Published in final edited form as:
*Semin Oncol*. 2012 June ; 39(3): 305–310. doi:10.1053/j.seminoncol.2012.02.013.

# Novel Recombinant Alphaviral and Adenoviral Vectors for Cancer Immunotherapy

**Takuya Osada**[a], **Michael A. Morse**[b], **Amy Hobeika**[a], and **H. Kim Lyerly**[a,c]
[a]Department of Surgery, Duke University Medical Center, Durham, NC
[b]Department of Medicine, Duke University Medical Center, Durham, NC
[c]Pathology and Immunology, Duke University Medical Center, Durham, NC

## Abstract

Although cellular immunotherapy based on autolgous dendritic cells (DCs) targeting antigens expressed by metastatic cancer has demonstrated clinical efficacy, the logistical challenges in generating an individualized cell product create an imperative to develop alternatives to DC-based cancer vaccines. Particularly attractive alternatives include in situ delivery of antigen and activation signals to resident antigen-presenting cells (APCs), which can be achieved by novel fusion molecules targeting the mannose receptor and by recombinant viral vectors expressing the antigen of interest and capable of infecting DCs. A particular challenge in the use of viral vectors is the well-appreciated clinical obstacles to their efficacy, specifically vector-specific neutralizing immune responses. Because heterologous prime and boost strategies have been demonstrated to be particularly potent, we developed two novel recombinant vectors based on alphaviral replicon particles and a next-generation adenovirus encoding an antigen commonly overexpressed in many human cancers, carcinoembryonic antigen (CEA). The rationale for developing these vectors, their unique characteristics, the preclinical studies and early clinical experience with each, and opportunities to enhance their effectiveness will be reviewed. The potential of each of these potent recombinant vectors to efficiently generate clinically active anti-tumor immune response alone, or in combination, will be discussed. Semin Oncol 39:305-310

Cancer immunotherapy has entered a new era in which the conceptual basis of tumor immunotherapy has been clinically proven using autologous cellular products enriched for dendritic cells (DCs)[1] as shown in Table 1, Strategy 1. This strategy, illustrated by sipuleucel-T, isolates autologous antigen-presenting cells (APCs), including DC from peripheral blood, loads cells with a tumor-associated antigen, activates cells, and re-infuses the activated cell product to individual patients.[1]

## Delivery of Antigens to Dendritic Cells in Situ

Due to the logistical challenges of manufacturing autologous cellular products, alternatives that are effective but that can be efficiently produced and provided without patient-specific manufacturing are urgently needed. An option shown in Table 1 as Strategy 2, is to use a vaccine strategy that avoids the ex vivo manipulation of DCs, and attempts to directly deliver antigen to DCs in situ, which then activate tumor-specific T-cell and antibody responses. Recent studies of antigen-delivery to resident APCs along with in situ activation



Address correspondence to H. Kim Lyerly, MD, Duke University Medical Center, 2424 Erwin Rd, Durham, NC 27710. lyerl001@mc.duke.edu.

Financial disclosures and conflicts of interest: H. Kim Lyerly is a scientific advisory board member of Alphavax and Etubics.

signals has demonstrated the immunogenicity of this approach. Specifically, we have clinically tested the concept of in vivo targeted delivery of a soluble, tumor-associated self-antigen to APCs through the mannose receptor (MR) alone and in combination with APC activation by Toll-like receptor (TLR) agonists.[2] Two phase I studies were performed with CDX-1307, a vaccine composed of a common tumor-associated antigen, human chorionic gonadotropin beta chain (hCG-β) fused to a DC-targeting ligand, specifically MR-specific monoclonal antibody, administered either locally (intradermally/intracutaneously) or systemically (intravenously). Although this strategy was shown to be effective in delivering antigen to the APCs, it did not provide activation signals, and vaccinations were subsequently conducted with APC activation using various combinations of granulocyte-macrophage colony-stimulating factor (GM-CSF) and TLR agonists, such as the TLR-3 agonist poly-ICLC and the TLR7/8 agonist resiquimod. We observed that this strategy of in vivo APC-targeting combined with TLR activation induced adaptive immunity against otherwise poorly immunogenic self-antigens. Although this is a promising approach to delivering antigen to DCs in vivo, it is complicated to generate the fusion proteins and it may be more difficult to deliver multiple antigens simultaneously.

Another alternative is to deliver antigen to DCs in vivo using recombinant viral vectors that are capable of either infecting DCs directly or expressing antigen in a form that can be processed by resident APCs as shown in Table 1, Strategy 3. We will focus on our interest in two such promising viral vector-based vaccines.

## Viral Vector Vaccines

The ideal viral vector for immunotherapy would be simple to produce, would be capable of delivering antigen to DCs, and would contain sufficient transgene capacity to express multiple genes of interest. Poxvectors, such as recombinant vaccinia, have been of particular interest due to their large genomes and ability to infect DCs. Nonetheless, repeated immunization with recombinant vaccinia may not boost immunity because the vector expresses a variety of viral proteins that are highly immunogenic. The vector-specific immune response to the Pox gene products then limits subsequent administration and expression of the antigen of interest.[3] In practical terms, a patient that has had a previous smallpox vaccination will have a robust immune response to the vaccinia components of an administered vaccine that will prevent it from entering cells and expressing the antigen target at a level sufficient to elicit the vaccine response of interest.

The presence of pre-existing anti-vector immune responses is felt to be a major contributor to the poor performance and/or failure of other recombinant vectors used in vaccine strategies. Some vectors are based on viruses that are natural human pathogens, and significant numbers of patients have a previous history of viral infection and immunity. For example, an adenovirus-specific immune response was found in a significant percentage of subjects in a large clinical trial of a human immunodeficiency (HIV) vaccine composed of a first-generation adenoviral vaccine, and these subjects had limited immune responses to the vaccine.[4] Thus, new recombinant vectors that address this key obstacle are needed.

Although a single improved viral vector system would be useful, there are compelling arguments to generate a series of novel vectors. For example, a particularly potent strategy to generate immune responses against a specific antigen is the use of a priming vaccine followed by a series of booster vaccines using a heterologous vector encoding the same antigen.[5] This heterologous prime and boost strategy has been demonstrated to be highly effective in preclinical models and in clinical studies. The efficacy of the PROSTVAC pox-vectors in prime boost immunizations for patients with prostate cancer[6] and preliminary

evidence of activity for the poxvectors encoding carcinoembryonic antigen (CEA) and a triad of costimulatory molecules in patients with colon cancer.[7]

Thus, we sought to generate at least two new recombinant viral vectors to be used alone, and in the future in combination in a heterologous prime and boost vaccine strategy.

## Alphaviral Vectors

Alphaviruses, such as Venezuelan equine encephalitis virus (VEE), are attractive vectors to express oncogenic antigens as they can replicate the RNA of interest in the cell cytosol, expressing the heterologous protein to high levels.[8] In addition, they are cytopathic, thereby significantly reducing the risk of integration of vector sequences into the cellular genome and avoiding the persistence of infected cells. Finally, it has been reported that alphaviruses preferentially infect DCs,[9] and thus have been found to be capable of inducing both humoral and cellular immune responses to the delivered transgene products. Consequently, alphavirus-based replicon particles, in particular VEE replicon particles (VRPs) have been designed and developed as models of viral vaccines that infect DCs, a preferred site for the induction of immunity.

An additional benefit of using alphaviral-based vectors is that they are not natural human pathogens, and pre-exisiting immunity to the wild-type virus does not exist. Therefore, priming immunizations in the majority of humans may be possible, since there is no widespread existing immunity in humans.

Therefore, we evaluated a series of VEE-based vectors encoding CEA, both alone and in combination with the established poxvirus-based CEA vaccine we have previously tested in preclinical and clinical settings. We inserted the CEA gene into a VEE replicon vector and generated virus-like replicon particles (VRP) and have shown that (1) cells infected with CEA-VRP express CEA to high levels; (2) the CEA expression product is appropriately modified and transported within infected cells; (3) CEA-VRP efficiently infect human DCs in vitro (up to 70%); (4) apoptosis in VRP-infected DCs is induced slowly, suggesting DC migration and function would be adequately maintained; (5) mice immunized with CEA-VRP rapidly develop anti-CEA antibody responses; and (6) mice immunized with CEA-VRP develop high-level CEA-specific T-cell responses.

Although the alphavirus strategy is very exciting, these vectors are early in their clinical development. Prior to performing clinical trials of heterologous prime and boost strategies with the CEA-VRP in combination with other vaccination strategies, we were required to perform phase I clinical trials of CEA-VRP as a single agent. We reported that an alphavirus vector could be packaged in VRP under Good Manufacturing Practice conditions for clinical use, and that these were capable of efficiently infecting human DCs and could be administered repeatedly to patients with metastatic cancer expressing the tumor antigen CEA. We noted that we could induce CEA-specific immune responses even in the presence of high titers of neutralizing antibodies that were induced by repeated immunization with VRP. Furthermore, the elevated regulatory T cells (Tregs) levels associated with advanced cancer patients did not inhibit the ability of the VRP to induce clinically relevant CEA-specific T-cell and antibody responses.[10] The CEA-specific antibodies mediated antibody-dependent cellular cytotoxicity (ADCC) against tumor cells from human colorectal cancer metastases. In addition, patients with CEA-specific T-cell responses exhibited longer overall survival.

These data suggest that VRP-based vectors can overcome the presence of neutralizing antibodies to break tolerance to self-antigen and may be clinically useful for immunotherapy in the setting of tumor-induced immunosuppression. Although alphaviral vectors remain

promising, there has been much attention directed toward recombinant adenoviral vectors in the past, which we will discuss below.

## Adenoviral Vectors

First-generation recombinant serotype 5 adenovirus (Ad5) vectors lacking E1 expression can be manufactured to high titer and quantity, and have been extensively tested in human trials of gene transfer and in vaccine studies.[11] These vectors induced robust immune responses against encoded transgenes in preclinical models but had muted responses in human trials. Because adenovirus is a natural human pathogen, widespread pre-existing anti-adenovirus immunity exists and the presence of this immunity correlates with a lack of immune response to vaccinations based on these first-generation adenoviral vectors.[12]

One early strategy to overcome this obstacle was to modify adenoviral vectors using a "stealth" strategy, specifically identifying the key antigens recognized by the naturally induced human immune response, and substituting these antigens on new vectors. Specifically, stealth type adenoviral vectors were generated using different serotypes of adenovirus (which are known to be immunologically recognized by different antibodies),[13] or using recombinant technology to modify the viral capsid components, which are key targets of the natural immune response to adenovirus.[14] Unfortunately, neither of these strategies has yielded profound improvement in immunogenicity in clinical trials, and alternative strategies were needed.

## E2b-Deleted Adenoviral Vectors

Although neutralizing antibodies were felt to limit the ability of Ad vectors to immunize, we were aware that Ad-specific T-cell responses also could have an impact on vaccination. Therefore, we have explored a novel alternative strategy, specifically reducing the expression of structural Ad5 genes in the vaccines by creating E1- and E2b-deleted recombinant Ad5 vectors.[15] In these studies, we and our colleagues constructed a novel Ad5 vector with unique deletions of the viral DNA polymerase and the pre-terminal protein region (Ad5 [E1-, E2b-]).[16–18] As a model system, we created vectors with a CEA gene insert.[19] The CEA-specific immune response and in vivo anti-tumor effects of repeated immunizations with Ad5 [E1-, E2b-]-CEA were compared to the current generation Ad5 [E1-]-CEA in wild type and Ad5 pre-immunized mice (13).

Our data show that [E1-, E2b-] vectors retaining the Ad5 serotype are potent immunogens in preclinical models despite the presence of significant Ad5-specific immunity. This was in contrast to [E1-] vectors, which were not as effective in generating immune responses in the presence of pre-existing anti-Ad immunity. Specifically, we demonstrated that Ad5-immune mice immunized multiple times with Ad5 [E1-, E2b-]-CEA induced CEA-specific cell-mediated immune responses that were significantly increased over those detected in Ad5-immune mice immunized multiple times with a current-generation Ad5 [E1-]-CEA.

The preclinical studies with these vectors were then extended to models of anti-tumor immunity. Ad5 immune mice bearing CEA-expressing tumors that were treated with Ad5 [E1-, E2b-]-CEA had increased anti-tumor responses as compared with Ad5 [E1-]-CEA–treated mice. These results demonstrate that Ad5 [E1-, E2b-]-CEA can induce CEA-specific immune responses that result in tumor growth inhibition despite the presence of pre-existing Ad5 immunity.

These preclinical studies with E1- and E2b-deleted recombinant Ad5 vectors suggest that anti-Ad immunity will no longer be a limiting factor, and that clinical trials to evaluate their performance are warranted. We are currently completing a phase I study of the Ad5 [E1-, E2b-]-CEA candidate vaccine ETBX-011.

## Engineering Improvements in Vectors

Despite the promise of recombinant viral vectors, additional barriers to effective immunization exist in cancer patients. For example, significant strategies have been undertaken to address the immunosuppressive environment found in cancer patients, including blocking Tregs, vascular endothelial growth factor (VEGF), and interactions with regulatory receptor on T cells, such as CTLA4 and PD-L1. Although many of these strategies include systemic delivery of agents to overcome the immunosuppressive environment, the systemic effects of these agents has led to significant immunologic effects, including autoimmunity. An alternative afforded by the use of recombinant vectors is to enhance local immune responses by engineering vectors not only for expression of costimulatory molecules but also the regional delivery of cytokines such as interleukin (IL)-12 to enhance vaccine responses dramatically.[20]

### TLR Signaling/TLR Adaptor Proteins—MyD88

Although critical for initiating and regulating immune responses, the therapeutic use of individual cytokines as anticancer immunotherapeutic agents has achieved only modest clinical success. Consequently, many current strategies have focused on the use of specific immunotherapeutic agonists that engage individual receptors of innate immune networks, such as the TLR system, each resulting in specific patterns of gene expression, cytokine production, and inflammatory outcome; however, these immunotherapies are constrained by variable cellular TLR expression and responsiveness to particular TLR agonists, as well as the specific cellular context of different tumors.

We hypothesized that overexpression of MyD88, a pivotal regulator of multiple TLR signaling pathways, could circumvent these constraints and mimic coordinated TLR signaling across all cell types in a ligand-independent fashion.[21] To explore this hypothesis, we generated an adenoviral vector expressing MyD88 and showed that Ad-MyD88 infection elicits extensive Th1-specific transcriptional and secreted cytokine signatures in all murine and human cell types tested in vitro and in vivo. Importantly, intratumoral injection of Ad-MyD88 into established tumor masses in vivo enhanced adaptive immune responses and inhibited local tumor immunosuppression, resulting in significantly inhibited local and systemic growth of multiple tumor types. Finally, Ad-MyD88 infection of primary human DCs, tumor-associated fibroblasts, and colorectal carcinoma cells elicited significant Th1-type cytokine responses, resulting in enhanced tumor cell lysis and expansion of human tumor antigen-specific T cells. Thus, Ad-MyD88 initiated robust anti-tumor activity in established murine tumor microenvironments and in human contexts, suggesting its potential effectiveness as a clinical immunotherapeutic strategy.

## Future Clinical Trial Design and Endpoints to Develop Novel Vectors

### Clinical Endpoints: Relapse-Free Versus Overall Survival Endpoints in Cancer Vaccine Studies

Although traditional clinical development of anti-cancer agents often rely on surrogate markers of patients benefit, such as tumor response, or relapse-free survival (RFS), these endpoints may not reliably predict the clinical benefit of contemporary cancer vaccines. For example, two prostate cancer immunotherapies, sipuleucel-T and PROSTVAC, increase survival of men with metastatic prostate cancer in the absence of improvements in RFS or response rates.[1,6]

There have been a number of postulated explanations for this observation but no definitive conclusions. Some have suggested that immunotherapy alone may not alter whether tumor recurs but rather reduces future tumor cell behavior alone or in synergy with subsequent

therapies. For example, if persistent tumor cells had their invasive behavior modified by the vaccination response, recurrences could occur, but they would have a more indolent behavior.

### Immune Endpoints

The reported biologic activity of a cancer vaccine is typically based on the induced antigen-specific immune response. In general, this follows the observation that strategies that activate more potent immune responses also are associated with more potent anti-tumor effects in preclinical models. Although there have been some reports that are mixed, this strategy was justified as previous studies have suggested immune response correlates with improved patient outcome. For example, Barth immunized patients with resected metastases of colorectal cancer with tumor lysate pulsed DCs either matured or not with CD40L and observed a better survival in patients with an ELISPOT-positive immune response.[22] There is controversy with this approach as the immune responses are usually measured in the peripheral blood, whereas other relevant immune responses within the tumor microenvironment are not sampled. In addition, the actual antigen to which the response is documented is not always the native antigen. For example, sipuleucel-T activated immune responses (antibody and T cell) against the fusion molecule of prostatic acid phosphatase (PAP)–GM-CSF (PA2024) at a greater rate than native PAP.[1] Finally, the function of the immune response that specifically is associated with clinical benefit (eg, catalytic activity, cytokine secretion) remains to be elucidated.

### Non-classic Endpoints: Altering Cell Behavior and/or Signaling

Although antibody responses classically include ADCC and complement-mediated cytotoxicity (CDC), in situations where the target antigen also has a function related to the malignant phenotype, antibody responses may mediate other anticancer activities. For example, we hypothesized that the polyclonal anti-HER2 antibody responses induced by HER2-containingcancer vaccines could inhibit HER2 function, even in trastuzumab- and lapatinib-refractory tumors. Furthermore, we hypothesized that the antibody response could synergize with lapatinib to enhance tumor inhibition. We developed a recombinant adenoviral vector expressing a kinase-inactive HER2 (Ad-HER2-ki) to use as a cancer vaccine. Ad-HER2-ki vaccine-induced potent T-cell and antibody responses in mice and the vaccine-induced polyclonal HER2-specific antiserum-mediated HER2 internalization (and thus, a decrease in HER2 expression) and degradation more effectively than trastuzumab. When combined with lapatinib, the polyclonal HER2 serum caused significant inhibition of HER2 signaling, decreased pERK and pAKT levels and reduced breast cancer cell proliferation. In addition, a known mechanism of resistance to lapatinib, induction of survivin, was inhibited. The combination of Ad-HER2-ki plus lapatinib also showed superior anti-tumor efficacy in vivo. Based on these results, we feel clinical studies using this approach to target HER2-overexpressing breast cancer, including trastuzumab- and lapatinib-resistant tumors is warranted.

## Acknowledgments

Supported in part by NIH P01, Susan G. Komen for the Cure, DOD, and K12.

## References

1. Kantoff PW, Higano CS, Shore ND, et al. Sipuleucel-T immunotherapy for castration-resistant prostate cancer. N Engl J Med. 2010; 363:411–22. [PubMed: 20818862]

2. Morse MA, Chapman R, Powderly J, et al. Phase I study utilizing a novel antigen-presenting cell-targeted vaccine with Toll-like receptor stimulation to induce immunity to self antigens in cancer patients. Clin Cancer Res. 2011; 17:4844–53. [PubMed: 21632857]
3. Marshall JL, Hoyer RJ, Toomey MA, et al. Phase I study in advanced cancer patients of a diversified prime and boost vaccination protocol using recombinant vaccinia virus and recombinant nonreplicating avipox virus to elicit anti-carcinoembryonic antigen immune responses. J Clin Oncol. 2000; 18:3964–73. [PubMed: 11099326]
4. Buchbinder SP, Mehrotra DV, Duerr A, et al. Efficacy assessment of a cell-mediated immunity HIV-1 vaccine (the Step Study): a double-blind, randomised, placebo-controlled, test-of-concept trial. Lancet. 2008; 372:1881–93. [PubMed: 19012954]
5. Paris RM, Kim JH, Robb ML, Michael NL. Prime-boost immunization with poxvirus or adenovirus vectors as a strategy to develop a protective vaccine for HIV-1. Expert Rev Vaccines. 2010; 9:1055–69. [PubMed: 20822348]
6. 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. [PubMed: 20100959]
7. Marshall JL, Gulley JL, Arlen PM, et al. Phase I study of sequential vaccinations with fowlpox-CEA(6D)-TRI-COM alone and sequentially with vaccinia-CEA(6D)-TRICOM, with and without granulocyte-macrophage colony-stimulating factor, in patients with carcinoembryonic antigen-expressing carcinomas. J Clin Oncol. 2005; 23:720–31. [PubMed: 15613691]
8. Pushko P, Parker M, Ludwig GV, Davis NL, Johnston RE, Smith JF. Replicon-helper systems from attenuated Venezuelan equine encephalitis virus: expression of heterologous genes in vitro and immunization against heterologous pathogens in vivo. Virology. 1997; 239:389–401. [PubMed: 9434729]
9. MacDonald GH, Johnston RE. Role of dendritic cell targeting in Venezuelan equine encephalitis virus pathogenesis. J Virol. 2000; 74:914–22. [PubMed: 10623754]
10. Morse MA, Hobeika AC, Osada T, et al. An alphavirus vector overcomes the presence of neutralizing antibodies and elevated numbers of Tregs to induce immune responses in humans with advanced cancer. J Clin Invest. 2010; 120:3234–41. [PubMed: 20679728]
11. Gallo P, Dharmapuri S, Cipriani B, Monaci P. Adenovirus as vehicle for anticancer genetic immunotherapy. Gene Ther. 2005; 12(1):S84–91. [PubMed: 16231059]
12. McCoy K, Tatsis N, Korioth-Schmitz B, et al. Effect of preexisting immunity to adenovirus human serotype 5 antigens on the immune responses of nonhuman primates to vaccine regimens based on human- or chimpanzee-derived adenovirus vectors. J Virol. 2007; 81:6594–604. [PubMed: 17428852]
13. Abbink P, Lemckert AA, Ewald BA, et al. Comparative seroprevalence and immunogenicity of six rare serotype recombinant adenovirus vaccine vectors from subgroups B and D. J Virol. 2007; 81:4654–63. [PubMed: 17329340]
14. Schoggins JW, Falck-Pedersen E. Fiber and penton base capsid modifications yield diminished adenovirus type 5 transduction and proinflammatory gene expression with retention of antigen-specific humoral immunity. J Virol. 2006; 80:10634–44. [PubMed: 16943295]
15. Seregin SS, Amalfitano A. Overcoming pre-existing adenovirus immunity by genetic engineering of adenovirus-based vectors. Expert Opin Biol Ther. 2009; 9:1521–31. [PubMed: 19780714]
16. Osada T, Yang XY, Hartman ZC, et al. Optimization of vaccine responses with an E1, E2b and E3-deleted Ad5 vector circumvents pre-existing anti-vector immunity. Cancer Gene Ther. 2009; 16:673–82. [PubMed: 19229288]
17. Gabitzsch ES, Xu Y, Yoshida LH, et al. Novel Adenovirus type 5 vaccine platform induces cellular immunity against HIV-1 Gag, Pol, Nef despite the presence of Ad5 immunity. Vaccine. 2009; 27:6394–8. [PubMed: 19559110]
18. Gabitzsch ES, Xu Y, Yoshida LH, et al. A preliminary and comparative evaluation of a novel Ad5 [E1-, E2b-]recombinant-based vaccine used to induce cell mediated immune responses. Immunol Lett. 2009; 122:44–51. [PubMed: 19073216]

19. Gabitzsch ES, Xu Y, Balint JP Jr, Hartman ZC, Lyerly HK, Jones FR. Anti-tumor immunotherapy despite immunity to adenovirus using a novel adenoviral vector Ad5 [E1-, E2b-]-CEA. Cancer Immunol Immunother. 2010; 59:1131–5. [PubMed: 20361185]

20. Osada T, Berglund P, Morse MA, et al. Co-delivery of antigen and IL-12 by Venezuelan equine encephalitis virus replicon particles enhances antigen-specific immune responses and antitumor effects. Cancer Immunol Immunother. In press.

21. Hartman ZC, Osada T, Glass O, et al. Ligand-independent toll-like receptor signals generated by ectopic overexpression of MyD88 generate local and systemic antitumor immunity. Cancer Res. 2010; 70:7209–20. [PubMed: 20823152]

22. Barth RJ Jr, Fischer DA, Wallace PK, et al. A randomized trial of ex vivo CD40L activation of a DC vaccine in colorectal cancer patients: tumor-specific immune responses are associated with improved survival. Clin Cancer Res. 2010; 16:5548–56. [PubMed: 20884622]

## Table 1
### Strategies to Generate Antigen-Loaded Dendritic Cells for Cancer Immunotherapy

| |
|---|
| Strategy 1 |
| Ex vivo antigen loading, activation, and re-infusion of autologous dendritic cells for cancer immunotherapy. |
| Strategy 2 |
| Delivery of antigen and costimulatory molecules to resident dendritic cells by targeting antigen delivery to mannose receptors, and delivering costimulatory signals. |
| Strategy 3 |
| Recombinant viral vectors that express antigen and costimulatory molecules to resident dendritic cells and infect them directly or express antigen that is processed and presented by dendritic cells. |