Published OnlineFirst July 8, 2015; DOI: 10.1158/2326-6066.CIR-15-0112

Cancer Immunology at the Crossroads: Engineering

Cancer Immunology Research

Engineering New Approaches to Cancer Vaccines

Naveen K. Mehta[1,2], Kelly D. Moynihan[1,2,3], and Darrell J. Irvine[1,2,3,4,5]

Abstract

Recently, a number of promising approaches have been developed using synthetic chemistry, materials science, and bioengineering-based strategies to address challenges in the design of more effective cancer vaccines. At the stage of initial priming, potency can be improved by maximizing vaccine delivery to lymph nodes. Because lymphatic uptake from peripheral tissues is strongly size dependent, antigens and adjuvants packaged into optimally sized nanoparticles access the lymph node with much greater efficiency than unformulated vaccines. Once primed, T cells must home to the tumor site. Because T cells acquire the necessary surface receptors in the local lymph node draining the tissue of interest, vaccines must be engineered that reach organs, such as the lung and gut, which are common sites of tumor lesions but inaccessible by traditional vaccination routes. Particulate vaccine carriers can improve antigen exposure in these organs, resulting in greater lymphocyte priming. Immunomodulatory agents can also be injected directly into the tumor site to stimulate a systemic response capable of clearing even distal lesions; materials have been designed that entrap or slowly release immunomodulators at the tumor site, reducing systemic exposure and improving therapeutic efficacy. Finally, lessons learned from the design of biomaterial-based scaffolds in regenerative medicine have led to the development of implantable vaccines that recruit and activate antigen-presenting cells to drive antitumor immunity. Overall, these engineering strategies represent an expanding toolkit to create safe and effective cancer vaccines. *Cancer Immunol Res; 3(8); 836–43. ©2015 AACR.*

Motivation for Cancer Vaccine Engineering

Therapeutic vaccination is one of the oldest and most studied concepts in cancer immunotherapy. Yet, in contrast with prophylactic vaccines against infectious disease, which have had a major impact on public health, therapeutic vaccines against cancer have generally been much less successful, and only a single cancer vaccine has been FDA approved to date (1, 2). This is likely due to a variety of factors, including a paucity of truly foreign antigens expressed by tumor cells, lack of infection-associated inflammatory cues that drive productive immunity, chronic antigen exposure, the presence of a highly immunosuppressive microenvironment in solid tumors, and our as yet still poor understanding of how to induce strong and sustained T-cell–mediated immune responses in humans. However, there are at least three reasons why cancer vaccines should see renewed interest as part of the cancer immunotherapy armamentarium, based on recent rapid advances in the field: To begin, the advent of clinical-stage therapeutics that can directly influence the immunological status of the tumor microenvironment, such as checkpoint blockade antibodies (3), regulatory T cell (Treg)–modulating chemotherapy (4), and indoleamine 2,3-dioxygenase (IDO) inhibitors (ref. 5; for example), now provide a number of ways to overcome immunosuppressive pathways in patients. Secondly, the availability of an ever-growing array of targeted drugs that can dramatically (but transiently) lower tumor burden provides a window of opportunity for vaccines to act in a setting of minimal disease, and some of these drugs may act synergistically with the immune response (6). Third, the powerful genomic sequencing capabilities are enabling the possibility of patient-specific vaccines targeting defined neoantigens, which have the potential for alleviating the safety and efficacy challenges of targeting unmutated self-antigens (7–10). Together, these recent developments in cancer therapy strongly motivate renewed efforts to develop effective therapeutic cancer vaccine approaches.

How might we enhance the vaccines themselves to enable therapeutic immunization to reach its full potential in this new era of cancer immunotherapy? The first concern is vaccine potency, as measured by the number, functionality, and avidity of antigen-specific T cells induced by cancer vaccines. A number of experimental and licensed infectious disease vaccines induce robust multifunctional $CD4^+$ and $CD8^+$ T-cell responses in humans that can be detected directly *ex vivo* and measured even by relatively low-sensitivity methods, such as peptide–MHC tetramer staining (11, 12). By contrast, with a few exceptions (13, 14), the response to cancer vaccines is often only robustly detected by expanding/stimulating patient T cells over 1 to 2 weeks *ex vivo* (15–17)—a direct indicator of the low frequency of responding cells. These results may be partly due to issues of tolerance to self-antigens and systemic immunosuppression in cancer patients, but also may reflect the common use of minimal-epitope peptide vaccines and weak adjuvants that are known to have immunologic shortcomings (18). Equally important is for vaccines to be capable of promoting T-cell responses enriched in high-avidity, polyfunctional T cells

[1]Koch Institute for Integrative Cancer Research, Massachusetts Institute of Technology, Cambridge, Massachusetts. [2]Department of Biological Engineering, Massachusetts Institute of Technology, Cambridge, Massachusetts. [3]Ragon Institute of MGH, MIT and Harvard University, Boston, Massachusetts. [4]Department of Materials Science and Engineering, Massachusetts Institute of Technology, Cambridge, Massachusetts. [5]Howard Hughes Medical Institute, Chevy Chase, Maryland.

N.K. Mehta and K.D. Moynihan contributed equally to this article.

Corresponding Author: Darrell J. Irvine, MIT, MIT Room 76-261, 77 Massachusetts Avenue, Cambridge, MA 02139. Phone: 617-452-4174; Fax: 617-452-3293; E-mail: djirvine@mit.edu

doi: 10.1158/2326-6066.CIR-15-0112

Published OnlineFirst July 8, 2015; DOI: 10.1158/2326-6066.CIR-15-0112

with high proliferative capacity that avoid induction of an exhausted/terminally differentiated phenotype. Finally, devising vaccine strategies that prime effective trafficking of effector cells to tumor sites is critical, which, in cases such as mucosal tumors, could be directly influenced by vaccines that program expression of appropriate tissue-homing receptors (19). Thus, a number of strategies exist to enhance current vaccine approaches to increase the efficacy of therapeutic antitumor immune responses.

There are many ways to improve therapeutic vaccines rooted in traditional vaccinology principles, such as microbial vector development, molecular biology, and adjuvant design. In this brief Cancer Immunology at the Crossroads perspective, we will review promising recent preclinical and early clinical developments derived from approaches based in immune engineering—bringing methods from chemistry, chemical engineering, materials science, and biological engineering to bear on the problem of therapeutic vaccine design. Such approaches are particularly well suited to augmenting vaccines based on subunit antigen (defined protein, peptide, or polysaccharide epitopes) and tumor-cell lysate-based vaccines, and we focus on these two ubiquitous classes of cancer vaccine antigens.

Targeting Vaccines to Lymph Nodes

A fundamental issue in generating robust immunity with cancer vaccines is efficient delivery of vaccine components to lymphoid tissues, the sites of immune response orchestration. Nearly all vaccines are administered parenterally, either intramuscularly or subcutaneously. Following injection of soluble protein/peptide vaccines, an antigen arrives in the draining lymph nodes (LN) in two phases: First, LN-resident dendritic cells (DC) directly access the antigen as it drains through the afferent ducts and present the antigen to T cells to initiate an immune response. This response is sustained during the second phase, when migratory DCs or monocytes that have phagocytosed additional antigens at the site of injection arrive in the LN (20). In some settings, however, only the first phase may be necessary: in mice vaccinated with protein antigens fused to an anti-CD205 antibody to target cross-presenting LN-resident DCs, migratory DC depletion actually enhanced T-cell priming (21). Migratory DCs were shown to contain expression signatures enriched in genes associated with immune suppression, compared with the expression signatures in cross-presenting LN-resident DCs. That LN delivery is key to vaccine potency is shown by studies of intra-LN injections, which demonstrated that peptide or DNA vaccines injected directly into LNs are at least 100-fold more potent than the same vaccine administered subcutaneously (22, 23).

Although the fate of injected vaccines is a complex interplay of numerous parameters, the physical size of vaccine components—whether they be particulates or individual molecules—plays a significant role in determining the outcome, as shown in Fig. 1A (24, 25). Molecules or particles injected in tissue can be cleared by either entering the blood or the lymph. In classic studies in sheep comparing the biodistribution of a series of molecules of varying molecular weight from tissues following injection, Supersaxo and colleagues showed that large proteins preferentially convected to the LN rather than being lost to systemic circulation (26). This is because the lymphatic endothelium has valve-like openings enabling the entry of large particles, whereas the capillary endothelium is lined by an uninterrupted basement membrane that blocks the transit of large macromolecules. A linear correlation is observed between molecular weight and the fraction of LN uptake up to a threshold of 45 kDa (corresponding to a size of approximately 4–5 nm in diameter for a globular protein), at which point nearly 100% of protein is delivered to the lymph and downstream draining LN (27). Consistent with this finding, unformulated peptides (28), molecular adjuvants (28–30), and small protein antigens show very poor uptake in LNs, and soluble small-molecule adjuvants often show significant systemic inflammatory toxicity (28–30). Although both preclinical and clinical studies have often sought to solve this problem by administering vaccines in "depot"-based adjuvants, such as incomplete Freund's adjuvant, it has been shown that passive, noninflammatory depots of antigens at the injection site become a decoy for effector cells that leads to deletion of the very T cells that are meant to be primed by the vaccine (31).

The size-dependent physiology of lymphatic trafficking has motivated studies of synthetic nanoparticles larger than individual proteins as carriers to efficiently deliver small antigens/molecular adjuvants to the LN. Maximal LN targeting is a size optimization problem, as particles that are larger than the average pore size in the extracellular matrix may become entrapped in the tissue rather than convecting to lymphatics. A series of studies by three different groups demonstrated that nanoparticles with diameters under approximately 50 nm target LNs much more efficiently than larger particles. Reddy and colleagues injected labeled 20-nm, 45-nm, and 100-nm poly(propylene sulfide) nanoparticles intradermally and sampled the draining LNs up to 120 hours later; although the 20-nm and 45-nm ultra-small nanoparticles were present in the LN throughout the time points sampled, the 100-nm particles could not be detected (32). Manolova and colleagues (33) and Fifis and colleagues (34) reached similar conclusions using virus-like particles or synthetic polystyrene nanoparticles of different sizes as carriers for vaccines, demonstrating that particles in the 20- to 40-nm size range were much more effective for LN delivery and subsequent vaccine responses than larger particles. Nanoparticles can also be used to deliver potent molecular adjuvants to LNs, and promising data in both mice (30, 35–40), and nonhuman primates (36, 41) suggest that this is an approach that should be moved toward clinical testing, especially for small-molecule adjuvants, for which such approaches can increase both the safety and potency (42). Finally, upon arrival in LNs, nanoparticles have the potential to affect multiple aspects of antigen processing and presentation by enabling antigen and adjuvant to be codelivered into recipient antigen-presenting cells (APC; ref. 43), promoting cross-presentation of antigens (35, 44), and acting as intracellular/extracellular vaccine depots (44, 45). An exciting recent study demonstrated a method to coat polymer nanoparticles with native tumor cell–derived plasma membranes, leading to cross-presentation of tumor membrane–associated antigens, thus providing a means to combine complex tumor-derived antigen mixtures with particle-based LN targeting (46).

A second strategy to target vaccines to LNs is to exploit reversible binding to proteins naturally meeting the size-dependent criteria for effective lymphatic uptake. For example, albumin, the most prevalent protein in blood and interstitial fluid, is a 66-kDa globular protein with a hydrodynamic diameter of approximately

Published OnlineFirst July 8, 2015; DOI: 10.1158/2326-6066.CIR-15-0112

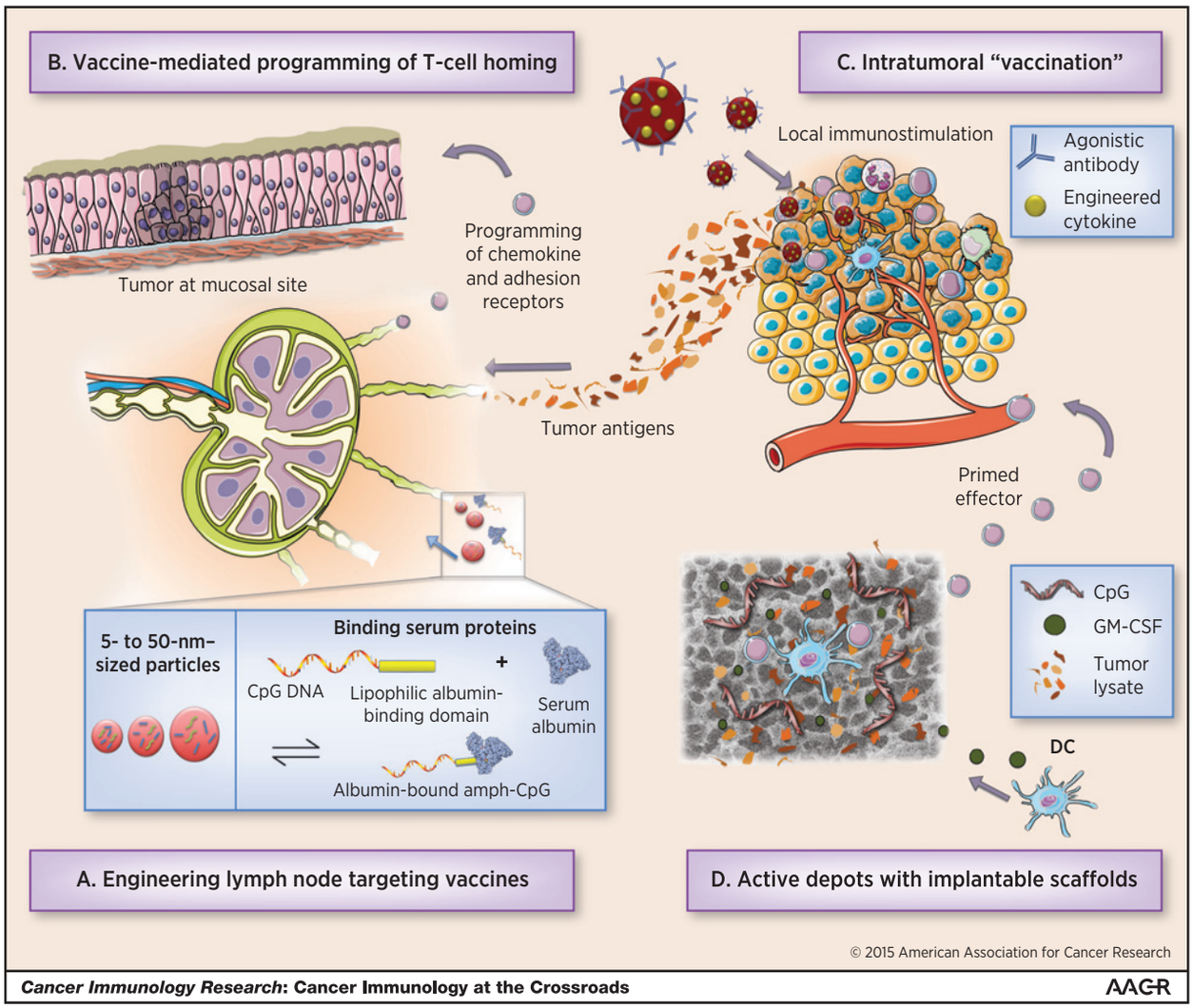

Figure 1.
A schematic diagram showing four strategies for engineering more effective cancer vaccines. A, vaccines are engineered to drain efficiently to LNs. Particles between 5 and 50 nm in size drain more effectively than particles of larger sizes, and molecular vaccines can be engineered to bind serum proteins to meet this size criterion for effective lymphatic drainage. B, T-cell homing to specific sites can be directed by the route of administration. For sites like the lung and the gut, engineering of biomaterial carriers may facilitate delivery. C, immunomodulatory therapies can be introduced directly into the tumor to generate antitumor responses. D, implantable biomaterial scaffolds can be loaded with tumor antigens and inflammatory signals to create an *in situ* dendritic cell vaccine.

5 nm, and thus traffics one way from the blood to the lymph in the interstitial space. Liu and colleagues conjugated peptide antigens and CpG adjuvant to saturated hydrocarbon lipid tails chosen to promote binding to fatty acid–binding pockets of albumin (28). Importantly, these conjugates comprised an albumin-binding tail linked to the antigen via a highly water-soluble poly(ethylene glycol; PEG) chain. This PEG spacer solubilized the conjugates and prevented them from stably inserting into cell membranes, an important distinction from traditional lipopeptide vaccines. Upon injection, these vaccine amphiphiles bound to albumin, leading to >10-fold increases in LN accumulation relative to the parent vaccine molecules. In therapeutic melanoma and cervical cancer tumor models, lipid-conjugated vaccines were able to significantly delay growth of established tumors at doses at which traditional peptide/adjuvant vaccines were completely ineffective.

Vaccine-Mediated Programming of T-cell Homing to Tumor Sites

It has been observed in many clinical studies that the presence of circulating tumor antigen–specific T cells does not correlate with clinical outcome, and this is consistent with the expectation that activated T cells must home to tumor sites to affect disseminated disease. Cancer vaccines can affect this phase of the immune response by ensuring induction of appropriate tissue-homing receptor profiles on newly primed tumor-specific lymphocytes, as seen in Fig. 1B. A key strategy to control tissue-specific effector-cell trafficking is via choice of vaccination site, because DCs in LNs draining different tissue sites express factors regulating the expression of tissue-homing receptors on T cells primed in these sites. Thus, T cells primed in mediastinal LNs express $\alpha_4\beta_1$ integrins and home to the lungs; DCs in skin-draining LNs induce

Published OnlineFirst July 8, 2015; DOI: 10.1158/2326-6066.CIR-15-0112

T cells to express cutaneous lymphocyte antigen (CLA) and CCR4 to home to the skin; and DCs of the gut-associated lymphoid tissues secrete retinoic acid, programming expression of $\alpha_4\beta_7$ and CCR9 on T cells for homing to the gut lamina propria (47). While these tissue-specific homing patterns have all been defined in the setting of T-cell trafficking to normal tissues, they are also critical in the therapeutic setting of effector T cells homing to tumor sites. For example, in orthotopic tumor models of head and neck cancer and lung cancer, Sandoval and colleagues demonstrated that mucosal, but not intramuscular, delivery of vaccines can promote CD8-mediated rejection of mucosal tumors (19). Human papillomavirus 16 E7-expressing TC-1 cells were engrafted in the submucosal lining of the tongue or in the lung as model mucosal tumors. Shiga toxin 1 subunit B (STxB) E7 fusions in combination with αGalCer adjuvant were administered intranasally or intramuscularly, and while both vaccines generated systemic $CD8^+$ T-cell responses, intranasal delivery resulted in more efficient tumor clearance in both models. Mechanistically, this was traced to mucosal imprinting of activated antigen-specific T cells, as measured by CD49a and CD103 expression, which allowed for effective homing and infiltration at the tumor site. Thus, strategies to enhance local tissue immunization may have a significant impact on the efficacy of cancer vaccines.

To this end, nanoparticle formulations discussed above for parenteral immunization have also been shown to enhance vaccine antigen/adjuvant uptake across pulmonary and nasal mucosa, which could promote tumor-homing T-cell responses in the setting of lung carcinoma, head and neck cancer, and treatment of lung metastases in a variety of other cancers. Nanoparticles can codeliver antigen and molecular adjuvants to DCs in the airway mucosa and promote uptake by DCs prior to mucociliary clearance (38, 48, 49). For example, exploiting the high density of DCs lining alveoli in the lungs, pulmonary vaccination with lipid nanocapsules carrying a protein antigen and Toll-like receptor (TLR) agonists led to increased persistence of antigen in the lungs 24 hours after administration, and subsequently greatly increased trafficking of antigens to lung-draining LNs several days later. This enhanced antigen delivery translated to >10-fold increases in T-cell priming compared with soluble forms of the same antigen and adjuvant, and enabled pulmonary nanocapsule vaccination to be 100% protective in a lung metastasis model, compared with only 20% protection elicited by the equivalent soluble vaccine (49). In a similar vein, intranasal vaccination with antigen-carrying poly(γ-glutamic acid) nanoparticles enhanced therapeutic protection against melanoma lung metastases (50). Pulmonary vaccination with PEGylated poly(propylene sulfide) nanoparticles conjugated to antigens using a reduction-sensitive linker combined with soluble CpG has been shown to enhance antigen uptake in lung-draining LNs and subsequent lung-homing antigen-specific T-cell populations (51, 52). Thus, several types of nanoparticle formulations have shown efficacy in enhancing mucosa-homing T-cell responses and mucosal antitumor immunity.

Analogous to pulmonary vaccination for protection of airway mucosal tissues, oral vaccination may facilitate antitumor immunity in the gastrointestinal tract and could thus help protect against cancers of the throat, stomach, intestine, and colon. A nonobvious benefit of vaccinating the gastrointestinal tract is that T-cell priming in the lymphoid organs of the large intestine can induce protection of rectal and vaginal mucosa, which are difficult to vaccinate directly (53). The design of effective oral vaccines that reach the large intestine has been challenging largely because of the low pH and destructive enzymatic activity characteristic of the gut. To solve this problem, Zhu and colleagues developed poly (lactide-*co*-gyolide; PLGA) nanoparticles that encapsulated peptide antigen and three TLR agonists: MALP-2, poly(I:C), and CpG (53). These nanoparticles were subsequently encapsulated within anionic pH-responsive polymer capsules. The capsules were designed to have mean diameters >10 μm to prevent nonspecific phagocytosis and uptake by Peyer's patches in the small intestine, and to dissolve at pH values greater than 7, characteristic of the terminal ileum of the large intestine, to allow for vaccine release only in this localized region of interest. Significantly stronger T-cell responses in the large intestine were generated when capsules of appropriate pH responsiveness were used as a coating rather than an alternative polymer that dissolved at more acidic pH. This general delivery strategy may thus hold potential in the treatment of colorectal tumors and establishes a paradigm for targeting other regions of the gastrointestinal tract. Together, these studies demonstrate that physically programmable properties of particulate vaccine carriers can be used to specifically target different organs to direct the immune response to the required site of protection.

Exploiting the Tumor Site as an Antigen Source

A seminal observation in cancer immunology was William B. Coley's discovery in 1893 that repeated intratumoral injection of bacteria could induce tumor rejection. Nearly 100 years later, trials in humans revealed that intratumoral administration of Bacillus Calmette–Guérin (BCG) in metastatic melanoma lesions resulted not only in the regression of 90% of the injected lesions, but also 17% of distal tumors (54). Although intratumoral injections of immunomodulators are intended to be local treatments, in many cases they can generate a systemic immune response capable of targeting distal tumors in a vaccine-like manner, turning the tumor itself into an *in situ* vaccine, as depicted in Fig. 1C. Importantly, this strategy does not depend on the discovery of tumor-specific antigens, and instead exploits the tumor itself as a source of antigens.

Local administration of diverse immunostimulatory agents to an accessible lesion has been effective at promoting systemic tumor rejection in animal models and in human patients. For example, intratumoral injection of CpG, anti-OX40, and anti-CTLA-4 in mouse lymphoma models can eradicate Tregs from tumors (55). Topical application of a cream prepared with 5% imiquimod, a TLR7 agonist, has been shown to induce 80% histologic clearance in human patients with superficial basal cell carcinoma (56). In some cases, local administration of immunomodulators increases susceptibility to subsequent systemic therapy. In mouse melanoma models with tumors on both the right and left flanks, Zamarin and colleagues showed that oncolytic virus injections into one tumor site increased lymphocytic infiltration in the contralateral tumor site, improving the efficacy of systemically administered anti–CTLA-4 therapy (57). Analogously in humans, results from early clinical trials suggest that stereotactic body radiotherapy, in which radiation is precisely delivered to tumor sites to enhance local inflammation, can improve responses to IL2 therapy in patients with metastatic lesions (58). Despite the promise of intratumoral injections in promoting antitumor immunity, one deficiency in intratumoral administration of soluble therapeutics is that locally applied drugs can still rapidly leak into the systemic circulation. This has been observed

Published OnlineFirst July 8, 2015; DOI: 10.1158/2326-6066.CIR-15-0112

in many studies in small animals (59–62) and with immunotherapy in humans, in whom intratumorally injected cytokines have been measured in the systemic circulation within minutes (63). Such systemic dissemination both weakens the potency of the therapy by clearing the drug from the tumor and gives rise to systemic inflammatory toxicity.

To promote such "*in situ* vaccination," biomaterials have been designed to trap immunomodulatory molecules in the tumor microenvironment. For example, slow-release particles or hydrogels have been injected peritumorally or intratumorally to allow local permeation of tumors with immunostimulatory drugs released from localized depots. This has been demonstrated with biodegradable microspheres releasing IL12 (62) and hydrogel matrices releasing an IL15 superagonist (64), both of which led to nontoxic but potent induction of $CD8^+$ T-cell responses against treated tumors. Such approaches can enable otherwise toxic treatments to be safely administered while eliciting systemic antitumor immunity. For example, anti-CD137 and IL2 administered directly into solid melanoma tumors disseminated into the systemic circulation, inducing systemic inflammation, including IL6 and TNFα in serum and major weight loss in mice (60). However, intratumoral injection of these same immunomodulators covalently anchored to liposomes prevented their dissemination outside of the local microenvironment (60, 61), eliminating their toxicity and enabling the drugs to remain concentrated at the tumor site for 96 hours after injection. These intratumoral immunoliposomes acted as vaccines and elicited systemic T-cell responses; mice that rejected treated tumors on one flank could also substantially delay the growth of an untreated tumor on the contralateral flank in the complete absence of supporting systemic therapy. Thus, even relatively simple strategies can be employed to significantly alter the efficacy and safety of immunotherapeutic drugs in this setting.

Active Depots with Implantable Vaccine Scaffolds

The only FDA-approved cancer vaccine to date is Provenge (sipuleucel-T; Dendreon), an autologous cell-based therapeutic vaccine against castration-resistant metastatic prostate cancer (65). Although this vaccine was shown to extend survival in prostate cancer patients by 4 months, its implementation is clinically complex. Briefly, peripheral blood is first collected from patients, shipped to a cell preparation facility, treated with antigen *ex vivo*, shipped back to the clinical site, and subsequently reinfused into the patient. Clinical trials of related processes based on the isolation of precursor cells, differentiation of these cells into DCs *in vitro*, activation and antigen loading of the resulting DCs, and injection as cellular vaccines have also shown promise (66, 67) but with the same logistical concerns. In an attempt to harness the power of DC vaccines without the practical limitations of cell therapy, several strategies have been developed to create implantable or injectable implants that would mimic this series of *ex vivo* treatment steps directly in patients. The common premise of these approaches is to employ a synthetic matrix or scaffold that when placed *in vivo* (e.g., following a minor subcutaneous implantation procedure) would release/present cues in the local tissue that enable the processes of attracting, differentiating, activating, and antigen loading of DCs, which would subsequently traffic to local draining LNs to initiate an antitumor immune response. This concept leverages a large body of experience from the tissue engineering and regenerative medicine field, where biomaterial scaffolds designed to attract and program cell fate have been studied for more than 20 years (68). These biodegradable scaffolds may release immunomodulatory agents with defined spatial and temporal profiles that can be engineered by manipulating the material properties of the implant. Multiple agents can be loaded into a single immunomodulatory scaffold, including antigen, adjuvant, and cytokine support, and they can be designed to promote cell recruitment and modulation within the scaffold.

One of the first reports of a DC-programming vaccine system utilized millimeter scale polymer rods that released the chemoattractant CCL19 along with tumor lysate as an antigen preparation (69). These attractant-releasing implants recruited APCs to the vaccine site, which correlated with enhanced tumor regression in a therapeutic lung carcinoma model. More recently, Ali and colleagues designed centimeter-scale porous polymer disks composed of PLGA, the same polymer used in resorbable sutures; these disks were loaded with three components: GM-CSF, CpG DNA, and tumor lysate, as shown in Fig. 1D (70). These scaffolds released GM-CSF to recruit and differentiate DCs into the structure and CpG as a danger signal to activate DCs internalizing antigens in the tumor lysate. These scaffolds were capable of protecting mice from B16F10 melanoma challenge in a prophylactic setting. In a follow-up study, this scaffold vaccine, in combination with vaccination using irradiated tumor cells transduced to express GM-CSF, was shown to also greatly enhance protection relative to nonscaffolded vaccines or GM-CSF–producing tumor cell–based vaccination alone in the therapeutic setting, results that correlated with enhanced recruitment of plasmacytoid DCs, cross-presenting $CD8^+$ DCs, and elevated IL12 production in the scaffold implants (71). In a rat glioma model, PLGA scaffold vaccines implanted after partial tumor resection resulted in significantly enhanced survival over control blank PLGA matrices (72). Efficacy in this model was only seen when scaffolds were placed next to the resection site but not within the resection site, highlighting the importance of implantation site for these implantable scaffold-based vaccines. Based on these encouraging preclinical results, this promising PLGA scaffold vaccine system was recently moved into a first-in-human phase I trial in patients with melanoma (73).

A number of strategies have sought to generate an *in situ*–forming immunomodulatory depot that does not require surgical implantation like the PLGA-based scaffolds described above. A recent report described a study in which antigen and adjuvant were mixed with chitosan and hydroxyapatite and coinjected with cross-linking agent tripolyphosphate and chondroitin sulfate via a two-needle aligned injection (74). These two aqueous solutions cross-linked *in vivo* to form a biodegradable hydrogel vaccine that was capable of inducing humoral responses durable for more than a year after implantation following a single injection; this type of sustained release implant may also be of interest for driving antitumor T-cell responses. In a second example, Kim and colleagues demonstrated that biodegradable mesoporous silica rods could nonspecifically coalesce to form a scaffold-like structure following subcutaneous injection (75). When formulated with GM-CSF, CpG, and antigen, these injectable scaffolds recruited DCs and primed T-cell responses that were capable of delaying the outgrowth of ovalbumin-expressing tumors in a prophylactic setting. Although still in early stages of preclinical development, these "injectable-scaffold" approaches may provide a facile strategy to repeatedly prime and boost antitumor immunity. Both

Published OnlineFirst July 8, 2015; DOI: 10.1158/2326-6066.CIR-15-0112

implanted and injectable matrix-based vaccines are powerful technological platforms for examining the importance of timing, dosing, and physical localization of immunostimulatory cues on the output immune response, making these systems potential therapeutics and valuable tools for determining how these factors quantitatively influence the immune response.

Conclusions

Although traditional techniques inspired by prophylactic vaccines activate immune responses, new vaccine concepts are of interest to overcome tumor antigen tolerance and tumor-induced immunosuppression in the setting of advanced cancer and to drive immune responses of the appropriate magnitude and quality to treat large metastatic tumor burdens. Approaches grounded in engineering methods for creating synthetic materials and synthesizing new molecules offer a number of strategies to enhance cancer immunotherapy and cancer vaccines in particular, including improving the delivery of vaccine components to lymphoid organs, optimally programming activated T cells to home to tumor sites, prolonging immunomodulation of lesions following intratumoral injection, and programming sequential events in immunization from a single injectable or implantable device. Overall, such engineering-based approaches have shown great promise in preclinical models, and the next few years should see a number of these approaches moving into clinical testing in patients.

Disclosure of Potential Conflicts of Interest

D.J. Irvine has ownership interest (including patents) in and is a consultant/advisory board member for Vedantra Pharmaceuticals. No potential conflicts of interest were disclosed by the other authors.

Grant Support

This work was supported by grants from the NIH (CA174795 and CA172164), the V Foundation, and the Bridge Project of the Koch Institute and the Dana-Farber/Harvard Cancer Center. K.D. Moynihan is supported by a graduate fellowship from the Hertz Foundation. K.D. Moynihan and N.K. Mehta are supported by graduate research fellowships from the NSF.

Published OnlineFirst July 8, 2015.

References

1. Rosenberg SA, Yang JC, Restifo NP. Cancer immunotherapy: moving beyond current vaccines. Nat Med 2004;10:909–15.
2. Melero I, Gaudernack G, Gerritsen W, Huber C, Parmiani G, Scholl S, et al. Therapeutic vaccines for cancer: an overview of clinical trials. Nat Rev Clin Oncol 2014;11:509–24.
3. Sharma P, Allison JP. The future of immune checkpoint therapy. Science 2015;348:56–61.
4. Ghiringhelli F, Menard C, Puig PE, Ladoire S, Roux S, Martin F, et al. Metronomic cyclophosphamide regimen selectively depletes CD4+CD25+ regulatory T cells and restores T and NK effector functions in end stage cancer patients. Cancer Immunol Immunother 2007;56:641–8.
5. Vacchelli E, Aranda F, Eggermont A, Sautes-Fridman C, Tartour E, Kennedy EP, et al. Trial watch: IDO inhibitors in cancer therapy. Oncoimmunology 2014;3:e957994.
6. Vanneman M, Dranoff G. Combining immunotherapy and targeted therapies in cancer treatment. Nat Rev Cancer 2012;12:237–51.
7. Fritsch EF, Hacohen N, Wu CJ. Personal neoantigen cancer vaccines: the momentum builds. Oncoimmunology 2014;3:e29311.
8. Hacohen N, Fritsch EF, Carter TA, Lander ES, Wu CJ. Getting personal with neoantigen-based therapeutic cancer vaccines. Cancer Immunol Res 2013;1:11–5.
9. Gubin MM, Zhang X, Schuster H, Caron E, Ward JP, Noguchi T, etal. Checkpoint blockade cancer immunotherapy targets tumour-specific mutant antigens. Nature 2014;515:577–81.
10. Yadav M, Jhunjhunwala S, Phung QT, Lupardus P, Tanguay J, Bumbaca S, et al. Predicting immunogenic tumour mutations by combining mass spectrometry and exome sequencing. Nature 2014;515:572–6.
11. Barnes E, Folgori A, Capone S, Swadling L, Aston S, Kurioka A, et al. Novel adenovirus-based vaccines induce broad and sustained T cell responses to HCV in man. Sci Transl Med 2012;4:115ra1.
12. Miller JD, van der Most RG, Akondy RS, Glidewell JT, Albott S, Masopust D, et al. Human effector and memory CD8+ T cell responses to smallpox and yellow fever vaccines. Immunity 2008;28:710–22.
13. Speiser DE, Lienard D, Rufer N, Rubio-Godoy V, Rimoldi D, Lejeune F, et al. Rapid and strong human CD8+ T cell responses to vaccination with peptide, IFA, and CpG oligodeoxynucleotide 7909. J Clin Invest 2005;115:739–46.
14. Rosenberg SA, Sherry RM, Morton KE, Scharfman WJ, Yang JC, Topalian SL, et al. Tumor progression can occur despite the induction of very high levels of self/tumor antigen-specific CD8+ T cells in patients with melanoma. J Immunol 2005;175:6169–76.
15. Walter S, Weinschenk T, Stenzl A, Zdrojowy R, Pluzanska A, Szczylik C, et al. Multipeptide immune response to cancer vaccine IMA901 after single-dose cyclophosphamide associates with longer patient survival. Nat Med 2012;18:1254–61.
16. Odunsi K, Matsuzaki J, Karbach J, Neumann A, Mhawech-Fauceglia P, Miller A, et al. Efficacy of vaccination with recombinant vaccinia and fowlpox vectors expressing NY-ESO-1 antigen in ovarian cancer and melanoma patients. Proc Natil Acad Sci 2012;109:5797–802.
17. Schwartzentruber DJ, Lawson DH, Richards JM, Conry RM, Miller DM, Treisman J, et al. gp100 peptide vaccine and interleukin-2 in patients with advanced melanoma. N Engl J Med 2011;364:2119–27.
18. Melief CJM, van der Burg SH. Immunotherapy of established (pre)malignant disease by synthetic long peptide vaccines. Nat Rev Cancer 2008;8:351–60.
19. Sandoval F, Terme M, Nizard M, Badoual C, Bureau MF, Freyburger L, et al. Mucosal imprinting of vaccine-induced CD8(+) T cells is crucial to inhibit the growth of mucosal tumors. Sci Transl Med 2013;5:172ra20.
20. Itano AA, McSorley SJ, Reinhardt RL, Ehst BD, Ingulli E, Rudensky AY, et al. Distinct dendritic cell populations sequentially present antigen to CD4 T cells and stimulate different aspects of cell-mediated immunity. Immunity 2003;19:47–57.
21. Anandasabapathy N, Feder R, Mollah S, Tse SW, Longhi MP, Mehandru S, et al. Classical Flt3L-dependent dendritic cells control immunity to protein vaccine. J Exp Med 2014;211:1875–91.
22. Johansen P, Haffner AC, Koch F, Zepter K, Erdmann I, Maloy K, et al. Direct intralymphatic injection of peptide vaccines enhances immunogenicity. Eur J Immunol 2005;35:568–74.
23. Maloy KJ, Erdmann I, Basch V, Sierro S, Kramps TA, Zinkernagel RM, et al. Intralymphatic immunization enhances DNA vaccination. Proc Natl Acad Sci U S A 2001;98:3299–303.
24. Bachmann MF, Jennings GT. Vaccine delivery: a matter of size, geometry, kinetics and molecular patterns. Nat Rev Immunol 2010;10:787–96.
25. Swartz MA. Immunomodulatory roles of lymphatic vessels in cancer progression. Cancer Immunol Res 2014;2:701–7.
26. Supersaxo A, Hein WR, Steffen H. Effect of molecular weight on the lymphatic absorption of water-soluble compounds following subcutaneous administration. Pharm Res 1990;7:167–9.
27. McLennan DN, Porter CJH, Charman SA. Subcutaneous drug delivery and the role of the lymphatics. Drug Discov Today Technol 2005;2:89–96.
28. Liu H, Moynihan KD, Zheng Y, Szeto GL, Li AV, Huang B, et al. Structure-based programming of lymph-node targeting in molecular vaccines. Nature 2014;507:519–22.

Published OnlineFirst July 8, 2015; DOI: 10.1158/2326-6066.CIR-15-0112

29. Smirnov D, Schmidt JJ, Capecchi JT, Wightman PD. Vaccine adjuvant activity of 3M-052: an imidazoquinoline designed for local activity without systemic cytokine induction. Vaccine 2011;29: 5434–42.
30. Hanson MC, Crespo MP, Abraham W, Moynihan KD, Szeto GL, Chen SH, et al. Nanoparticulate STING agonists are potent lymph node-targeted vaccine adjuvants. J Clin Invest 2015;125:2532–46.
31. Hailemichael Y, Dai Z, Jaffarzad N, Ye Y, Medina MA, Huang XF, et al. Persistent antigen at vaccination sites induces tumor-specific CD8(+) T cell sequestration, dysfunction and deletion. Nat Med 2013;19:465–72.
32. Reddy ST, Rehor A, Schmoekel HG, Hubbell JA, Swartz MA. In vivo targeting of dendritic cells in lymph nodes with poly(propylene sulfide) nanoparticles. J Control Release 2006;112:26–34.
33. Manolova V, Flace A, Bauer M, Schwarz K, Saudan P, Bachmann MF. Nanoparticles target distinct dendritic cell populations according to their size. Eur J Immunol 2008;38:1404–13.
34. Fifis T, Gamvrellis A, Crimeen-Irwin B, Pietersz GA, Li J, Mottram PL, et al. Size-dependent immunogenicity: therapeutic and protective properties of nano-vaccines against tumors. J Immunol 2004;173:3148–54.
35. Moon JJ, Suh H, Bershteyn A, Stephan MT, Liu H, Huang B, et al. Interbilayer-crosslinked multilamellar vesicles as synthetic vaccines for potent humoral and cellular immune responses. Nat Mater 2011; 10:243–51.
36. Kasturi SP, Skountzou I, Albrecht RA, Koutsonanos D, Hua T, Nakaya HI, et al. Programming the magnitude and persistence of antibody responses with innate immunity. Nature 2011;470:543–7.
37. St John AL, Chan CY, Staats HF, Leong KW, Abraham SN. Synthetic mast-cell granules as adjuvants to promote and polarize immunity in lymph nodes. Nat Mater 2012;11:250–7.
38. de Titta A, Ballester M, Julier Z, Nembrini C, Jeanbart L, van der Vlies AJ, et al. Nanoparticle conjugation of CpG enhances adjuvancy for cellular immunity and memory recall at low dose. Proc Natl Acad Sci U S A 2013;110:19902–7.
39. Reddy ST, van der Vlies AJ, Simeoni E, Angeli V, Randolph GJ, O'Neil CP, et al. Exploiting lymphatic transport and complement activation in nanoparticle vaccines. Nat Biotechnol 2007;25:1159–64.
40. Thomas SN, Vokali E, Lund AW, Hubbell JA, Swartz MA. Targeting the tumor-draining lymph node with adjuvanted nanoparticles reshapes the anti-tumor immune response. Biomaterials 2014;35:814–24.
41. Fraser CC, Altreuter DH, Ilyinskii P, Pittet L, LaMothe Ra, Keegan M, et al. Generation of a universal CD4 memory T cell recall peptide effective in humans, mice and non-human primates. Vaccine 2014; 32:2896–903.
42. Wu TYH, Singh M, Miller AT, De Gregorio E, Doro F, D'Oro U, et al. Rational design of small molecules as vaccine adjuvants. Sci Transl Med 2014;6:12.
43. Kourtis IC, Hirosue S, de Titta A, Kontos S, Stegmann T, Hubbell JA, et al. Peripherally administered nanoparticles target monocytic myeloid cells, secondary lymphoid organs and tumors in mice. PloS One 2013;8: e61646.
44. Shen H, Ackerman AL, Cody V, Giodini A, Hinson ER, Cresswell P, et al. Enhanced and prolonged cross-presentation following endosomal escape of exogenous antigens encapsulated in biodegradable nanoparticles. Immunology 2006;117:78–88.
45. Krishnamachari Y, Geary SM, Lemke CD, Salem AK. Nanoparticle delivery systems in cancer vaccines. Pharm Res 2011;28:215–36.
46. Fang RH, Hu CM, Luk BT, Gao W, Copp JA, Tai Y, et al. Cancer cell membrane-coated nanoparticles for anticancer vaccination and drug delivery. Nano Lett 2014;14:2181–8.
47. von Andrian UH, Mackay CR. T-cell function and migration: two sides of the same coin. N Engl J Med 2000;343:15.
48. Nochi T, Yuki Y, Takahashi H, Sawada S, Mejima M, Kohda T, et al. Nanogel antigenic protein-delivery system for adjuvant-free intranasal vaccines. Nat Mater 2010;9:572–8.
49. Li AV, Moon JJ, Abraham W, Suh H, Elkhader J, Seidman MA, et al. Generation of effector memory T cell-based mucosal and systemic immunity with pulmonary nanoparticle vaccination. Sci Transl Med 2013;5: 204ra130.
50. Matsuo K, Koizumi H, Akashi M, Nakagawa S, Fujita T, Yamamoto A, et al. Intranasal immunization with poly(γ-glutamic acid) nanoparticles entrapping antigenic proteins can induce potent tumor immunity. J Control Release 2011;152:310–6.
51. Stano A, Nembrini C, Swartz MA, Hubbell JA, Simeoni E. Nanoparticle size influences the magnitude and quality of mucosal immune responses after intranasal immunization. Vaccine 2012;30:7541–6.
52. Nembrini C, Stano A, Dane KY, Ballester M, van der Vlies AJ, Marsland BJ, et al. Nanoparticle conjugation of antigen enhances cytotoxic T-cell responses in pulmonary vaccination. Proc Natl Acad Sci U S A 2011;108: E989–97.
53. Zhu Q, Talton J, Zhang G, Cunningham T, Wang Z, Waters RC, et al. Large intestine-targeted, nanoparticle-releasing oral vaccine to control genitorectal viral infection. Nat Med 2012;18:1291–6.
54. Morton DL, Eilber FR, Holmes EC, Hunt JS, Ketcham AS, Silverstein MJ, et al. BCG immunotherapy of malignant melanoma: summary of a seven-year experience. Ann Surgery 1974;180:635–41.
55. Marabelle A, Kohrt H, Sagiv-Barfi I, Ajami B, Axtell RC, Zhou G, et al. Depleting tumor-specific Tregs at a single site eradicates disseminated tumors. J Clini Invest 2013;123:2447–63.
56. Schulze HJ, Cribier B, Requena L, Reifenberger J, Ferrandiz C, Garcia Diez A, et al. Imiquimod 5% cream for the treatment of superficial basal cell carcinoma: results from a randomized vehicle-controlled phase III study in Europe. Br J Dermatol 2005;152:939–47.
57. Zamarin D, Holmgaard RB, Subudhi SK, Park JS, Mansour M, Palese P, et al. Localized oncolytic virotherapy overcomes systemic tumor resistance to immune checkpoint blockade immunotherapy. Sci Transl Med 2014;6:12.
58. Seung SK, Curti BD, Crittenden M, Walker E, Coffey T, Siebert JC, et al. Phase 1 study of stereotactic body radiotherapy and interleukin-2: tumor and immunological responses. Sci Transl Med 2012;4:7.
59. Le Mercier I, Poujol D, Sanlaville A, Sisirak V, Gobert M, Durand I, et al. Tumor promotion by intratumoral plasmacytoid dendritic cells is reversed by TLR7 ligand treatment. Cancer Res 2013;73:4629–40.
60. Kwong B, Gai SA, Elkhader J, Wittrup KD, Irvine DJ. Localized immunotherapy via liposome-anchored Anti-CD137 + IL-2 prevents lethal toxicity and elicits local and systemic antitumor immunity. Cancer Res 2013; 73:1547–58.
61. Kwong B, Liu H, Irvine DJ. Induction of potent anti-tumor responses while eliminating systemic side effects via liposome-anchored combinatorial immunotherapy. Biomaterials 2011;32:5134–47.
62. Egilmez NK, Jong YS, Sabel MS, Jacob JS, Mathiowitz E, Bankert RB. In situ tumor vaccination with interleukin-12-encapsulated biodegradable microspheres: induction of tumor regression and potent antitumor immunity. Cancer Res 2000;60:3832–7.
63. Herpen V, Huijbens R, Looman M, de Vries J. Pharmacokinetics and immunological aspects of a phase Ib study with intratumoral administration of recombinant human interleukin-12 in patients with head and neck squamous cell carcinoma: a decrease of T-bet in peripheral blood mononuclear cells. Clin Cancer Res 2003; 9:2950–6.
64. Hori Y, Stern PJ, Hynes RO, Irvine DJ. Engulfing tumors with synthetic extracellular matrices for cancer immunotherapy. Biomaterials 2009; 30:6757–67.
65. Kantoff PW, Higano CS, Shore ND, Berger ER, Small EJ, Penson DF, et al. Sipuleucel-T immunotherapy for castration-resistant prostate cancer. N Engl J Med 2010:363:411–22.
66. Mitchell DA, Batich KA, Gunn MD, Huang MN, Sanchez-Perez L, Nair SK, et al. Tetanus toxoid and CCL3 improve dendritic cell vaccines in mice and glioblastoma patients. Nature 2015;519:366–9.
67. Palucka K, Banchereau J. Dendritic-cell-based therapeutic cancer vaccines. Immunity 2013;39:38–48.
68. Langer R, Vacanti JP. Tissue engineering. Science 1993;260:920–6.
69. Kumamoto T, Huang EK, Paek HJ, Morita A, Matsue H, Valentini RF, et al. Induction of tumor-specific protective immunity by in situ Langerhans cell vaccine. Nat Biotechnol 2002;20:64–9.
70. Ali OA, Huebsch N, Cao L, Dranoff G, Mooney DJ. Infection-mimicking materials to program dendritic cells in situ. Nat Mater 2009; 8:151–8.
71. Ali OA, Emerich D, Dranoff G, Mooney DJ. In situ regulation of DC subsets and T cells mediates tumor regression in mice. Sci Transl Med 2009;1: 8ra19–8ra.

Published OnlineFirst July 8, 2015; DOI: 10.1158/2326-6066.CIR-15-0112

72. Ali OA, Doherty E, Bell WJ, Fradet T, Hudak J, Laliberte M-T, et al. The efficacy of intracranial PLG-based vaccines is dependent on direct implantation into brain tissue. J Control Release 2011;154:249–57.
73. Hodi SF. Dendritic cell activating scaffold in melanoma. 2014. [last updated: 2015 May 8; last verified: May 2015]. Available from: https://clinicaltrials.gov/ct2/show/NCT01753089.
74. Chua BY, Sekiya T, Al Kobaisi M, Short KR, Mainwaring DE, Jackson DC. A single dose biodegradable vaccine depot that induces persistently high levels of antibody over a year. Biomaterials 2015;53:50–7.
75. Kim J, Li WA, Choi Y, Lewin Sa, Verbeke CS, Dranoff G, et al. Injectable, spontaneously assembling, inorganic scaffolds modulate immune cells in vivo and increase vaccine efficacy. Nat Biotechnol 2015;33:64–72.

Published OnlineFirst July 8, 2015; DOI: 10.1158/2326-6066.CIR-15-0112

Cancer Immunology Research

Engineering New Approaches to Cancer Vaccines

Naveen K. Mehta, Kelly D. Moynihan and Darrell J. Irvine

Cancer Immunol Res 2015;3:836-843. Published OnlineFirst July 8, 2015.