Review Article

Next generation approaches for tumor vaccination

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Abstract: Tumor vaccines have been an attractive concept in the immunotherapy of cancer based on the central role of tumor-associated antigens in allowing the immune system to recognize cancer cells and the large variety of platforms in which to present such antigens to the immune system. Early clinical studies of vaccines, however, were largely disappointing. Recent evidence that cancer-mediated T cell suppression may prevent T cell activation is leading to renewed interest in vaccine development. The use of T cell checkpoint inhibitors alone has revolutionized the contemporary treatment of human cancer, and has suggested that the emergence of neoantigens may be an important biomarker of therapeutic response. Thus, the possibility of using more personalized vaccines targeting relevant neoantigens alone and in combination with T cell checkpoint blockade is a new area of active clinical investigation. In this review we will discuss the central role of antigens in tumor immunotherapy, describe how vaccines may be developed in the context of modern genomic profiling of tumor cells and provide a forward looking perspective on how tumor vaccines may be incorporated into the current landscape of cancer therapy.

Keywords: Antigen; cancer; neoantigen; oncolytic virus; vaccine

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Introduction

The concept of immunization, in which previous exposure to some segment or form of a pathogenic substance provides protection against the full course of a disease caused by the pathogen, has been known for centuries. The use of immunization is best defined in the setting of infectious disease prevention. Eastern civilizations were reported to have scratched active pox lesions onto normal individuals as a method of preventing smallpox. The modern era of vaccination is largely credited to Edward Jenner who made the seminal observation that in many households devastated by smallpox outbreaks, the milkmaids often survived the epidemic. Jenner identified a minor illness attributed to cowpox in these individuals and eventually isolated the virus known as vaccinia virus, thought to be a hybrid between cowpox and smallpox, as the vaccine used in the smallpox eradication campaign. The success of smallpox vaccinations is clearly evident, as the disease has been essentially eliminated from the planet. The discovery that tumor cells encode a number of highly expressed differentiation proteins, cancer-testis antigens or neoantigens due to emerging mutations characteristic of malignant cells, suggested that cancer might also be amenable to vaccination.

The general approach to cancer vaccination has been to identify a putative antigen or antigenic epitope(s) and present them to patients through a variety of platforms, including MHC-specific peptides, whole or partial proteins, encoded in RNA or DNA, in recombinant viral or bacterial vectors and expressed in dendritic cells or as whole tumor cell preparations. In many protocols, additional vaccine adjuvants were added to help boost the host immune

response against the tumor antigen(s). Historically, cancer vaccines for the treatment of metastatic cancer have not met with much success. Despite a significant effort in translational research, clinical trial results with tumor vaccines were largely disappointing. In 2004, Rosenberg *et al.* reported an overall objective response rate of only 2.6% from several highly selected cancer vaccine clinical trials, including cell-, peptide- and viral-based approaches (1). These results led to considerable pessimism in the field, but also provoked a search for why vaccines were not effective in cancer. Several factors could have led to these unsuccessful results. While the reasons are likely multifactorial, a major difference between cancer and infectious disease vaccine development is the use of vaccines in normal individuals prior to exposure to a pathogen in infectious disease clinical development compared to testing cancer vaccines in patients who have very advanced disease and typically have already failed standard therapy. This is particularly relevant as emerging data in the early part of the 21st Century strongly supported the notion that established cancers have evolved a variety of mechanisms designed to suppress the host immune responses. Could it be possible that the failure of vaccination was related to tumor-mediated immune suppression? If so, overcoming the suppressive mechanisms utilized by cancer might need to be addressed prior to considering a vaccine.

Cancer mediates immune suppression through several mechanisms, and these have become better defined in recent years. For example, many types of suppressor host and immune cells exist within the tumor microenvironment, such as CD4+FoxP3+ regulatory T cells, tumor-associated macrophages, fibroblasts, adipocytes and myeloid-derived suppressor cells, which can inhibit tumor-specific, cytotoxic CD8+ T cells and tumor-reactive natural killer (NK) cells. Soluble factors, such as tumor growth factor-β (TGF-β), interleukin-10 (IL-10), vascular endothelial growth factor A (VEGF-A), and other can also block effector T cell function within the tumor microenvironment. The most important finding, however, may be the role of CD8+ T cell intrinsic factors that promote T cell exhaustion and inhibit T cell function. This includes the cytotoxic T lymphocyte antigen 4 (CTLA-4) receptor that is mobilized to the surface of T cells following recognition of peptide-MHC complexes by the cognate T cell receptor (referred to as Signal 1 in T cell activation) and engagement of T cell surface CD28 (signal 2) by the B7.1 or B7.2 complexes on antigen-presenting cells. This two-signal system promoted T cell proliferation, cytokine production and blocks apoptosis. CTLA-4 is mobilized to the cell surface after CD28 signaling and competes with CD28 for binding to the B7 ligands. CTLA-4 then inhibits T cell proliferation, blocks cytokine production and promotes T cell anergy thereby acting as a T cell checkpoint, likely a normal homeostatic mechanism to prevent overactive T cell stimulation and autoimmunity. In 2011, the first randomized clinical trial of an anti-CTLA-4 monoclonal antibody demonstrated an overall survival (OS) benefit in patients with metastatic melanoma resulting in FDA approval of ipilimumab for the treatment of melanoma (2). Similarly, the programmed cell death 1 (PD-1) receptor on T cells is a maker of early T cell activation and at higher levels indicates T cell exhaustion. When PD-1 binds to its ligand, PD-L1, the T cell is eliminated. Many tumors express PD-L1, which may serve as an important mechanism for eradicating tumor-reactive T cells within the tumor microenvironment. Monoclonal antibodies targeting PD-1 and PD-L1 are demonstrating significant therapeutic activity against many cancers, including melanoma, non-small cell lung cancer, renal cell carcinoma, head and neck cancer, bladder cancer and Hodgkin's lymphoma (3).

The success of T-cell checkpoint inhibitors has allowed for further studies to identify the most important patient and tumor factors that play a role in the anti-tumor clinical response. In an analysis of tumor genomic data from melanoma patients treated with CTLA-4-targeted checkpoint inhibitors, Snyder *et al.* characterized the somatic mutation burden and emergence of neoantigens generated from those mutations as highly associated with clinical benefit. An important observation in this trial was that certain somatic neoepitopes were shared by patients who benefited from treatment and were absent in patients who did not demonstrated therapeutic benefit, suggesting that neoantigens may be appropriate targets for immune recognition (4). Other groups have also reported that the recognition of neoantigens by T cells plays a substantial role in T cell checkpoint therapy (5). These findings have important implications for cancer vaccines. First, the ability to therapeutically block T cell exhaustion provides a powerful new strategy to limit T cell suppression and could be envisioned as an adjuvant to tumor vaccination. Second, the identification of tumor cell neoantigen emergence provides potentially new targets for vaccine development.

In this review, we will discuss the central role of antigens in mediating anti-tumor immune responses, describe the emerging concept of neoantigens and personalized vaccine development, detail other strategies for vaccination, such

as oncolytic viruses, and provide our opinion on the most promising future approaches for tumor vaccines. The progress in immunotherapy has been exciting over the last decade and renewed interest in tumor vaccines for the treatment of cancer is appropriate given our improved understanding of how the immune system recognizes and mediates tumor regression. Further studies in using vaccines in the setting of cancer prevention may be especially interesting given the ability to rapidly interrogate the cancer genome and develop precision vaccines for clinical delivery. Finally, it is tempting to hypothesize that combination approaches in which tumor vaccines are used to direct a T cell response, and checkpoint inhibitors are used to prevent T cell suppression, will be especially useful in the treatment of advanced cancers. Further clinical and translational investigation will be needed to confirm this hypothesis. The application of validates biomarkers will also become a high priority to fully realize the promise of precision immunology and cancer vaccines.

The central role of antigen in mediating anti-tumor immunity

The steps in immune-mediated recognition and eradication of tumor cells have been nicely summarized by Chen and Mellman in what they described as the "cancer-immunity cycle" (6). In this cycle, immune responses begin when professional antigen-presenting cells, such as dendritic cells, engulf soluble tumor-associated antigens or whole, necrotic tumor cells. This likely occurs within the tumor microenvironment, and following antigen exposure, the dendritic cells mature and likely traffic to secondary lymphoid organs where they present tumor antigens to their cognate T cells as processed peptides bound to the major histocompatibility complex 1 (MHC-1) for CD8+ T cells or MHC-II for CD4+ T cells. The activated T cells must then circulate back to the tumor microenvironment, likely guided by chemokine gradients and other inflammatory cues. The antigen-specific T cells must then interact with MHC-I-peptide complexes on the surface of tumor cells and then they release cytotoxic granules in which enzymes, such as granzyme B and perforin, mediate tumor cell lysis. The lytic effect on tumor cells results in release of more antigens to begin the cycle again, and may release other antigens causing an expanded response, which has been referred to as antigen spreading (6). This process is complicated and, while many steps can be targeted for immunotherapy drug development, cancers may also evolve various mechanisms for thwarting the process at each step. Nonetheless, the importance of antigen in initiating the cycle is clear since immunotherapy does not elicit therapeutic responses in the absence of T cells.

The central role of T cells is well established through murine studies in which T cell deficient mice are unable to eradicate established tumors, and in human clinical trials where a strong association between T cell rich tumors and clinical responses to immunotherapy has been documented (7). The magnitude of immune response during the cycle further depends on the balance between the activation of effector cells versus the activation of regulatory cells. A major barrier to successful immunotherapy has been the absence of tumor antigen-specific T cell responses. This may occur because the antigens are self-proteins and clonal selection may delete the cognate T cell, peripheral tolerance may prevent T cell activation, or some antigens may elicit regulatory T cell responses. The balance between effector and regulatory T cells may also be affected by soluble factors, such as local cytokines and chemokines, as well as T cell intrinsic factors, such as T cell co-stimulatory and co-inhibitory molecules, often referred to as T cell checkpoints. The finding that many cancers express PD-L1 provides yet another mechanism in which tumor cells may eliminate PD-1 + antigen-specific effector T cells. Thus, overall cancer immunotherapy seeks to initiate and amplify the cancer-immunity cycle while blocking the suppressive effects often regulated by cancer cells or other features of the tumor microenvironment.

The role of vaccines may be especially important since vaccination can initiate and/or expand an antigen-specific T cell response when the tumor has not been able to do so. While earlier work suggested that there might be different classes of antigens, the optimal antigen for tumor vaccines has been elusive. In general, antigens can be categorized as normal self-proteins that are overexpressed in cancer cells [e.g., prostate-specific antigen (PSA)], differentiation antigens (e.g., gp100, tyrosinase), cancer-testis antigens which are normally expressed only in immune privileged sites (e.g., NY-ESO-1) or mutated antigens (e.g., neoantigens). To date, all such antigens have been targeted through a variety of delivery platforms, including peptides or protein in emulsified vehicles, direct targeting to dendritic cells, RNA and DNA plasmids, recombinant viral and bacterial vectors and use of whole tumor cells. Vaccine adjuvants have also been used to help enhance antigen recognition and T cell activation. Commonly used adjuvants have included Freund's incomplete adjuvant, BCG,

granulocyte-macrophage colony-stimulating factor (GM-CSF), IL-2, toll-like receptor agonists, and others. Although antigen-directed vaccines have shown limited promise to date, it is important to remember that most vaccines have been tested in advanced, metastatic cancer patients who have already failed standard therapy and this may not be the ideal patient population for vaccination alone. Furthermore, little is known about the optimal dosing, route, schedule, and booster strategies needed to develop the most effective induction of T cell responses.

To date, the only vaccine that has achieved regulatory approval for the treatment of advanced cancer is Sipuleucel-T, which was approved by the FDA in 2010 for treatment of asymptomatic or minimally symptomatic hormone-refractory prostate cancer (HRPC). Sipuleucel-T therapy consists of dendritic cells derived from autologous peripheral blood mononuclear cells obtained by leukapheresis and loaded with recombinant human fusion protein encoding the prostatic acid phosphatase (PAP) antigen and GM-CSF, a cytokine that helps mature dendritic cells and prime T cell responses. In a double blind, placebo controlled, prospective phase III clinical trial in 512 patients with minimally symptomatic HRPC, Sipuleucel-T treatment was associated with a relative reduction of 22% in the risk of death when compared to patients treated with placebo (8). Overall, the median survival was 25.8 months in the Sipuleucel-T group and 21.7 months in the placebo group, with an overall of 4.1 months improvement in median OS in the Sipuleucel-T group. Adverse events that were reported more frequently in the Sipuleucel-T group included fevers, chills, headaches, influenza-like illness, myalgia, hypertension, hyperhidrosis, and groin pain. The most common adverse events within one day after infusion were chills (51.2%), fevers (22.5%), fatigue (16.0%), nausea (14.2%), and headache (10.7%). Only 0.9% of patients in the Sipuleucel-T group were not able to receive all three infusions secondary to infusion-related adverse events (8). While the therapeutic impact was modest, the approval represents a landmark in cancer vaccine development and Sipuleucel-T is now being evaluated in various combination clinical trials.

Another recent prostate cancer vaccine strategy with promising early clinical data is the PROSTVAC-VF vaccine. PROSTVAC-VF is a prime-boost regimen that uses a priming dose of recombinant vaccinia virus encoding PSA and a triad T cell co-stimulatory molecules (B7.1, ICAM-1, and LFA-3) followed by booster immunizations with a non-replicating fowlpox virus encoding PSA and the co-stimulatory molecules. The PROSTVAC-VF vaccine is currently being tested in a phase III clinical trial for treatment of metastatic castration-resistant prostate cancer. In a previous phase II clinical trial, PROSTVAC-VF was tested in 125 men with minimally symptomatic castration-resistant metastatic prostate cancer (mCRPC) (9). In this trial, 82 patients received PROSTVAC-VF and 40 received control vectors. The primary endpoint was progression-free survival (PFS), which was found to be similar in both groups. However, patients who received PROSTVAC-VF had a better OS with 30% being alive at 3 years compared to 17% of the control patients. Overall, there was a median OS improvement of 47% or 8.5 months in the treatment group compared to the control group (25.1 *vs.* 16.6 months respectively). PROSTVAC-VF was well tolerated with a subset of patients experiencing injection site reaction, fever, fatigue and nausea (9). In addition to the phase III randomized trial; PROSTVAC-VF is also being evaluated in several combination clinical trials with T cell checkpoint inhibitors (i.e., ipilimumab), androgen deprivation therapy (i.e., enzalutamide) and radiation therapy.

The results with Sipuleucel-T and PROSTVAC-VF demonstrate how defined tumor antigens can be utilized in vaccine therapy. The advantage of this approach is that the vaccine vectors can be mass-produced, immune responses can be easily monitored for antigen-specific T cell expansion and the tolerable safety profile allows for combination trials. The recent advances in genomic profiling, however, have illuminated the frequency of mutations that can give rise to neoantigens and an emerging T cell repertoire that might represent a better target for vaccine development.

Personalized vaccines for cancer therapy

As mentioned, clinical studies of T cell checkpoint inhibitors have suggested an association between therapeutic responses and the emergence of neoantigens presumably arising from new mutations within the cancer cell genome. If substantiated, this might indicate that the better antigens for vaccine targeting are neoantigens. This is perhaps logical since widely expressed tumor antigens may undergo immune editing resulting in antigen-specific suppression in established cancers. The new epitopes appear due to the genetic instability inherent in tumor progression, and these would not have time for editing and T cell suppression (4). This possibility is also supported by recent data demonstrating effective T cell targeting with adoptively transferred T cells recognizing neoantigens in patients with

cancer (1). The real challenge is whether information about new antigenic epitopes can be incorporated into a vaccine in a feasible timeframe that will allow for clinical development. The advent of high-throughput screening techniques and computerized algorhythms that predict epitope-MHC binding characteristics may allow for such personalized vaccine approaches. Several studies are in development to test this strategy.

A phase I open-label study was recently initiated to evaluate the safety and immunogenicity of a personalized polyepitope DNA vaccine strategy in persistent triple-negative breast cancer patients following neoadjuvant chemotherapy. The hypothesis of this trial is that personalized polyepitope DNA vaccines will be safe and effective in generating measurable CD8+ T cell responses to tumor-specific neoantigens (10). In this study, resected tumor prior to neoadjuvant therapy is being assessed for genomic mutations. Each mutation is being evaluated for HLA-A2 binding affinity and several epitopes will be selected for synthesis based on mutation status and high affinity for MHC binding. The vaccine will be given following definitive tumor resection in a high-risk population for tumor recurrence. Although ambitious, this trial will help establish the clinical feasibility of such an approach and improvements in all steps will likely be required before this can be more broadly developed as a cancer therapeutic approach.

Oncolytic virus immunotherapy

Oncolytic viruses are native or genetically modified viruses that preferentially infect and/or replicated in tumor cells causing them to lyse and leading to *in situ* tumor antigen release. Thus, oncolytic viruses differ from standard vaccines in having a dual mechanism of action in which tumor cells are directly killed by virus and immune responses are initiated using a multitude of tumor-specific antigens. Oncolytic viruses likely further promote anti-tumor immunity by release of other cell-associated danger factors and through interferon signaling, which orchestrate potent T cell responses (11).

A first-in-class oncolytic virus based on an attenuated herpes simplex virus, type 1 (HSV-1) encoding GM-CSF, and designated as Talimogene laherparepvec (T-VEC) demonstrated an improved durable and objective response rate in patients with metastatic melanoma leading to regulatory approval in the United States and Europe (11). T-VEC was studied in a prospective phase III trial where 436 unresectable stage IIIB, IIIC, and IV melanoma patients were randomized in a 2:1 manner to treatment with T-VEC or recombinant GM-CSF. The durable response rate, which was defined as an objective response beginning within 12 months of treatment and lasting for at least 6 months, was found to be 16.3% in patients treated with T-VEC compared to 2.1% in patients given GM-CSF. The objective response rate was 26.4% for the T-VEC group compared to 5.7% for the GM-CSF group. Overall, 10.9% patients had a complete response to T-VEC treatment and responses were observed in both injected and un-injected tumors. Overall, T-VEC was well tolerated with major adverse events being low-grade fevers, chills, nausea, fatigue, and local injection site reactions (12). T-VEC is now being studied in combination with T cell checkpoint inhibitors, including ipilimumab and pembrolizumb for treatment of melanoma, and plans are in development to test T-VEC in other types of cancer. While early data suggests that T-VEC treatment is associated with detection of MART-1-specific CD8+ T cells, further research is needed to better understand if neoantigen responses may also be emerging following T-VEC therapy.

Another oncolytic therapy known as H101 was recently approved when used in combination with chemotherapy for treatment of nasopharyngeal carcinoma in China. H101 is a genetically modified oncolytic adenovirus that was evaluated in a randomized phase III clinical trial with 160 patients with advanced squamous cell carcinoma of the head and neck or esophagus. Patients were randomized to chemotherapy only or chemotherapy + H101 treatment. Chemotherapy consisted of cisplatin and 5-FU for chemotherapy-naïve patients or Adriamycin and 5-FU for patients who received previous platinum-based chemotherapy. The chemotherapy with or without H101 was given for 5 consecutive days every 3 weeks. 123 of the patients completed the treatment and went through evaluation for response. Patients treated with H101 and cisplatin/5-FU had a response rate of 78.8% compared to a response rate of 39.6% in the cisplatin/5-FU alone group. For patients assigned to Adriamycin/5-FU with or without H101, a comparable 50% response rate was seen but the overall sample size was small with only 18 participants in this cohort. The major adverse effects were fever, injection site reactions, and flu-like symptoms (13).

The use of an oncolytic, native coxsackievirus A (CVA21) is also in clinical trials for treatment of melanoma. Coxsackievirus is a non-enveloped single-stranded RNA enterovirus that has been shown to promote the infiltration of immune effector cells such as NK cells and CD8+

T cells. CVA21 also activates dendritic cells leading to increased antigen presentation and has demonstrated lysis of multiple tumor cell types *in vitro*. (14,15). CVA21 was recently evaluated in a phase II study for safety and efficacy of CVA21 in patients with advanced melanoma. The trial involved 57 patients with treated and untreated unresectable stage III-IVM1c melanoma. Primary end-point of the study was to achieve more than 9 of 54 evaluable patients with immune-related progression-free survival (irPFS) at 6 months. The primary endpoint was achieved with 21 evaluable patients showing irPFS at 6 months. The secondary endpoint of overall response rate (irRECIST) was 28.1% with durable response rate of >6 months in 19.3% (11 of 57 patients). Overall, CVA21 was well tolerated with the most common adverse event of grade 1 fatigue, fever, chills, and local injection site reactions (16).

Future priorities for oncolytic viruses will include an assessment of tumor cell neoantigen repertoires following treatment, determining if expression of tumor antigen in the virus might improve therapeutic responses, and studies of combination strategies in melanoma and other cancers. The potential immune adjuvant effects of oncolytic viruses, such as release of danger-associated molecular factors and stimulation of local interferon signaling pathways, must be confirmed in clinical trials.

Bacterial and yeast vectors for tumor vaccine development

The presentation of tumor antigens to the immune system can also be enhanced by expression in bacterial vectors, and several have advanced into clinical trials, including *Listeria monocytogenes*, *Escherichia coli*, *shigella*, *and salmonella*. Bacterial vectors have several benefits including low cost of production as well as the ability to generate more diverse immune responses by activating both innate and adaptive immune responses.

Listeria monocytogenes is a gram-positive bacterium that upon infection gets engulfed by phagocytic cells, such as macrophages and dendritic cells. Most of the bacteria get degraded within phagolysosomes; however, about 10% of bacteria escape the phagosomal compartment and enter the cytosol of the cell. The bacteria in the cytosol polymerize host cell actin filaments allowing bacteria to spread from one cell to another. Since *Listeria* goes through degradation in both cytosol and the phagolysosome, it makes it a unique vector for expressing antigens that are degraded by phagolysosomes and presented on the antigen-presenting cell preferentially by MHC II molecules, and antigens that are processed in the cytosol are preferentially expressed by MHC I molecules, thus promoting both CD4+ and CD8+ T cell responses. In addition, *Listeria* can activate the innate immune system by engaging toll-like receptor dependent mechanisms through bacterial ligands, such as peptidoglycan, lipoprotein, lipoteichoic acid, and nucleotide-binding oligomerization domain. *Listeria* degraded by phagolysosomes can induce an IFN-β-mediated transcription response that eventually leads to IL-8 production and NFκB activation. The ability of *Listeria* to activate the immune system through several different pathways makes it a unique and effective vector for vaccine development (17).

A live-attenuated *Listeria monocytogenes* vaccine, Lm-LLO-HPV E7, was studied in a phase I safety trial in patients with advanced carcinoma of the cervix. Lm-LLO-HPV E7 secreted HPV-16 E7 antigen that was fused with a non-hemolytic fragment of Lm protein listeriolysin O (LLO) to enhance immune responses. The study involved 15 patients who were previously treated with metastatic, refractory, or recurrent invasive carcinoma of the cervix with historical median survival of 180 days and a one year survival rate of approximately 5%. Patients were equally divided into 3 groups (5 patients per group) with each group getting a different dose of vaccine (1×10^9, 3.3×10^9, or 1×10^{10} colony forming units) with a total of 2 doses at 3 weeks intervals. All patients developed a flu-like syndrome but responded to non-prescription symptomatic treatment. No grade 4 adverse events were observed. No patients required antibiotic treatment suggesting that the side effects were most likely secondary to cytokine release rather than bacterial infection. At the high dose of 1×10^{10} CFU, patients did have severe fever and dose limiting hypotension. Two patients died during the study. From the evaluable patients, 5 had progressive disease, 7 had stable disease, and 1 had a partial response. For the treated patients, median survival was reported to be 347 days. Based on the efficacy and safety of the treatment, Lm-LLO-E7 is now being studied in a phase II trial in patients with advanced cervical cancer (18). Other antigens are also being used in *Listeria* for vaccination, including PSA for prostate cancer and mesothelin for pancreatic cancer with intriguing preliminary results in early phase clinical trials.

In a group of patients with previously treated metastatic pancreatic cancer, patients were randomly assigned at a 2:1 ratio to treatment with a priming doe of GVAX, a whole cell cancer vaccine encoding GM-CSF with cyclophosphamide

for two doses followed by *Listeria monocytogenes* encoding mesothelin for four doses, or six GVAX/cyclophosphamide vaccines alone. In this study, 61 patients received the prime/boost regimen and 29 patients received only G-VAX (19). 97% of the patients had received prior chemotherapy with over half having two or more prior regimens for treatment of metastatic disease. The study found an OS benefit for patients in the prime/boost arm (6.1 *vs.* 3.9 months; HR 0.59; P=0.02). The most common grade 3 or greater treatment-related adverse events were transient fever, lymphopenia, increase in hepatic transaminases and fatigue. The trial also saw a correlation between survival and an increase in mesothelin-specific CD8+ T cells.

Yeast derived vaccines are also currently being studied as potential cancer immunotherapy. Using yeast as a vector has several advantages, including easy engineering of tumor antigens and simple storage and transportation of the vector since heat-killed yeast are very stable. Yeast-CEA (GI-6207) is a genetically modified vaccine that expresses carcinoembryonic antigen (CEA) protein using heat-killed yeast *Saccharomyces cervisiae* as a vector to target against CEA expressing tumors. *In vitro* studies have shown that yeast-CEA can increase surface expression of MHC class I and II molecules, increases cytokine release, and upregulates expression of genes involved in antigen uptake, antigen presentation, and chemokine/cytokine production. In a phase I study, yeast-CEA was evaluated for safety, tolerability, and clinical response in patients with metastatic CEA-expressing carcinoma who failed standard treatment. Twenty-five patients were enrolled in the study, of which 20 had colon adenocarcinoma, 2 had rectal adenocarcinoma, one had pancreatic adenocarcinoma, one had NSCLC, and one had medullary thyroid cancer. Patients who were eligible required serum CEA >5 ng/mL or >20% CEA+ tumor block, ECOG PS 0 to 2, and no history of autoimmune disease. Median baseline CEA was 107. Patients received subcutaneous vaccines every 2 weeks for 3 months and then one dose monthly. The vaccine was well tolerated with the most common adverse effects being grade 1 or grade 2 injection-site reactions. Only three grade 3 toxicities were reported which were injection-site reaction, vigorous immune response in one patient that resolved with high dose corticosteroids, and pain (possibly confounded by progressing disease). The median survival after enrollment was 7 months. In 24 evaluable patients, 7 patients had declines in their serum CEA levels. Five patients had stable disease beyond 3 months, and each of these 5 patients had a decrease in CEA levels. In some patients, post-vaccination results showed increased in CD4+ and CD8+ T cells with decrease in regulatory T cells (20).

Combination of tumor vaccines and T cell checkpoint inhibitors

Given the success of T cell checkpoint inhibitors alone, and in combination, as well as the emergence of neoantigens following T cell checkpoint blockade, support the concept of combining tumor vaccines with T cell checkpoint inhibitors (21). While perhaps logical, there have been relatively few studies completed to date. Although the original clinical trial of ipilimumab used a gp100 peptide vaccine arm alone as a control, and also included a combination arm, no significant impact of the vaccine was demonstrated (2). This may be due to the limited strength of gp100 peptide vaccine and new trials with more contemporary antigens, such as neoantigens and better vectors regimens, should be considered. To date, most of the encouraging data with combination studies using T cell checkpoint inhibitors have come from studies with oncolytic viruses.

In a small phase Ib clinical trial, 18 patients with stage IIIB-IV melanoma were treated with T-VEC and ipilimumab (22). In this study, 9 of the 18 patients treated with the combination had an objective response with 4 having a complete response (22%). Treatment was well tolerated with no treatment-related dose-limiting toxicities reported but 32% of patients did have grade 3–4 adverse events. A single grade 5 event related to CNS disease progression was also seen. A larger 200 patient randomized trial comparing the combination to ipilimumab alone has now been completed and results are pending. A large, randomized phase III clinical trial of T-VEC and pembrolizumab versus pembrolizumab alone is also under way in patients with advanced melanoma.

Combination of the coxsackievirus CVAA21 and ipilimumab is currently being studied in a phase 1b trial in patients with advanced melanoma. The primary objective of this study will be to evaluate the safety and tolerability of multiple intratumoral injections of the combination, which will be assessed by incidence of dose-limiting toxicities in treatment. The secondary objective of the study is to investigate the objective response rate to the combination in patients with advanced melanoma (23). A single-arm phase II trial of CVA21 and pembrolizumab is also underway in patients with advanced melanoma.

Combination of vaccines and cytokines

Other interesting combination trials would include vaccines and other T cell stimulants, such as cytokines. Using a similar HLA-A2-resricted gp100 peptide vaccine as used in the ipilimumab trial, Schwartzentruber and colleagues evaluated the vaccine in combination with high-dose interleukin-2 (IL-2) in patients with advanced melanoma. In this prospective, randomized, phase III clinical trial, 185 patients with stage IV or locally advanced stage III cutaneous melanoma, expressing HLA*A0201, an absence of brain metastases, and suitability for high-dose IL-2 therapy, were randomized to treatment with combination vaccine and IL-2 or IL-2 alone. The combination showed a significant improvement in objective response rate and PFS. Combination treated patients had a significant improvement in objective response rate by central review compared to patients treated with IL-2 alone (16% *vs.* 6%, P=0.03). PFS was 2.2 months in the combination group compared to 1.6 months in IL-2 group alone (P=0.008). The median OS was 17.8 months in patients in the combination group compared to 11.1 months in IL-2 therapy group (P=0.06). The adverse effects were similar in both groups with most toxicity attributed to expected IL-2-related effects (24).

Other T cell agonists, such as interleukin-15 (IL-15), 4-1BB and OX40 agonists, would be expected to be of high interest with vaccines that can elicit antigen-specific T cell responses. While such combinations have shown therapeutic benefit in animal models, clinical trials have not been conducted to date.

Combinations of vaccines and adoptive cell transfer therapy (ACT) therapy

An emerging and promising therapy for cancer is ACT. In ACT, genetically engineered or tumor-reactive T cells, which may be collected from peripheral blood or tumor-infiltrating lymphocytes, are used to treat patients. ACT therapy requires selection of an appropriate antigen for T cell targeting, optimizing the T cell phenotype for transfer, inclusion of non-myeloablative conditioning regimens, and post-transfer T cell support with IL-2 or related immune stimulating factors. The use of chimeric antigen receptors (CARs) utilize single chain antibody Fab fragments with associated TCR signaling elements have shown significant promise in hematologic malignancies.

The use of CAR-modified T cells targeting the B cell-specific antigen CD19 have shown remarkable success in treatment of acute lymphoblastic leukemia. Complete remission rates as high as 90% have been reported in patients including children and adults with relapsed and refractory ALL (25). A recent abstract by Grupp *et al.* at the ASH conference showed 90% complete response in 30 children with CD19+ relapsed, refractory ALL. At the median follow up of 8 months, 16 patients still had ongoing complete response showing the possibility of a long-term response rate (26). The most common toxicity associated with CAR-modified T cell therapy is cytokine release syndrome (CRS), which is an inflammatory process that can lead to a range of clinical presentations from flu-like constitutional symptoms to multisystem organ failure (25). Neurologic toxicity has also been reported.

The use of vaccines encoding similar antigens to stimulate adoptively transferred or CAR T cells have been suggested by combination studies in animal models. Further, T cell persistence has been a hallmark of therapeutic response in small clinical trials, and thus the use of vaccines to boost T cell responses and maintain an expanded population of tumor-antigen specific T cells is a logical strategy. To date, these studies have not yet been done.

Combination of vaccines and radiation therapy

Strategies to combine radiation therapy and immunotherapy are also currently being developed based on observations of an abscopal effect when radiation was used in combination with T cell checkpoint inhibitors (27). Although the mechanisms are not completely understood, it is possible that radiation increases release of tumor-associated antigens, which results in enhanced antigen presentation and induction of T cell immune responses (28). Furthermore, radiation may enhance the expression of MCH class I molecules that may allow for increased exposure of antigens to cytotoxic T cells through peptide-MHC complexes. Also in response to radiation, there is an increased degradation of proteins and generation of new peptides that enables new peptides to be presented on MHC class 1 molecule (29). In addition to these mechanisms, radiation can damage cancer cells leading to the release of immunostimulatory molecules, such as the high mobility group box 1 (HMGB1) protein. HMGB1 molecules interact with toll-like receptor 4 (TLR4) antigen presenting dendritic cells leading to a more enhanced presentation of dying tumor cell antigens (30,31).

The effects of radiation may be synergistic when used in combination with immunotherapy. For example, PD-1 blockade in combination with localized radiation therapy resulted in significantly longer median survival in mice with intracranial gliomas compared to animals treated with either monotherapy (32). In breast cancer mouse models, radiation and CTLA-4 blockade was found to have a greater abscopal effect against secondary sites than either treatment alone (33). Combination of radiation and immunotherapy is being explored in clinical trials as well. Gulley *et al.* conducted a randomized Phase II clinical trial using a combination of radiation therapy and the PROSTVAC-VF vaccine in 30 patients with prostate cancer who were randomized in a 2:1 ratio to combination treatment or radiotherapy alone. Seventeen of 19 patients in the combination group completed their full course of treatment. Thirteen of 17 patients were reported to have a 3-fold increase in PSA-specific CD8+ T cells while there was no detectable increase in the radiotherapy alone group (34). Chi *et al.* conducted an open-label, single arm phase I study of a dendritic cell vaccine and radiotherapy combination in patients with advanced hepatoma not suitable for surgery or transarterial embolization. Patients were injected with autologous immature dendritic cells into the tumor along with radiotherapy. Twelve of 14 patients finished the vaccinations. The treatment was well tolerated with no evidence of autoimmune disease. Two partial responses and four minor responses were reported, and a decrease of AFP of more than 50% was found in three patients. Ten patients showed complete responses 2 weeks after receiving the vaccine. An AFP-specific T cell response was seen in 8 patients analyzed by cytokine release assay and in 7 patients when analyzed by ELISPOT assay. Six patients showed increased NK cell cytotoxic activity (35).

Combination of vaccines and cytotoxic chemotherapy

Similar to radiation, chemotherapy can also help induce an immune response against cancer by promoting antigen release among other mechanisms. Cyclophosphamide has been known for years to potentially help promote anti-tumor immunity by targeting regulatory T cells. For example, Lutsiak *et al.* have reported that administration of cyclophosphamide not only decreases the number of regulatory CD4+ T cells but also decreased the suppressive capability of regulatory T cells. This effect might be mediated by down-regulation of GITR and FoxP3 expression (36). Furthermore, cyclophosphamide may have the ability to enhance higher avidity T cell responses against specific tumors, although inclusion of cyclophosphamide in vaccine regimens has not been associated with prolonged survival in vaccine studies (37).

Lutz *et al.* have demonstrated that in patients with pancreatic ductal adenocarcinoma (PDAC), combination of cyclophosphamide and the allogenic PDAC vaccine (GVAX) can alter the tumor microenvironment by allowing formation of vaccine-induced intratumoral tertiary lymphoid aggregates. These results were particularly impressive since PDAC is considered a non-immunogenic" on-immunogenic gates. These immunotherapies have not been able to induce any significant immune activity. The study provided the first example of converting a Pnon-immunogenic "on-immunogenic have immunogenic" neoplasm (38). It is important to note, however, that the cyclophosphamide could not be directly linked to the formation of the tertiary aggregates in this trial. The role of cyclophosphamide remains controversial and there is the theoretical risk that it may be deleterious due to the non-specific mechanism of action and potential for depletion of effector T cells in addition to regulatory T cells.

In a dose-ranging study, Emens *et al.* established that allogeneic, HER2-positive, GM-CSF-secreting breast tumor vaccine alone or with cyclophosphamide and doxorubicin is safe and can induce a HER2 specific immune response in patients with metastatic breast cancer (39). Based on preclinical and clinical studies, the combination of chemotherapy and vaccine therapy may play a substantial role in future cancer treatments. This general concept has also been demonstrated in the setting of T cell checkpoint blockade resistance, in which tumor cells expressing oncogenic Kras and lacking p53 were not sensitive to checkpoint inhibition (40). In this study, treatment with chemotherapy restored sensitivity to checkpoint blockade through TLR4 simulation and therapeutic responses were dependent on CD8+ T cell responses. These studies support a possible role for chemotherapy in enhancing host anti-tumor immunity, but further clinical studies will be required to confirm the efficacy of the approach and better define the optimal agents and schedule of administration.

Other chemotherapy agents, as well as targeted therapies, may also mediate tumor cell antigen release and enhance T cell priming. Interestingly, in some studies of melanoma tumors following exposure to BRAF inhibitor drugs, an

increase in infiltrating CD4+ and CD8+ T cells was seen (41). These findings suggest that cytotoxic drugs might be able to enhance T cell infiltration into established tumors and support the hypothetical strategy of combining cytotoxic agents with vaccines that can help prime and expand such T cell responses. Further studies of individual cytotoxic drugs with vaccines may be a fruitful avenue for future research.

Vaccines for cancer prevention

Since infectious disease vaccination is more effective in prophylaxis against disease, one approach to consider is to use cancer vaccines in the preventive setting prior to tumor-induced immune suppression. Historically, most tumor vaccines have been used to try to suppress already established and growing tumors. Few clinical trials have been done with tumor vaccines prior to cancer progression. Early prophylactic treatment may show better outcomes since it can potentially prepare the immune system to detect tumor cells before tumors cells have the chance to activate immune suppression mechanisms. Vaccines against viruses such as the hepatitis B virus (HBV) and human papillomavirus (HPV) have shown significant success in liver cancer and cervical cancer prevention, respectively (42,43). In spite of the success of viral antigen based vaccines, the use of non-viral antigens against cancers has not made similar successful leaps to the prevention setting despite preliminary evidence of T cell priming and an acceptable safety profile. This may relate to concerns over regulatory pathways to approval, the large samples sizes and follow-up times needed to conduct such studies or the economic costs associated with prevention studies in oncology. Nevertheless, several murine cancer models have demonstrated significant benefit in tumor prevention with specific vaccine approaches, whereas vaccination with the same vaccine against metastatic cancer had negligible therapeutic impact (44,45).

Kimura *et al.* conducted a clinical trial using a MUC1 peptide vaccine in patients with recent diagnosis and removal of colorectal adenomas. In the trial, 44% of patients developed a MUC-1-specific T cell response with evidence of a memory phenotype. The authors hypothesized that the T cell responders may have already had some degree of immune memory against MUC-1 at baseline and vaccination served as a booster expanding the MUC-1 T cell population (46). The authors also reported that there were few adverse events but some patients did have evidence of high myeloid-derived suppressor cells suggesting that even in patients with advanced adenomas, the immune system may already be compromised. Although the number of patients treated was small (n=39), the authors concluded that the safety and induction of MUC-1-specific T cell responses supported further studies in high-risk populations. An emphasis on defining appropriate high-risk populations and methods for limiting samples sizes and financially supporting prevention trials may be an appropriate path forward for tumor vaccines.

Conclusions

Great progress has been made in the clinical implementation of tumor immunotherapy for the treatment of cancer. Antigens likely play an important role in most forms of immunotherapy, and recent discovery of a correlation between neoantigen emergence and therapeutic response with T cell checkpoint inhibitors has provided renewed interest in the development of vaccines for cancer therapy. Although the optimal antigen, dose, delivery vector, route, schedule, and adjuvant approaches are not established, the ability to rapidly screen the cancer genome in individual patients and development of personalized vaccines based on the results are being actively investigated for feasibility and effectiveness. A variety of new and more potent vectors, such as oncolytic viruses, bacteria and yeast are leading to new strategies for vaccine development. The concept of combination immunotherapy has also gained enthusiasm and the use of vaccines to generate antigen-specific immunity while adding other forms of immunotherapy to boos T cell responses or limit T cell exhaustion are especially exciting. Insights from infectious disease studies suggest that the use of vaccines in prevention approaches may be more meaningful, and the availability of many vaccine regimens with an acceptable safety profile, suggests that the time is right to reconsider vaccines in cancer prevention studies. The next generation of vaccines is likely to become increasingly important in the management of patients with and at risk for cancer.

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Footnote

Conflicts of Interest: Dr. Kaufman has served as a consultant for Alkermes, Amgen, EMD Serono, Merck, Prometheus, and Sanofi and receives research funding from Bristol-Myers- Squibb, Merck and Viralytics. And other authors have no conflicts of interest to declare.

References

1. Rosenberg SA, Yang JC, Restifo NP. Cancer immunotherapy: moving beyond current vaccines. Nat Med 2004;10:909-15.
2. Hodi FS, O'Day SJ, McDermott DF, et al. Improved survival with ipilimumab in patients with metastatic melanoma. N Engl J Med 2010;363:711-23.
3. Sharma P, Allison JP. The future of immune checkpoint therapy. Science 2015;348:56-61.
4. Snyder A, Makarov V, Merghoub T, et al. Genetic basis for clinical response to CTLA-4 blockade in melanoma. N Engl J Med 2014;371:2189-99.
5. Joyce JA, Fearon DT. T cell exclusion, immune privilege, and the tumor microenvironment. Science 2015;348:74-80.
6. Chen DS, Mellman I. Oncology meets immunology: the cancer-immunity cycle. Immunity 2013;39:1-10.
7. Bindea G, Mlecnik B, Tosolini M, et al. Spatiotemporal dynamics of intratumoral immune cells reveal the immune landscape in human cancer. Immunity 2013;39:782-95.
8. Kantoff PW, Higano CS, Shore ND, et al. Sipuleucel-T immunotherapy for castration-resistant prostate cancer. N Engl J Med 2010;363:411-22.
9. Kantoff PW, Schuetz TJ, Blumenstein BA, et al. Overall survival analysis of a phase II randomized controlled trial of a Poxviral-based PSA-targeted immunotherapy in metastatic castration-resistant prostate cancer. J Clin Oncol 2010;28:1099-105.
10. Gillanders WE, Mardis E, Fleming T, et al. Safety and immunogenicity of a personalized polyepitope DNA vaccine strategy in breast cancer patients with persistent triple-negative disease following neoadjuvant chemotherapy. ClinicalTrials.gov 2015:NCT02348320. Available online: https://clinicaltrials.gov/ct2/show/NCT02348320
11. Kaufman HL, Kohlhapp FJ, Zloza A. Oncolytic viruses: a new class of immunotherapy drugs. Nat Rev Drug Discov 2015;14:642-62.
12. Kaufman HL, Bines SD. OPTIM trial: a Phase III trial of an oncolytic herpes virus encoding GM-CSF for unresectable stage III or IV melanoma. Future Oncol 2010;6:941-9.
13. Xia ZJ, Chang JH, Zhang L, et al. Phase III randomized clinical trial of intratumoral injection of E1B gene-deleted adenovirus (H101) combined with cisplatin-based chemotherapy in treating squamous cell cancer of head and neck or esophagus. Ai Zheng 2004;23:1666-70. Chinese.
14. Shafren DR, Dorahy DJ, Ingham RA, et al. Coxsackievirus A21 binds to decay-accelerating factor but requires intercellular adhesion molecule 1 for cell entry. J Virol 1997;71:4736-43.
15. Shafren DR, Au GG, Nguyen T, et al. Systemic therapy of malignant human melanoma tumors by a common cold-producing enterovirus, coxsackievirus a21. Clin Cancer Res 2004;10:53-60.
16. Andtbacka RH, Curtie BD, Kaufman H, et al. Final data from CALM: A phase II study of Coxsackievirus A21 (CVA21) oncolytic virus immunotherapy in patients with advanced melanoma. J Clin Oncol 2015;33:abstr 9030.
17. Shahabi V, Maciag PC, Rivera S, et al. Live, attenuated strains of Listeria and Salmonella as vaccine vectors in cancer treatment. Bioeng Bugs 2010;1:235-43.
18. Maciag PC, Radulovic S, Rothman J. The first clinical use of a live-attenuated Listeria monocytogenes vaccine: a Phase I safety study of Lm-LLO-E7 in patients with advanced carcinoma of the cervix. Vaccine 2009;27:3975-83.
19. Le DT, Wang-Gillam A, Picozzi V, et al. Safety and survival with GVAX pancreas prime and Listeria Monocytogenes-expressing mesothelin (CRS-207) boost vaccines for metastatic pancreatic cancer. J Clin Oncol 2015;33:1325-33.
20. Bilusic M, Heery CR, Arlen PM, et al. Phase I trial of a recombinant yeast-CEA vaccine (GI-6207) in adults with metastatic CEA-expressing carcinoma. Cancer Immunol Immunother 2014;63:225-34.
21. Larkin J, Chiarion-Sileni V, Gonzalez R, et al. Combined nivolumab and ipilimumab or monotherapy in untreated melanoma. N Engl J Med 2015;373:23-34.
22. Puzanov I, Milhem MM, Andtbacka RH, et al. Primary analysis of a phase 1b multicenter trial to evaluate safety and efficacy of talimogene laherparepvec (T-VEC) and ipilimumab (ipi) in previously untreated, unresected stage IIIB-IV melanoma. J Clin Oncol 2014:32abstr 9029^.
23. Curti B. Study of intratumoral CAVATAK (Coxsackievirus A21) and Ipilimumab in patients with advanced melanoma. ClinicalTrials.gov 2015:NCT02307149. Available online: https://clinicaltrials.gov/ct2/show/NCT02307149
24. Schwartzentruber DJ, Lawson DH, Richards JM, et al.

gp100 peptide vaccine and interleukin-2 in patients with advanced melanoma. N Engl J Med 2011;364:2119-27.
25. Maude SL, Teachey DT, Porter DL, et al. CD19-targeted chimeric antigen receptor T-cell therapy for acute lymphoblastic leukemia. Blood 2015;125:4017-23.
26. Maude SL, Shpall EJ, Grupp SA. Chimeric antigen receptor T-cell therapy for ALL. Hematology Am Soc Hematol Educ Program 2014;2014:559-64.
27. Zeng J, Harris TJ, Lim M, et al. Immune modulation and stereotactic radiation: improving local and abscopal responses. Biomed Res Int 2013;2013:658126.
28. Demaria S, Formenti SC. Sensors of ionizing radiation effects on the immunological microenvironment of cancer. Int J Radiat Biol 2007;83:819-25.
29. Formenti SC, Demaria S. Radiation therapy to convert the tumor into an in situ vaccine. Int J Radiat Oncol Biol Phys 2012;84:879-80.
30. Apetoh L, Ghiringhelli F, Tesniere A, et al. Toll-like receptor 4-dependent contribution of the immune system to anticancer chemotherapy and radiotherapy. Nat Med 2007;13:1050-9.
31. Hagemann T, Balkwill F, Lawrence T. Inflammation and cancer: a double-edged sword. Cancer Cell 2007;12:300-1.
32. Zeng J, See AP, Phallen J, et al. Anti-PD-1 blockade and stereotactic radiation produce long-term survival in mice with intracranial gliomas. Int J Radiat Oncol Biol Phys 2013;86:343-9.
33. Dewan MZ, Galloway AE, Kawashima N, et al. Fractionated but not single-dose radiotherapy induces an immune-mediated abscopal effect when combined with anti-CTLA-4 antibody. Clin Cancer Res 2009;15:5379-88.
34. Gulley JL, Arlen PM, Bastian A, et al. Combining a recombinant cancer vaccine with standard definitive radiotherapy in patients with localized prostate cancer. Clin Cancer Res 2005;11:3353-62.
35. Chi KH, Liu SJ, Li CP, et al. Combination of conformal radiotherapy and intratumoral injection of adoptive dendritic cell immunotherapy in refractory hepatoma. J Immunother 2005;28:129-35.
36. Lutsiak ME, Semnani RT, De Pascalis R, et al. Inhibition of CD4(+)25+ T regulatory cell function implicated in enhanced immune response by low-dose cyclophosphamide. Blood 2005;105:2862-8.
37. Le DT, Jaffee EM. Regulatory T-cell modulation using cyclophosphamide in vaccine approaches: a current perspective. Cancer Res 2012;72:3439-44.
38. Lutz ER, Wu AA, Bigelow E, et al. Immunotherapy converts nonimmunogenic pancreatic tumors into immunogenic foci of immune regulation. Cancer Immunol Res 2014;2:616-31.
39. Emens LA, Asquith JM, Leatherman JM, et al. Timed sequential treatment with cyclophosphamide, doxorubicin, and an allogeneic granulocyte-macrophage colony-stimulating factor-secreting breast tumor vaccine: a chemotherapy dose-ranging factorial study of safety and immune activation. J Clin Oncol 2009;27:5911-8.
40. Pfirschke C, Engblom C, Rickelt S, et al. Immunogenic Chemotherapy Sensitizes Tumors to Checkpoint Blockade Therapy. Immunity 2016;44:343-54.
41. Wilmott JS, Long GV, Howle JR, et al. Selective BRAF inhibitors induce marked T-cell infiltration into human metastatic melanoma. Clin Cancer Res 2012;18:1386-94.
42. Lowy DR, Schiller JT. Prophylactic human papillomavirus vaccines. J Clin Invest 2006;116:1167-73.
43. Kane MA. Global implementation of human papillomavirus (HPV) vaccine: lessons from hepatitis B vaccine. Gynecol Oncol 2010;117:S32-5.
44. DuPage M, Jacks T. Genetically engineered mouse models of cancer reveal new insights about the antitumor immune response. Curr Opin Immunol 2013;25:192-9.
45. Koebel CM, Vermi W, Swann JB, et al. Adaptive immunity maintains occult cancer in an equilibrium state. Nature 2007;450:903-7.
46. Kimura T, McKolanis JR, Dzubinski LA, et al. MUC1 vaccine for individuals with advanced adenoma of the colon: a cancer immunoprevention feasibility study. Cancer Prev Res (Phila) 2013;6:18-26.

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