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Cancer Immunoprevention—The Next Frontier

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Abstract

Cancer immunotherapy is a rapidly developing field, but limited in its success by a high tumor burden and immune tolerance. In contrast, immunoprevention has the potential to prevent cancer before the development of immune tolerance, and to prevent cancer recurrence in the setting of minimal residual disease. Although immunoprevention for viral-induced cancers has been successful in the setting of hepatitis B and human papillomavirus vaccination, primary prevention of nonviral-induced cancers is in its infancy. In contrast, prevention of cancer recurrence after adjuvant treatment (secondary prevention) is gaining steam. This review provides an overview of the scope of research in cancer immunoprevention over the last three years and directions for future research.

Introduction

Cancer immunotherapy is currently undergoing exponential growth. The FDA has recently approved the dendritic cell (DC)–based vaccine Sipuleucel-T and the checkpoint inhibitor ipilimumab for the treatment of metastatic castrate-resistant prostate cancer and metastatic melanoma, respectively. Several other agents, including programmed cell death 1 (PD-1) pathway blocking agents (1–3), are currently under clinical investigation, and are showing promising results for the treatment of some advanced cancers. However, the success of immunotherapy for most patients with progressing cancers has been limited because of the established immunosuppressive milieu and a large tumor burden that prevent optimal immune activation and antitumor efficacy. Cancer immunoprevention has the potential to circumvent these problems, and is feasible if applied in the right setting.

Primary cancer immunoprevention is gaining attention because vaccines against hepatitis B and human papillomavirus (HPV) are successfully preventing viral-induced cancers. These

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vaccines are effective due to their ability to prevent primary infection following exposure to these viruses, thereby eliminating their oncogenic potential. Unlike cancers that are induced by viruses, the development of nonviral cancers involves progressive genetic alterations that accumulate over many years and drive the transition from normal tissue through programmed stages of premalignant transformation, and eventual development of the full malignancy. This provides a window of time for primary preventive intervention, especially for high-risk patients. Currently, chemopreventive agents for breast cancer (tamoxifen) and colon cancer (aspirin) are the only clinically proven methods of primary prevention of non-virus-associated cancers. These interventions have limited utility for several reasons, including lack of patient interest due to an unclear benefit, unwanted toxicities experienced by otherwise healthy individuals, need for daily dosing, and lack of physician comfort in prescribing these agents. In contrast to chemoprevention agents, vaccines induce memory T cells that are active for years and are nontoxic in most cases. Thus, immunization of otherwise healthy individuals for primary prevention of cancer offers advantages over drug-based approaches, with low toxicity and no need for daily dosing.

Although the role of immunotherapy in the treatment of cancer is rapidly increasing, the transition to a preventive paradigm has been slower. This review provides an overview of the scope of research in cancer immunoprevention over the last 3 years (Table 1) as well as directions for future research (Figure 1).

Primary Prevention

Vaccination as primary prevention is well established for both hepatitis B-associated hepatocellular carcinoma (4) and for HPV-associated cancers (5). Both the HPV-16/18 AS04-adjuvant vaccine (Cervarix) and the HPV-6/11/16/18 vaccine (Gardasil) are approved by the FDA for the prevention of cervical cancer. In addition to preventing cervical cancer and its precursors cervical intraepithelial neoplasia and adenocarcinoma *in situ* (6), vaccination against HPV also decreases the incidence of penile intraepithelial neoplasia (7) and anal intraepithelial neoplasia (8).

Vaccination as primary prevention for nonviral cancers is in the very early stages of development, with only a few clinical trials that have been initiated in patients. One recent trial targeted MUC1 in patients with premalignant colorectal adenomas and evaluated its immunogenicity, though no clinical endpoints were included in the study (9). MUC1 is a tumor-associated antigen that is highly expressed by both colon cancer and premalignant colorectal adenomas. A 100-amino acid-long synthetic MUC1 peptide with the adjuvant Toll-like receptor 3 agonist poly-ICLC was given to 39 patients with advanced colorectal adenomas. The vaccine was administered at week 0, 2, and 10 and a booster was given at 52 weeks. Seventeen patients (44%) had a measurable MUC1-specific antibody response (anti-MUC1 IgG titer increase by 2-fold by 12 weeks). The vaccine induced long-term memory, with 12 (75%) of the 16 patients who received a booster at 52 weeks responding with an increase in antibody titers. Of the 4 patients (25%) who did not respond, 3 already had persistent high levels of antibody. The authors noted that nonresponders had a significantly higher percentage of circulating CD11b⁺, CD33^{+/low}, and HLA-DR^{-/low} myeloid-derived suppressor cells (MDSC) compared with responders and healthy donors, showing that

immunosuppressive mediators can already be present at the stage of premalignancy. However, there was no difference in regulatory T cells (Tregs) between the groups. Furthermore, no association was found between HLA type and response to vaccination, precluding the need for HLA-typing. MUC1 is currently being investigated in a phase II randomized placebo-controlled clinical trial for patients with advanced colon polyps (NCT02134925). In addition to vaccine response and safety, the investigators will evaluate the association between the presence of MDSCs, immunologic vaccine response, and polyp recurrence. If the presence of MDSCs before treatment is confirmed to be associated with a lower rate of response and subsequent increased rate of progression, this may prove to be a useful marker for selecting the right patient population. It would also provide the rationale for incorporating MDSC-targeted immune modulation in future studies.

Secondary Prevention

Preventing recurrence of solid tumors has traditionally been done with postsurgery adjuvant chemotherapy and/or radiation or with maintenance therapy in patients with no evidence of disease (NED) after induction chemotherapy. The use of immunotherapy has largely been experimental in this setting. For melanoma, one of the more immunogenic tumor types, the FDA has approved interferon- α -2b as adjuvant immunotherapy for patients with high risk of recurrence. However, no other therapies using the immune system to improve progression-free survival (PFS) or overall survival (OS) in patients with NED have been approved.

Several vaccination strategies have shown promise for preventing recurrence in patients with high-risk melanoma. In one clinical trial, 22 patients with stage III melanoma and an unfavorable prognosis received a vaccine consisting of bone marrow and peripheral blood mononuclear cell (PBMC)-derived DC loaded with melanoma-associated peptides (tyrosinase, Melan A/MART-1, gp100, MAGE-1, and/or MAGE-3) or autologous tumor cell lysate, and keyhole limpet hemocyanin (KLH; ref. 10). Although 3-year disease-free survival (DFS) showed a trend toward improvement compared with pair-matched historical controls (40.9% vs. 14.5% in historical controls), only 3-year OS was significantly improved (68.2% vs. 25.7%). Melanoma antigen-specific interferon- γ (IFN γ) producing CD8-positive T lymphocytes were detected in peripheral blood from 13 of the vaccinated patients, including all of the patients who survived more than 5 years.

A peptide vaccine plus ipilimumab combination has also shown benefit in patients with high-risk melanoma. Ipilimumab inhibits signaling through cytotoxic T lymphocyte antigen-4 (CTLA-4), an inhibitory receptor that is expressed on T cells, and has been approved by the FDA for the treatment of advanced unresectable or metastatic melanoma. To investigate the role of ipilimumab in preventing recurrence, 75 patients with resected stage IIIc or metastatic melanoma with NED received ipilimumab with or without vaccination with tyrosinase, gp100, and MART-1 peptides in Montanide ISA 51 VG (11). Although 23% of patients stopped the treatment due to toxicity associated with ipilimumab treatment (e.g., hypophysitis, colitis, and dermatitis), the median recurrence-free survival (RFS) and OS had not been reached at a median follow-up of 29.5 months, which compared favorably with literature-reported DFS in resected stage IV or stage III patients of 7.2 and 8.8 months, respectively. Development of significant side effects, high C-reactive protein

(CRP) at baseline, and a larger change in Th17 inducibility from baseline to 6 months were associated with improved freedom from relapse. However, unlike the previously mentioned study, in this study, the presence of vaccine peptide-specific circulating T cells was not associated with clinical benefit.

Secondary prevention of breast cancer is a rapidly expanding arena with several trials targeting the oncogenic antigen Her-2/NEU (HER2). To date, the HER2-specific monoclonal antibody trastuzumab is the mainstay of adjuvant treatment for HER2-expressing breast cancer. More recently, the FDA approved pertuzumab, a monoclonal antibody that blocks HER2 dimerization, in the neo-adjuvant setting in combination with trastuzumab and docetaxel. In addition, several HER2 T-cell epitopes are being used as targets for vaccination. One of these (E75) has shown promise in a recent trial for patients with lymph node-positive or high-risk lymph node-negative breast cancer (12). A synthetic E75 peptide combined with granulocyte-macrophage colony-stimulating factor (GM-CSF) was given to 106 patients after completion of surgery, chemotherapy, and radiation. A trend toward improvement in 2-year DFS in the vaccinated group was found compared with 76 control patients (94.3% vs. 86.8%, respectively; $P = 0.08$). Subset analysis showed higher 2-year DFS in vaccinated patients who were lymph node-positive (90.2% vs. 79.1%), and who had HER2-low-expressing (94% vs. 79.4%) or low-grade (98.4% vs. 86%) tumors. On the basis of these results, a phase III trial evaluating E75 with GM-CSF as adjuvant treatment for patients with node-positive HER2-low/intermediate breast cancer is currently recruiting patients (NCT01479244). Furthermore, there was no disease recurrence in the group of patients with HER2-high-expressing tumors who received the vaccine in addition to trastuzumab compared with 20% in the group of patients receiving the vaccine alone, prompting initiation of a phase II trial with trastuzumab plus E75/GM-CSF (NCT01570036). Several other HER2 peptides are also being evaluated as vaccine targets (NCT00524277 and NCT01632332). HER2 is not the only antigen being targeted for the secondary prevention of breast cancer. There is a phase Ib trial targeting folate-binding proteins E39 and J65 currently under way for patients with breast or ovarian cancer with NED (NCT02019524). There are also several ongoing trials targeting MUC1: in non-small cell lung cancer (NSCLC; NCT01720836), triple-negative breast cancer (NCT00986609 and NCT00004156), and ovarian cancer, either alone (NCT00006041) or in combination with a HER2 peptide vaccine (NCT00640861).

Immunotherapy for hematologic malignancies is more established than for solid tumors. Aggressive hematologic malignancies often require allogeneic hematopoietic stem cell transplantation (HSCT) to be cured, relying not on the cytotoxic effects of the chemotherapy but on the graft-vs.-tumor effect. Techniques to optimize allogeneic HSCT are currently being explored and are beyond the scope of this review. However, notable developments include addition of tumor-specific vaccines as an adjuvant to either allogeneic (13) or autologous HCST (14). Vaccination approaches for patients in remission after treatment for low-grade hematologic malignancies are less established. A tumor isotype-matched Id (IgM or IgG) protein conjugated to KLH was recently evaluated for the prevention of recurrence of follicular lymphoma after PACE chemotherapy (prednisone, doxorubicin, cyclophosphamide, and etoposide; ref. 15). Median DFS was 44.2 months in the vaccinated

group compared with 30.6 months in the unvaccinated control group (GM-CSF plus KLH). Median OS was not reached. Subset analysis (although the trial was not powered for this) showed a significant difference in DFS in the group vaccinated with IgM isotype versus control (52.9 vs. 28.7 months). Although these results appear promising, this trial was conducted before the addition of rituximab to the standard treatment of follicular lymphoma. The Id vaccine induced both humoral and cellular immunity, and although rituximab delays induction of the humoral response, it does not affect tumor-specific cellular immunity. In contrast to previous negative trials, this trial with an Id vaccine did demonstrate a clinical benefit. However, the authors note that patient selection and vaccine production was different in previous trials, which could explain the different findings.

Other approaches being investigated for secondary prevention include viral vectors encoding tumor antigens and GM-CSF secreting whole tumor cell vaccines (GVAX). One recent trial compared viral vectors (poxvirus and recombinant vaccinia virus) encoding CEA, MUC1, and costimulatory molecules CD80, CD54, and CD58 (PANVAC) given with GM-CSF to an autologous PANVAC-modified DC vaccine in patients with resectable metastatic colorectal (16). RFS and OS were similar in both groups (RFS: 22.9 vs. 28.9 months with median OS not reached in either group of 37 patients each). When the vaccinated group as a whole was compared with historical controls, the vaccinated group showed similar RFS (21.9 vs. 25.7 months), but showed improved OS (not reached in study group vs. 44.1 months). Although the comparison was made with a historical group, if a similar difference is again found in a randomized controlled trial, this would have implications for future trial design, as investigators more often look at PFS than at OS.

Our group has been developing GVAX for pancreatic ductal adenocarcinoma (PDA), and previously demonstrated that mesothelin-specific CD8⁺ T-cell responses induced by GVAX are associated with longer DFS and OS in a phase II study of adjuvant GVAX for resectable PDA (17). However, the increased OS compared with historical controls (24.8 months for vaccinated patients vs. 20.3 months for controls) was modest, indicating that GVAX by itself is generally not sufficient. Because we have seen improved activity in patients with advanced unresectable PDA when GVAX is combined with low-dose cyclophosphamide to inhibit Tregs (18), we conducted two trials investigating the use of GVAX combined with cyclophosphamide in patients with resectable PDA. In the first, we are evaluating the activity of GVAX with or without cyclophosphamide as (neo-) adjuvant treatment before and after surgery and chemoradiation (NCT00727441; ref. 19). In the second, we are evaluating the safety of integrating GVAX combined with cyclophosphamide with adjuvant chemoradiation (FOLFIRINOX) in this patient population (NCT01595321).

Although survival analyses for these ongoing studies are pending, we have observed the development of vaccine-induced intratumoral lymphoid aggregates within 2 weeks of GVAX treatment in 85% of patients evaluated thus far who were treated in our neo-adjuvant study (19). These aggregates not only confirm activity of the vaccine, but also show that the tumor microenvironment undergoes significant changes in response to GVAX treatment, and demonstrate that treatment with GVAX can convert an immunologically quiescent tumor into an immunologically active tumor. Some of the changes induced by GVAX, such as the upregulation of the PD-1 ligand PD-L1, may represent targetable resistance

mechanisms. Our data suggest that multiple immune-evasion mechanisms with the potential to counter GVAX are active in PDA tumors, and could explain the inefficacy of GVAX alone. These data also suggest that the reason immune checkpoint inhibitors fail as single agents against PDA is because there are too few tumor-specific immune effectors for these agents to act on without a vaccine. In support of this idea, we have already shown that combining GVAX with ipilimumab is capable of inducing PDA tumor regression, and is associated with improved survival compared with ipilimumab alone (5.7 vs. 3.6 months) in the metastatic setting (20). Thus, this combination may be effective in the adjuvant setting as well but the significant toxicities of ipilimumab may limit its usefulness in this setting. Additional studies are planned to evaluate GVAX in combination with other immune modulators, including PD-1 pathway inhibitors, to determine which modulators are most capable of enhancing the activity of GVAX and inducing clinically relevant antitumor protection. Thus far, the data suggest that in most patients with PDA, multiple immune checkpoint pathways may need to be inhibited to achieve optimal immune activation and clinical efficacy.

Although the above-mentioned trials are encouraging, there have also been trials that have shown no benefit, or even a detrimental effect from vaccination. In 2005, a phase III trial evaluating Canvaxin for stage III melanoma was stopped because of lack of clinical benefit. More recently, vaccination against MAGE-A3 (ref. 21 and the recently stopped MAGRIT trial) has shown no effect on DFS in patients with resected NSCLC. In addition, two out of three independent studies evaluating Id vaccines for follicular lymphoma did not show signs of clinical benefit. There are numerous potential reasons for these failures, including the choice of vaccine vector, the choice of adjuvant, or the level of vaccine-targeted antigen presented by antigen-presenting cells. It is also possible that, in some cases, the vaccines did their job and induced an immune response, but the tumor evaded the immune response. In addition, the choice of antigens targeted may be less than optimal. The vaccines evaluated thus far target tumor-associated antigens that are not mutated and are expressed by normal tissues. Unlike the viral antigens targeted by vaccines for viral-associated cancers, tumor-associated antigens are not tumor specific, and most T cells capable of recognizing them with high avidity (like the T cells that recognize viral antigens) are either deleted or rendered inactive by peripheral tolerance mechanisms. Differences in the repertoire of T cells available and immune tolerance mechanisms at play are likely two of the more important reasons why the vaccines tested so far have induced weaker immune responses, why fewer patients have responded, and why overall these vaccines have been less effective than vaccines targeting viruses that cause cancer.

In addition to the above-mentioned trials that failed to show a benefit, one trial targeting GM2 ganglioside antigen was stopped because of improved survival in the observation control arm (22). Although the authors noted that this may have been due to the composition of their vaccine or the vaccination schedule, inflammation can drive tumor development (23) and the results of this study suggest that in some cases, vaccines may have the opposite effect and support tumor growth rather than provide protection. Thus, it will be important to establish biomarkers capable of predicting outcomes, good or bad, following treatment.

Primary Prevention Targeting the Earliest Genetic Changes and the Tumor Microenvironment

For preventive vaccination to be feasible, specific targets need to be identified that are expressed early, and ideally, are responsible for driving the change from normal cells to premalignant cells or from premalignant to malignant cells. Mutations provide particularly appealing targets for vaccines because they are specific to the tumor, differentiating them from normal tissue. Furthermore, because they are not encountered before the onset of tumor development, each mutation has the potential to generate a novel and essentially foreign antigen (termed neo-epitope). Thus, T cells recognizing mutant tumor neo-epitopes are likely to have higher avidities for antigen than T cells that recognize tumor-associated antigens such as mesothelin, MUC1, or HER2, and could therefore respond more robustly to vaccination. In addition, T cells recognizing mutant tumor neo-epitopes are capable of driving antitumor immune responses (24–27).

PDA is an example of a malignant transformation that is strongly associated with specific mutations in Kras. Mutated Kras is present in >90% of PDA and is one of the earliest genetic changes found in premalignant pancreatic intraepithelial neoplasm (PanIN) lesions (28). Mutated Kras is not only involved in the initiation of oncogenesis, but is also essential for the progression and maintenance of PDA (29). Mutated Kras therefore provides an example of an ideal target for vaccination in the preventive setting. However, immunosuppressive subsets of inflammatory cells, including Tregs and MDSCs, that are known to be present in the PDA tumor microenvironment (30) are also found in premalignant PanINs as contributors to tumor development and progression (31). Therefore, simply targeting mutations, such as mutated Kras, without countering these early immunoregulatory signals may be ineffective for primary prevention of PDA.

In support of this idea, we have shown in a mouse model that primary prevention of PDA is feasible, but requires simultaneous modulation of the immunosuppressive microenvironment (32). We vaccinated KPC mice (that express mutated Kras and p53 specifically in the pancreas that drive the development of PDA) with an attenuated intracellular *Listeria monocytogenes* (LM) vaccine genetically modified to express the entire mutant Kras^{G12D} gene product that drives PDA development in KPC mice (LM-Kras). *Listeria monocytogenes*-based vaccines expressing the entire mutated protein induce robust CD4⁺ and CD8⁺ T-cell immunity against multiple mutated antigenic epitopes independent of their major histocompatibility type (33) and have been shown to be safe and active as treatment for patients with metastatic PDA (34). One day before vaccination, we depleted Tregs with anti-CD25 antibody (PC61) in conjunction with low-dose cyclophosphamide. The combination of vaccination with Treg depletion slowed progression to PDA when administered at an early stage (PanIN 1). When mice were given the vaccine alone, PanIN progressed to PDA despite the induction of peripheral CD8⁺ T-cell responses against mutated Kras, demonstrating that the induction of cytotoxic T cells alone was in itself not sufficient, but that manipulation of the immunosuppressive environment was necessary to prevent PanIN progression to PDA. We also found that CD11b⁺ Gr-1⁺ cells from the pancreata of treated mice secreted higher levels of immunostimulatory cytokines and

chemokines than untreated mice, including chemokines responsible for recruiting myeloid and lymphoid cells, and M1/N1-associated cytokines responsible for promoting IFN γ and IL17 responses in T cells. Chemokine and cytokine production by Gr-1⁺ cells was the highest when LM-Kras was combined with Treg depletion. The data from this model demonstrate that targeting an early genetic driver mutation is feasible, and can be successful provided it is done at an early stage, and combined with appropriate immune modulation.

The Future of Cancer Immunoprevention: Challenges Influencing New Developments

Some vaccination strategies in the secondary prevention setting have shown promise. Additional progress will require the addition of immune-modulating agents that alter the procarcinogenic tumor microenvironment that is likely unique to micrometastases relative to premalignant and metastatic microenvironments. Additional studies are required to further delineate these differences and identify immune-modulating agents that improve vaccine activity and minimize toxicities. In contrast, there has been little progress to date in the development of successful primary prevention strategies. Although vaccination against HBV and HPV has proven to prevent HBV and HPV-associated cancers when given before exposure to the viruses, vaccines do not currently exist for the prevention of other oncoviruses, such as human T lymphotropic virus (HTLV), merkel cell polyomavirus, Kaposi-sarcoma-associated virus (HHV-8), and Epstein-Barr virus (EBV) that exhibit complex mechanisms of oncogenesis. In addition, due to the complex nature of the developing tumor microenvironment associated with initial oncovirus exposure or initial driver gene mutation, establishing a cancer immunopreventive paradigm for situations in which an individual has been either exposed to the oncovirus or expresses the initiating mutation of a non-oncovirus-induced malignancy has proven challenging. For example, there is little data supporting potential vaccine targets and platforms for preventive vaccination. In addition, there is a lack of well-defined high-risk populations and adequate numbers of subjects at risk for many types of cancer, which provide a barrier to conducting "proof of principle" clinical trials even if a vaccine were available for testing in this setting. Furthermore, in contrast to clinical trials in the secondary prevention setting, data on cancer-free survival or OS in the primary prevention setting will take many years to mature. Thus, adequate biomarkers of response are needed as well to test any new vaccine approach.

For each tumor type, a high-throughput approach is needed to identify potential targets. One approach that was recently used by Broussard and colleagues (35) is to combine expression array and siRNA screening to identify genes that are upregulated in adenoma and colorectal cancer cell lines. They found that silencing the most highly upregulated genes (*CDH3*, *CLDN1*, *KRT23*, and *MMP7*) resulted in decreased viability and proliferation of these cell lines, as well as increased apoptosis (*CDH3* and *KRT23*). They then determined the immunogenicity of these proteins by assessing serum IgG against these proteins in patients with colorectal cancer. One concern with determining the immunogenicity of upregulated antigens using sera from patients with cancer is that the immune system can be suppressed. Furthermore, if an immune response has been mounted against an antigen expressed on the tumor, this antigen may not have an essential role in oncogenesis. Thus, the best targets may

be antigens that a patient with cancer does not mount an immune response against, but has the potential to do so if properly activated.

Ideally, vaccine targets would be commonly mutated driver genes, such as Kras for PDA. However, one possible limitation to targeting only commonly mutated driver genes is that each of these mutations will be presented by a restricted subset of HLA molecules, and will therefore only be targetable in a subset of HLA compatible patients. Furthermore, it is estimated that cancer cells only carry a few driver mutations, and even when the same gene is mutated, the specific mutations are often variable between different patients' tumors (36). Thus, common driver mutations are relatively rare. Mutations uniquely specific to each patient's tumor may also provide ideal candidates. Targeting these antigens is currently not feasible in the primary prevention setting, but is now being considered in the secondary prevention setting, given the availability of cancer tissue and the feasibility of next-generation exome sequencing that can rapidly identify targets for patient-specific vaccines. Furthermore, in the secondary prevention setting, immune tolerance may already be established to these mutated antigens, and therefore the addition of immune-modulatory agents may be required to induce clinically potent antitumor immune responses.

Other targets that could be further explored are nonmutated oncoproteins (such as HER2), oncofetal proteins (e.g., CEA), cancer–testes antigens (e.g., MAGE-A3), translocations (e.g., bcr-abl), "tissue lineage" antigens (e.g., PAP, PSA, gp100, and tyrosinase), or mutated microenvironment antigens (e.g., CLEC14a, which has been found in the tumor vasculature). However, as mentioned before, the immune responses generated in the secondary prevention setting against these shared tumor-associated antigens have been weak and present only in a small percentage of patients. One concern with target selection is that antigens that are not essential for driving the malignant process could be down-regulated by the tumor and lead to resistance (as is the case with EGFR inhibitors in lung cancer or BRAF inhibitors in melanoma). Another concern, especially with "tissue lineage" antigens, is that targeting antigens that are also expressed on normal tissue could potentially cause autoimmunity. Although autoimmune side effects, such as hypophysitis and colitis, are seen following treatment with immune checkpoint inhibitors, to date very little autoimmunity has been seen following vaccination against cancer associated antigens. The nonspecific nature of checkpoint inhibitors makes them more toxic than other more targeted approaches such as antigen-specific vaccination. For this reason, checkpoint inhibitors may not be ideal for the primary preventive setting, but their inclusion with vaccines may be necessary for secondary prevention of cancers with a high risk of recurrence particularly those that recur within a short time following remission.

Many different platforms for vaccination delivery are currently being investigated in the immunotherapeutic setting. One problem with transferring successful vaccination techniques from the treatment to preventive setting is that the production of these vaccines is often complicated, making some of them less suitable for large scale use. Less complicated vaccines, such as peptide-based vaccines, would be ideal candidates for large-scale use, whereas more complicated vaccines, such as DC-based vaccines, could potentially be developed for a highly specific at-risk population. Other potential platforms for large-scale synthesis include poxvirus or *Listeria monocytogenes* vectors that give the option of

inserting multiple antigen constructs, and thereby targeting multiple antigens simultaneously. The use of monoclonal antibodies (e.g., rituximab and trastuzumab) is limited in the preventive setting due to their side effects and frequent dosing schedule, but newly designed constructs, such as semisynthetic peptibodies, bispecific T-cell engaging antibodies (e.g., peptide with anti-CD3), or chimeric antigen receptor (CAR)-modified T cells, are currently being developed and may provide additional options for the preventive setting.

Large-scale vaccination for the general population, such as is being done with HPV vaccination, is unlikely to be successful for nonviral-induced cancers given the low likelihood of any given person developing a specific type of cancer. Therefore, high-risk populations need to be identified. Carriers of BRCA1 or BRCA2 mutations are ideal candidates. Vaccination in this population provides a huge potential for decreased morbidity as preventive surgery is the currently recommended strategy. Another potential high-risk group includes individuals with p53 or mismatch repair gene mutations, such as hereditary nonpolyposis colorectal cancer (HNPCC or Lynch syndrome) or Li-Fraumeni syndrome, or individuals with a strong family history of a specific type of cancer. Given the potential wide range of mutated genes in the cancers that develop in these patients, identifying predictable mutated targets may be difficult. However, sensitive approaches are being developed for sequencing circulating tumor DNA (37). Thus, if we develop strategies for predicting the likelihood of a mutation to promote tumor development, and the likelihood of a mutation to be recognized by the immune system, it may be possible to combine these strategies to identify vaccine targets in high-risk patients early on, before a bulky tumor and immune tolerance have developed. Other populations that could benefit from preventive vaccination include individuals with already identified precancerous lesions, such as colorectal adenoma.

Clinical trial design is one of the challenging aspects of any preventive strategy. The drug approval process is much slower than in the therapeutic setting, because assessing the effect of a preventive intervention on event-free survival or OS takes many years. We therefore need surrogate endpoints that would be expected to correlate with a cancer-free survival benefit, but that can be measured at a much earlier stage. In the case of virus-causing cancers, antibody responses to viral proteins serve as surrogate endpoints. However, antibody responses are less likely to provide the same protection against genetically programmed cancers. Therefore, other surrogate endpoints need to be evaluated. Examples include measuring the development of precancerous lesions or cancer markers such as circulating DNA.

Conclusion

To allow for effective immune-prevention strategies, there should be a careful balance between the ability to prevent cancer, costs, and side effects. Ideal vaccines would target antigens that are essential for tumor survival and specific to cancer cells, such as driver mutations, and care should be taken to select an appropriate high-risk population. Ideally, a premalignant marker should be available to establish success of the therapy in a reasonable time frame. Immunotherapy as a single preventive treatment may be sufficient, but

depending on the timing and tumor type, may need to be combined with other agents capable of modulating the tumor microenvironment. The road to successful preventive immunization strategies for nonviral-induced cancer is long, and will require the development of high-throughput methods for antigen and biomarker identification, the development of proven vaccine constructs, and careful clinical trial design.

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Figure 1.

Timeline of malignant transformation and prevention. As transformation to malignancy progresses, an increasing amount of immunosuppressive mediators are present in the tumor microenvironment. Primary prevention vaccines should target early driver genes and could by themselves be sufficient to prevent further malignant transformation, although immunosuppressive mediators have been found in some premalignant lesions. Secondary prevention should include both driver gene targets and immune modulators. M, macrophage; N, neutrophil; Tc, cytotoxic T cell; Tr, regulatory T cell.

Table 1

Overview of recent nonviral cancer immunoprevention studies

Cancer	Vaccine type	Adjuvant	Target	Treatment effect	Reference
Primary prevention					
Colon cancer prevention for patients with colorectal adenomas	Peptide vaccine	Toll-like receptor 3 agonist (poly-ICLC)	MUC1	<ul style="list-style-type: none"> 44% MUC1-specific antibody response Vaccine induces long-term memory Nonresponders showed higher percentage of immunosuppressive mediators No clinical endpoints 	9
Secondary prevention					
Melanoma	DC vaccine	KLH	Melanoma-associated antigens (tyrosinase, Melan-A/MART-1, gp100, MAGE-1, and MAGE-3) or autologous tumor cell lysate	<ul style="list-style-type: none"> 3-year DFS 40.9% vs. 14.5% (historical controls, $P = 0.1083$) 3-year OS 68.2% vs. 25.7% (historical controls, $P = 0.0290$) 	10
Melanoma	Ipilimumab ± peptide vaccination	Montanide ISA 51 VG	Tyrosinase, gp100, MART-1	<ul style="list-style-type: none"> 23% stopped treatment due to toxicity related to ipilimumab Median RFS and OS not reached at 29.5 months. 	11
Breast cancer	Peptide vaccine	GM-CSF	HER2 peptide E75	<ul style="list-style-type: none"> 2-year DFS 94.3% vs. 86.6% (controls, $P = 0.08$) Subset analysis: improved DFS in node-positive, low HER2-expressing and low grade tumors. Improved DFS in HER2-high-expressing patients receiving vaccine plus trastuzumab compared with vaccine alone 	12
Follicular lymphoma	Protein vaccine	KLH	Tumor isotype matched Id (IgM and IgG)	<ul style="list-style-type: none"> Median DFS 44.2 vs. 30.6 months (controls, $P = 0.047$) Median OS not reached Subset analysis: improved DFS with IgM isotype vaccine Trial done pre-rituximab 	15
NSCLC	Protein vaccine	AS02 _B	MAGE-A3	<ul style="list-style-type: none"> 44-month recurrence rate 35% vs. 43% (controls, not significant). DFS or OS not significantly improved 	21
Melanoma	Peptide vaccine	KLH plus QS-21	GM2 ganglioside	<ul style="list-style-type: none"> Trial stopped after median follow up of 18 months due to increased survival in control group (HR, 1.66; $P = 0.02$) 	22
Colorectal cancer	Viral vector vs. DC vaccine	GM-CSF (viral vector)	CEA, MUC1, CD80, CD54, and CD58	<ul style="list-style-type: none"> RFS 22.9 vs. 28.9 months (DC vs. viral vector) 	16

Cancer	Vaccine type	Adjuvant	Target	Treatment effect	Reference
Pancreatic cancer	Whole cell vaccine (GVAX)	GM-CSF	Allogeneic pancreatic cancer cell line antigens (incl. mutated k-ras, mesothelin)	<ul style="list-style-type: none">• Median OS not reached in either group vs. 44.1 months (historic controls, $P < 0.0001$)• OS 24.8 vs. 20.3 months (historical controls, not significant)• Induction of mesothelin-specific CD8 T cells correlated with increased DFS	17