also demonstrated

improved survival that did not reach statistical significance, potentially due to the small sample size [34].

In a Phase II study, a vaccine composed of autologous breast tumor cells supplemented with allogeneic breast tumor cells, 3 additional TAAs antigens combined with IL-2 and GM-CSF was evaluated in 42 breast cancer patients [35]. Fifty-seven to 100% of patients demonstrated increased lymphoproliferative responses to the various antigens. Two of 16 patients with metastatic breast cancer experienced a partial response, while 7 of 16 experienced stable disease for 6 to 55 months [35].

In a related study, 37 breast cancer patients with depressed immunity received the vaccine as described above via 6 intradermal injections. The 10-year survival of vaccinated patients with depressed immunity (89%) was significantly greater than the 10-year historical survival of unvaccinated patients with depressed immunity (59%) [27]. These data underscore the potential of adjuvant ATCV to develop and maintain host anti-tumor immunity

There are two active clinical trials registered in the NIH Clinical Trial database investigating autologous breast cancer cells that have been transfected with an adenoviral vector engineered to express GM-CSF. Both trials are sponsored by the Dana-Farber Cancer Institute The first is a Phase I trial in metastatic breast cancer patients who are given three weekly vaccinations followed by biweekly vaccination until the vaccine supply is depleted [36] . The second study is a Phase I/II trial is nearly identical except that enrolled patients have operable, stage II or III breast cancer [37]. Expected completion dates for the metastatic and stage II-III breast cancer trials are January 2015 and April 2015, respectively.

ATCV Clinical Trials in Other Cancers

Although there have not been many ATCV clinical trials focusing on breast cancer, several promising studies in other cancers are highlighted here to further demonstrate the potential of ATCVs. A Phase III study in 254 patients with stage II or III colon cancer evaluated a vaccine (OncoVAX®) comprised of autologous colon cancer cells with immunostimulatory bacillus Callmette-Guerin (BCG) [30]. OncoVAX® was found to significantly prolong recurrence-free survival and 5-year overall in Stage II, but not Stage III patients. A related publication found a 61% risk reduction for recurrences in Stage II patients that were vaccinated compared to no treatment following curative surgery [38].

Several clinical studies have evaluated ATCVs engineered to secrete GM-CSF (reviewed in [39]). GM-CSF has been shown to enhance the recruitment, activation and maturation of dendritic cells [40]. Among the more intriguing clinical studies, a phase I/II multicenter trial in patients with early and advanced stage non-small-cell lung cancer (NSCLC) investigated the safety and efficacy autologous tumor cells genetically modified to secrete GM-CSF [41]. Three of 33 advanced NSCLC patients experienced durable complete responses lasting 6, 18 and at least 22 months. In addition, high levels of vaccine mediated GM-CSF secretion were associated with prolonged survival.

An ATCV comprised of formalin-fixed autologous hepatocellular carcinoma (HCC) fragments supplemented with GM-SF, IL-2 and tuberculin freeze-dried powder was

evaluated in 67 HCC patients following curative surgery [42]. More than 70% of patients receiving the vaccine developed DTH responses against HCC fragments. In addition, recurrence rates were significantly lower in patients receiving the vaccine (1 year, 12.6%; 2-year, 35.9%; 3 year, 54%) versus the untreated control cohort (1 year, 31.6%; 2-year, 61.3%; 3 year, 72.1%).

Preclinical Studies using ATCVs for Breast Cancer Therapy

To our knowledge, no preclinical study has evaluated an ATCV in the strict sense of using resected breast tumor tissue to create a therapeutic vaccine. However, several studies have approximated ATCVs with irradiated 4T1 murine mammary adenocarcinoma cells. On one hand, this approximation may overestimate vaccine efficacy as: 1) tumor cell lines are homogeneous whereas human breast cancer is highly heterogeneous, and 2) a significant fraction of tumor tissues are comprised of non-tumor cells such as fibroblasts, lymphocytes, endothelial cells, etc. On the other hand, 4T1 tumors are highly aggressive, spontaneously metastatic and result in 100% mortality 3-5 weeks after inoculation if untreated. Therefore, any immunotherapy capable of inhibiting 4T1 growth is deserving of additional consideration.

In one preclinical study, weekly immunization with irradiated IL-2-modified 4T1 cells significantly reduced spontaneous pulmonary metastasis in mice following footpad inoculation with parental 4T1 cells [43]. Separately, Ostrand-Rosenberg's group found that vaccination of mice bearing established 4T1 tumors with irradiated MHC II-transfected or B7.1-transfected 4T1 cells significantly reduced the number of spontaneous metastases with no effect on primary tumor growth [44]. In a related study, the addition of interleukin-12 (IL-12) further improved antitumor efficacy [45]. This study also determined that a combination of $CD4^+$, $CD8^+$ T cells and NK cells was responsible for antitumor activity. In another study by the same group, 4T1 cells engineered to express MHC II, B7.1 and the superantigen *Staphylococcal aureus* enterotoxin B (SEB) were found to reduce established metastases and extend survival when applied as an adjuvant immunotherapy following tumor resection [46].

Despite these intriguing data, no additional studies investigating tumor-cell based vaccines for breast cancer therapy have been published in nearly 15 years. It is likely that potential manufacturing obstacles, including *ex vivo* transfection of resected tumor cells, has dampened enthusiasm for clinical translation. However, given recent advances in the production of cellular immunotherapies, including the FDA approval of Provenge®, the time is ripe for further preclinical exploration of ATCVs for breast cancer, particular in the adjuvant setting.

Expert Commentary and Five Year View

Despite the fact that metastasis accounts for 9 of 10 breast cancer-related deaths, breast cancer is detected relatively early and standard-of-care tumor resection often results in remission or minimal residual disease. Therefore, post-resection breast cancer represents an ideal setting for implementation of ATCVs given the low burden of disease and the relative

immune-competence of these patients. Nevertheless, five key issues must be addressed in order for ATCVs for breast cancer to achieve widespread clinical application.

First, autologous breast tumor cells must be processed in a manner that reproducibly increases their immunogenicity. Engineering cancer cells to express antigen presenting or costimulatory molecules is effective, yet laborious in ensuring transfection in a heterogeneous primary culture. Alternative approaches to increase immunogenicity include: 1) inducing upregulation of immunogenic markers during the inactivation process, or 2) co-formulating breast cancer cells in a delivery system with one or more immunomodulators. Regarding the former, cytokines, especially interferons, have been shown to enhance expression of MHC II and B7.1. Autologous tumor cells may be exposed to cytokines during irradiation or other inactivation steps. Regarding the latter, the function of a well-designed delivery system would be to co-localize and co-deliver both antigen and adjuvant for optimal vaccine response. GM-CSF and IL-2 have been shown to be effective ATCV adjuvants [47]. These and other pro-inflammatory cytokines are worthy of exploration.

Second, methods for harvesting tumor cells and preparing vaccines must be standardized. There are numerous recipes for enzymatic digestion and mechanical disruption of solid tumors to create a single tumor cell suspension. However, the literature lacks a definitive comparison of these methods to guide the rational design of a standardized vaccine prep. Fortunately, processes for the manufacture of complex cellular immunotherapies for clinical applications are now routine and demonstrate that acquiring autologous tumor cells for use in immunotherapies is feasible. Preclinical models will be useful in helping engineer a clinically relevant manufacturing process which maximizes the yield of tumor cells that can be extracted from a resected tumor.

Third, potential limits regarding the size and pre-treatment of breast tumors must be clearly defined. Breast tumors are detected relatively early and are often less than 2 cm. It is likely that a lower tumor size limit exists such that it becomes impossible to harvest an adequate number of tumor cells to generate a high quality ATCV. Whether this limit is 0.1cm, 1.0cm or somewhere in between must be delineated in order to inform patient eligibility. Fortunately, fresh and frozen human breast tumor tissues are readily available to study the relationship between tumor size and tumor cell yield. For patients with tumors larger than 2 cm, neoadjuvant chemotherapy to shrink tumors prior to resection is recommended. Chemotherapy is likely to reduce the number and quality of harvested tumor cells, but it is not clear if this reduction would disqualify pre-treated patients from receiving ATCV. Once again, comparing tumor cell yields from size-matched pre-treated and non-treated tumors will be critical in determining the appropriate patient population for autologous tumor cell vaccination.

Fourth, the timing of vaccination relative to other therapies must be considered. Surgery, chemotherapy and radiotherapy all negatively impact a patient's immune system. Vaccinating patients too soon after any of these interventions would likely result in suboptimal vaccine response. Conversely, delaying vaccination for too long may not capitalize on a patient's minimal residual disease status. There exists an optimal window for administration of ATCVs with existing therapies. Preclinical studies aimed at understanding

optimal vaccine timing would help accelerate clinical translation ACTVs through combination with current standard-of-care therapies.

Fifth, the discovery of a prognostic indicator, in the form of an immunogenicity index or a gene signature, would be helpful in identifying patients that will benefit from vaccination. Due to the vast heterogeneity of breast cancer, one may expect that certain breast tumor phenotypes may be unsuitable for use as vaccines or that certain patients may not respond to vaccination. For instance, tumor cells which have extremely low levels of MHC expression or that express high levels of immunosuppressive cytokines, may be unacceptable for use in ATCV immunotherapy. An immunogenicity index can be developed, through a series of knockdown and depletion studies, to understand which tumor phenotypes are amenable for vaccination. Separately, patients with deficiencies in antigen presentation or severely depressed immunity may not be good candidates for vaccination until such deficiencies could be corrected. A recent study in patients with melanoma demonstrated differences in the expression of immune-related genes between immunotherapy responders and non-responders [48]. The discovery of a similar gene signature to predict which breast cancer patients have the best chance to respond to ATCVs would be highly beneficial.

Looking further into the future, efforts to combine ATCVs with immunomodulatory small molecular inhibitors, such as sunitinib, or monoclonal antibodies, such as ipilimumab and nivolumab, which target immune checkpoints should be explored to further enhance vaccine responses. In the next five years, the greatest hindrance to the development of an ATCV for breast cancer will continue to be the plethora of experimental treatment options available to the breast cancer patient. There are more clinical studies for breast cancer than any other cancer. The majority of these studies combine current adjuvant therapies with the latest small molecule inhibitor or explore unique combinations of existing pharmaceuticals. It is unlikely that this type of incremental approach will lead to a breakthrough capable of impacting progressive recurrences and therefore breast cancer mortality. In the meantime, ATCVs, because of their excellent safety profile and potential to induce a patient-specific, multivalent anti-tumor immune response, are deserving of more thorough consideration.

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Key Issues

- Approximately 40,000 women will die from breast cancer in the US in 2014. The vast majority of these deaths are due to progressive recurrences of non-metastatic breast cancer.
- Current adjuvant therapies, in addition to being toxic, fail to protect against recurrence in tens-of-thousands of breast cancer patients each year.
- ATCVs have a number of advantages as an adjuvant therapy for breast cancer, including being multivalent, patient-specific and safe.
- Several clinical trials in breast cancer and other cancers have demonstrated that ATCVs are safe and effective in extending survival for some cancer patients.
- Preclinical studies show that insertion of genes expressing IL-2, MHC II, B7.1 or SEB can enhance the immunogenicity of tumor cells and lead to their effective use as therapeutic vaccines.
- Investments in preclinical research aimed at overcoming key obstacles will likely help ATCVs achieve widespread clinical application as an adjuvant therapy for breast cancer.

Table 1

Summary of clinical trials using ATCVs for the management of breast cancer.

Phase	ATCV Composition	Stage	Significant Clinical Findings	Ref.
I	Irradiated autologous tumor cells expressing GM-CSF	IV	Study in progress	[36]
I/II	Irradiated autologous tumor cells expressing GM-CSF	II-III	Study in progress	[37]
*	Irradiated autologous tumor cell infected with Newcastle disease virus	I-IV	96% 4-year survival for breast cancer patients receiving high quality vaccine vs. 68% for low quality vaccine	[34]
II	Autologous and allogeneic (MCF-7) tumor cells with CA 15-3, CEA, CA 125 proteins and IL-2 and GM-CSF	I-IV	2/16 patients with metastatic breast cancer had a partial response; 7/16 had stable disease for 6 to 55 months	[35]
*	Autologous and allogeneic (MCF-7) tumor cells with CA 15-3, CEA, CA 125 proteins and IL-2 and GM-CSF	I-III	89% 10-year disease-specific survival of patients vaccinated compared to 59% for unvaccinated historical controls	[27]

* Phase could not be determined.