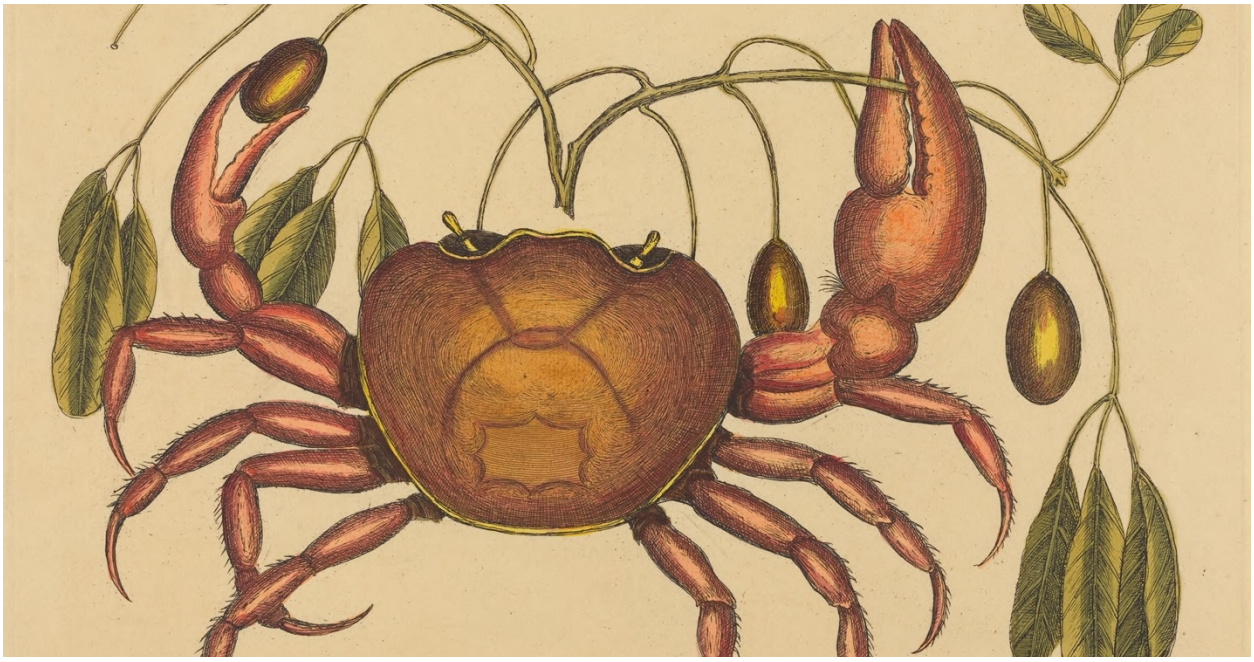

"The Land-Crab (Cancer ruricola)" by Mark Catesby. Courtesy National Gallery of Art, Washington.

Dendritic Cell Vaccines in Ovarian Cancer: Have We Reached Their Potential?

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Ovarian cancer is the most lethal gynecological malignancy, largely driven by high rates of relapse and chemoresistance. Ovarian cancer is thought to be immunogenic, making it amenable to immunotherapy. However, immunotherapies such as PD-L1 inhibitors and T cell transfers have produced modest, if any, survival benefit. One particular immunotherapy of interest is the dendritic cell vaccine, which delivers mature dendritic cells loaded with tumor antigens with the goal of mounting a T cell response against tumor cells. This review will focus on the role that dendritic cells play in the ovarian tumor microenvironment, general approaches to engineering dendritic cell vaccines and assessing their efficacy alone and in combination with other immunotherapies and systemic chemotherapies. Finally, we will discuss important areas of ongoing research in the field, including the development of personalized neoantigen-targeting DC vaccines.

INTRODUCTION

Ovarian cancer is the most lethal gynecological cancer, with an overall 5-year survival of 48% in 2020. Of those that present at an advanced stage, the 5-year survival is just 29% (1). The current standard treatment of epithelial ovarian cancer (EOC) is debulking surgery followed by platinum-

based chemotherapy. Despite good initial responses in most patients, chemoresistance and relapse are common (2, 3). For patients with resistance to platinum-based therapies, their treatment options remain limited.

Ovarian cancer is thought to be immunogenic as it expresses multiple well-known tumor-associated antigens (TAA). Some tumors are infiltrated by lymphocytes, which correlates positively with progression-free survival and overall survival (4, 5). These data suggest that ovarian cancer could be amenable to immunotherapy targeting. However, to date, immunotherapies have shown modest, if any, benefit. For example, PD-1 inhibitors have a response rate of 11.5% in advanced metastatic disease, which is thought in part due to poor T cell infiltration as well as poor antigen presenting function of antigen-presenting cells (APCs) (6, 7).

DENDRITIC CELLS IN OVARIAN CANCER

Dendritic cells in the tumor microenvironment take up and process tumor-associated antigens and present them on MHC I/II molecules to activate CD8+ and CD4+ cells, respectively. In comparison to other APCs such as B cells, mononuclear cells and macrophages, DCs are regarded as the most powerful cell type in its ability to capture, process and present antigens (8). In general, different DCs subtypes can play a multitude of roles in the tumor microenvironment. Conventional DCs (cDC) are the main subtype tasked with activating CD8+ T cells (particularly cDC type 1) and differentiation of CD4+ T cells (cDC type 2) through cytokine production (9). Conversely, plasmacytoid DCs (pDC), which are the main subtype of DCs in ovarian cancer, can exert both anti-tumor and immunosuppressive effects. Whether pDCs skew towards being tumor-protective or tumor-suppressive is largely determined by the signals they receive from their tumor microenvironment (9, 10).

Investigations into ovarian cancer have revealed that dendritic cells (DCs) make essential contributions to the depressed immune function observed in the ovarian tumor microenvironment. While ovarian cancer lesions have a high degree of DC infiltration, these DCs can have low efficacy of antigen presentation due to DC tolerance, which is characterized by downregulated expression of costimulatory molecules on the surface of DC cells and weaker antigen-presenting ability (11). Further, DCs can support the immunosuppressive milieu through their interactions with Tregs. For example, DC expression of indoleamine 2,3-deoxygenase, an essential enzyme in amino acid metabolism, can reduce the amount of tryptophan near Tregs and as a result maintain Tregs in an immunosuppressive state through mTORC-Akt signaling (12). DCs have also been shown to activate immunosuppressive Tregs by expressing ICOS ligand, leading to tumor progression (13).

Vaccines of functional DCs loaded with tumor-associated antigens have held promise for expanding tumor-specific T cell populations by restoring antigen presentation to T cells and bypassing the dysregulated milieu of the tumor microenvironment. There are different types of cancer vaccines, including cell-based vaccines, peptide/protein vaccines, epigenetic vaccines and genetic vaccines (14). This review will focus on the two most common DC vaccine types: cell-based and peptide/protein-based vaccines, which are designed to present T cells with tumor-associated antigens. Specifically, we will review the general approaches to engineering these vaccines as well as assessing their efficacy alone and in combination with other immunotherapies and systemic chemotherapies. Finally, we will discuss important areas of ongoing research

in the field, including the development on personalized neoantigen-targeting DC vaccines.

ENGINEERING DENDRITIC CELL VACCINES

Dendritic cell vaccines have long been an immunotherapy of interest for ovarian cancer, particularly given the demonstrated dysfunction of DCs surrounding ovarian tumors. Further, these vaccines are generally well-tolerated by patients and can induce long-term immunologic memory (15).

Currently, vaccines targeting DCs ex vivo are produced using three general steps. First, apheresis is performed to obtain either immune cells that have the potential to become DCs, such as monocytes, or immature DCs from peripheral blood. Among all cell types, monocyte-derived DCs (MoDCs) are most often used as immature DCs are typically not found in sufficient quantity in peripheral blood to produce a vaccine. Monocytes are subsequently cultured in vitro with a cytokine cocktail of GM-CSF and IL-4 that induces differentiation into immature DCs (16). However, from a functional standpoint, MoDCs have been shown to be inferior to cDCs in inducing long-lasting immune responses through T-cell activation, raising questions of their appropriateness as the DC subtype used in many EOC vaccines (17).

Second, once immature DCs have been obtained, they are loaded with tumor-associated antigens, ranging from specific peptides to proteins to multiple antigens from whole tumor lysates. To date, the most common approach has been to load DCs with one or several peptides known to be expressed on ovarian cancer cells. One example includes Wilms tumor 1 (WT-1), which is overexpressed in ovarian cancer along with many other solid tumors and can be targeted by cytotoxic T cells (CTLs). One group incubated DCs with an MHCI-restricted WT-1 peptide and a streptococcal primer and showed that these DCs elicited a CTL effect (18). This has been repeated with other peptides expressed by ovarian cancer cells, such as Her-2/neu, epithelial mucin 1, and p53. These vaccines reproducibly generated antigen-specific IFN-y secreting T cells (19, 20). However, the success of these single peptide/protein vaccines has been limited, resulting in short-term disease stabilization that ultimately gives way to progression after several months. One possible theory for this is that when a vaccine target is a non-mutated self-antigen or shared antigen that is overexpressed in the tumor, vaccine efficacy can be low because T cell recognition of self-antigens will be limited by central tolerance (21).

More recently, whole tumor cell lysates have been investigated as an antigen source for DC vaccines. In this scheme, DCs are pulsed with lysed ovarian tumor cells. These cells can be derived from ovarian cancer cell lines or even from a patient's own tissue sample (22). This has the benefit over single peptide vaccines in that lysates can elicit responses to more than one neoantigen, thus reducing avenues for tumor escape. Further, in the case of an autologous tumor cell lysate, the patient can produce a more "personalized" tumor-specific T cell pool by targeting their own unique set of tumor-associated antigens (23, 24). Previous studies with DC vaccines loaded with whole tumor lysate have demonstrated clinical benefit for patients with non-Hodgkin's lymphoma and melanoma (25, 26). There are multiple approaches to stimulating cell death to induce antigen release, including repetitive freeze-thaw cycles or exposing cells to hypochlorous acid (HOCl). Chiang et al. found that autologous ovarian tumor cells killed with oxidation and lysed with freeze-thaw cycles were superior to cells killed with irradiation or freeze-thaw lysis in priming T cell responses in vitro (27).

Finally, regardless of the antigen, immature antigen-presenting DCs are then matured in the presence of immunogenic substances like LPS and IFN-y to trigger expression of co-stimulatory molecules on the DC surface. These co-stimulatory molecules are essential for T cell activation upon antigen presentation in the lymph node. Once this step is complete, DCs are typically fractionated into multiple doses to be used as serial vaccines over a defined treatment period. Typically, vaccines are given intranodally, but can also be given with intramuscular or subcutaneous injection (16).

CLINICAL EFFICACY OF DC VACCINES

Since the early 2000s, multiple DC vaccines have been investigated in clinical trials, though most therapies have not progressed past phase II trials and most trials have consisted of small patient cohorts ranging from 3 to 56 patients at different stages of ovarian cancer (16).

Thus far, DC vaccine trials have primarily focused on patients with recurrence after standard therapy. One study investigated DC vaccines pulsed with WT1 peptide given to patients resistant to chemotherapy. Only one of three patients responded to the vaccines and reached stable disease (18). With regard to the efficacy of DC vaccines early in a patient's disease course, one retrospective study evaluated the potential benefit of early DC vaccines against multiple TAAs when given before recurrence as part of maintenance therapy. After 5-7 doses, the mean survival time from diagnosis was 30.4 months and 14.5 months from first vaccination. The authors argued that early DC vaccines could have the potential to elongate progression-free survival, although a larger prospective study is necessary to substantiate this (28).

In 2018, Tanyi et al. reported the results of their phase I trial of an ovarian cancer dendritic cell vaccine using autologous whole tumor cell lysate as an antigen source (29). In this trial of 25 patients with platinum-treated, immunotherapy-naive, recurrent ovarian cancer, intranodal injections of the vaccine was safe and feasible. Patients mounted T cell responses to autologous tumor antigens, and the vaccine was associated with significantly prolonged survival. Patients were randomized to either receive bevacizumab (a VEGF inhibitor) and cyclophosphamide, bevacizumab, cyclophosphamide and their personalized DC vaccine or just bevacizumab and their personalized DC vaccine. In the cohort receiving all three treatments, the median progression free survival was 11.1 months compared with 4.1 months in a historical control cohort. Remarkably, 24-month survival in patients with a confirmed immune response was 100% for vaccine/ cyclophosphamide/ bevacizumab. This was compared to 40% for vaccine/ bevacizumab and 40% for bevacizumab/ cyclophosphamide, indicating that low-dose cyclophosphamide was needed to improve survival. However, for patients without an immune response, no clinical benefit was observed.

Further, additional immunotherapies might act synergistically with DC vaccines. In one study evaluating DC vaccines and T cell transfer, seven recurrent advanced-stage ovarian cancer patients received DC vaccines (30). Of these patients, three had a partial disease response and went on to receive autologous T cell transfer after these T cells were expanded in vitro. This resulted in one complete response, one achieved stable disease and one unfortunately had disease progression after T cell transfer. With regards to immune checkpoint inhibitors, while there is a theoretical benefit to combining these therapies, no clinical trials are currently investigating these therapies in combination.

Of note, some trials have reported an increase in adverse side-effects from DC vaccines when used in combination with chemotherapies, however it is unclear how much is related to the DC vaccines themselves (29).

PERSONALIZED NEOANTIGEN-TARGETING VACCINES – THE NEXT BIG LEAP?

One key area of interest for DC vaccines is the development of neoantigen-targeted vaccines. Neoantigens are proteins expressed by tumors that differ from other tumor-associated antigens in several keys. First, neoantigens arise from DNA mutations within the tumor and thus produce peptides that are specific to tumor cells. Because these antigens are not expressed on other cell types in the body, they are often highly immunogenic in comparison to other tumor-associated antigens, which are typically non-mutated self-antigens that are simply overexpressed on cancer cells. This increased immunogenicity is in part driven by increased affinity for major histocompatibility complexes (MHCs) (31). Further, because neoantigens are only expressed by tumor cells, this limits potential off-target effects. Thus, neoantigens are ideal targets for an anti-tumor T cell response (31). While ovarian cancers have lower mutational burdens than most other cancer types, recent analyses have shown that some patients can express moderate to high levels of neoantigens (32). In other cancers, neoantigen-loaded DC vaccines have shown promising results in small phase I trials in melanoma patients and non-small cell lung cancers (33, 34).

Neoantigens can be classified as being shared or personalized. Shared neoantigens are common in some tumor types and can be used to broadly treat patients who have the same tumor type. However, not all patients express these shared neoantigens, and even if they are expressed, different patients may mount different immune responses to them (35). Thus, personalized neoantigens, which are specific to individual patients and tumors, have become a point of interest for DC vaccine design across all cancer types (22). However, identifying a patient's neoantigen repertoire has only recently become possible with the development of next-generation sequencing (NGS) such as whole exome sequencing, mass spectrometry analysis of the immunopeptidome (i.e., peptides associated with HLAs), as well as highly predictive bioinformatics tools (36-38).

Initial evidence suggests that recognition and targeting of tumor-specific neoantigens improves the effectiveness of dendritic cell vaccines in ovarian cancers. For example, patients with tumor lysate-pulsed DCs were found to have activated high-avidity CD8+ T cell clonal expansion specific for de novo neoantigens, which improved progression-free survival compared to patients without neoantigen-specific T cell responses (29).

However, multiple research groups are still investigating the true benefit of this approach compared to using overexpressed self-antigens or whole tumor lysates, particularly because the process of identifying a patient's neoantigens remains costly and resource-intensive (22). Currently, one trial is underway to assess a personalized neoantigen-pulsed DC vaccine in ovarian cancer patients. This trial will investigate the feasibility and safety of a personalized neoantigen-loaded DC vaccine in patients with ovarian cancer (ClinicalTrials.gov NCT04024878).

CONCLUSIONS

Dendritic cell vaccines have been shown to be an effective immunotherapy for ovarian cancer, however it remains an active area of innovation in the wake of new technological

advances in the realms of NGS and bioinformatics. Further, combinations of various immunomodulatory treatments with DC vaccines will likely be required to capitalize on the immunogenicity of ovarian malignancies and will be a crucial area of clinical investigation going forward.

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