3-2016

Micro- and Nanoparticulate Cancer Vaccines: A Vision for the Future

Lipika Chablani
St. John Fisher University, lchablani@sjf.edu

Publication Information

Chablani, Lipika (2016). "Micro- and Nanoparticulate Cancer Vaccines: A Vision for the Future." *AAPS Newsmagazine* , 14-18.

Micro- and Nanoparticulate Cancer Vaccines: A Vision for the Future

Abstract

In lieu of an abstract, here is the article's first paragraph:

Formulation scientists are constantly challenged with unique opportunities to deliver chemotherapeutic drug molecules to cancer patients. With the growing rate of cancer diagnoses, new therapies are being introduced to pave their way to the market. Along with drug delivery, these formulation approaches are being screened for formulation, design, and development of cancer vaccines. Can a vaccine be formulated to obtain a protective or therapeutic immune response against cancer?

Disciplines

Pharmacy and Pharmaceutical Sciences

MICRO- AND NANOPARTICULATE CANCER VACCINES: A VISION FOR THE FUTURE

Micro/nanoparticles show promise for immunotherapy against cancer.

By Lipika Chablani, Ph.D., St. John Fisher College

Formulation scientists are constantly challenged with unique opportunities to deliver chemotherapeutic drug molecules to cancer patients. With the growing rate of cancer diagnoses, new therapies are being introduced to pave their way to the market. Along with drug delivery, these formulation approaches are being screened for formulation, design, and development of cancer vaccines. Can a vaccine be formulated to obtain a protective or therapeutic immune response against cancer?

Research proves that micro/nanoparticles loaded with unique cancer antigens and decorated with immunostimulatory molecules are capable of activating the immune system against cancer. Priming the immune system provides a specific cytotoxic effect against the cancer cells. Thus, these microparticulate cancer vaccines activate the immune system and also limit the adverse effects on the healthy cells as seen with chemotherapeutic agents. Several research studies in this direction are progressing to promising preclinical outcomes.

CANCER VACCINES AND IMMUNOTHERAPY

The term cancer refers to about 200 diseases that share two common characteristics: an uncontrolled growth of cells and the ability to invade and damage normal tissues either locally or at distant sites in the body. Current advances in diagnostic methods allow health professionals to diagnose several cancer patients early and provide medical interventions. With the advent of chemotherapy in the 1940s, there was an increasing need for drug delivery systems/formulations for these relatively toxic drug molecules. Several of these chemotherapeutic drug molecules are given intravenously and are associated with systemic adverse effects. Most of these chemotherapeutic drugs are limited by virtue of their dose-related toxicity, leaving the patient to move on to an alternative chemotherapeutic regimen. Apart from chemotherapy, surgical removal of tumor masses and elimination of solid tumors via radiation is also commonly employed. However, all these methods are significantly invasive and debilitate the patient, impacting the quality of life. Additionally, many patients undergo a relapse and must revisit these challenges all over again.

Considering the drawbacks of chemotherapy, surgery, and radiation therapy, patients and health practitioners often seek noninvasive alternative therapies. A promising alternative is immunotherapy. Immunotherapy involves training patients' own immune systems to fight against cancer and eliminate it. These immunotherapeutic approaches include application of various kinds of cancer vaccines to prime the immune system against cancer. Many ongoing preclinical and clinical studies aim to achieve a successful cancer vaccine.

Formulating a cancer vaccine is much more challenging than formulating an infectious disease vaccine. A cancer vaccine is against a self-cell that has mutated and become cancerous. Thus, it is important to

The Formulation Design and Development section submitted this article.
Reprinted from the March 2016 issue of AAPS Newsmagazine.

prime the immune system judiciously to ensure an attack against cancer cells only and not healthy cells, which may resemble these cancerous cells in several aspects. Additionally, each cancer patient may be unique with a variable set of mutations requiring an individualized therapy to gain maximum efficacy. All these factors make formulation, design, and development of a cancer vaccine challenging and provide an opportunity for researchers to explore various vaccine delivery systems. These vaccine delivery systems can enable us to prime the immune cells with unique tumor-associated antigens (TAAs) that are expressed only by the tumor cells and thus generate an immune response against the cancer. To further boost the immune response generated by the vaccine, an adjuvant (an immunostimulant) is included in the formulation. Upon generation of the immune response, if the vaccine is capable of inducing the memory cells of the immune system to remember the cancer antigens, the benefits will be multifold. Such memory immune responses are beneficial when the cancer relapses. Unlike conventional therapies, immunotherapy can potentiate the immune response against a relapsed tumor (if it expresses the same antigens) and eliminate it before it forms a mass. Thus, these cancer vaccines can provide long-term protection versus temporary relief.

MICRO- AND NANOPARTICLES AS CANCER VACCINES

In the last decade, several preclinical studies have explored micro/nanoparticles for immunotherapeutic applications. These micro/nanoparticles have been formulated with different polymers, lipids, proteins, and

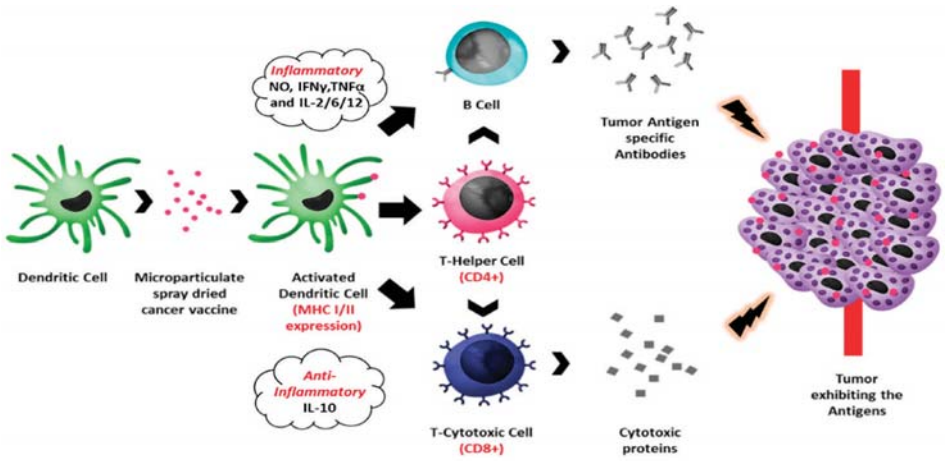


Figure 1: Graphical representation of a microparticulate cancer vaccine. Upon administration of the vaccine microparticles, the dendritic cells are stimulated releasing inflammatory and/or anti-inflammatory cytokines. These activated dendritic cells are then capable of activating humoral (B cells) and cellular (T cells) adaptive immune responses to inhibit tumor growth.

adjuvants. Examples of such polymers, lipids, and proteins include cyclodextrins, celluloses, phospholipids, poly (lactic-co-glycolic) acid (PLGA), and albumin.[1-4] Formulation scientists have employed techniques like spray drying and coacervation to prepare these particulate delivery systems, resulting in an optimized and reproducible formulation. These micro- and nanoparticles may either encapsulate the vaccine antigens (tumor-specific antigens) or exist as a matrix of the polymer embedded with the vaccine antigens. The formulation design permits the inclusion of adjuvants to boost the immune response. Adjuvants like interleukins, GM-CSF, alum, and CpG have been evaluated with such vaccines.

Irrespective of composition, these micro/nanoparticles are within the average size range of 100 nm to 5 µm. This unique size range allows these micro/nanoparticles to mimic the infectious disease agents like bacteria/viruses, thus allowing the dendritic and macrophage cells, in vivo, to phagocytose them preferentially. Upon phagocytosis, the particles are digested by the cellular enzymes, and the antigens are expressed by the dendritic cells via the major histocompatibility complexes (major histocompatibility complex [MHC] I or II). These dendritic cells are capable of releasing immunostimulatory cytokines to activate both innate and adaptive immune systems. The adaptive immune system responds specifically to the antigens expressed by the dendritic cells and prepares an assault against any cancer cells expressing these antigens (Figure 1). Multiple boosters of these microparticulate vaccines induce a series of immunostimulatory responses and assist in eliminating the tumor burden. The results from preclinical studies in this field depict that these micro/nanoparticulate vaccines are capable of generating tumor-specific adaptive immune responses and lead to the shrinkage of tumor mass extending the life span of diseased animals.[5-8] These results are promising and merit further investigation to prepare a cancer vaccine.

Such particulate delivery systems include a range of nanocarriers including liposomes, virus-like particles, virosomes, polymeric particles, and nanoemulsions.[9] Here are some of the notable advancements in the field of immunotherapy using these individual nanocarriers:

Liposomes

Liposomal delivery systems consist of a phospholipid bilayer structure with an aqueous core, thereby accommodating both hydrophilic and lipophilic bioactive molecules, either by encapsulating them or embedding them in the bilayers respectively. DepoVax is an example of such a liposome-in-oil emulsion composed of cancer antigens and an adjuvant. The DepoVax platform includes liposomes containing cancer antigens and Montanide ISA51 VG as the adjuvant in the oil phase. This system has been evaluated under phase 1 clinical trials and has been shown to be safe and capable of inducing immune response against ovarian cancer. Additional clinical studies using this platform are ongoing to evaluate the safety and immune response of the vaccine with low dose cyclophosphamide against ovarian cancer. Similarly, Tecemotide (Stimuvax or BLP25) was another liposomal cancer vaccine that paved its way through the phase 3 clinical trials. The vaccine included the MUC1 antigen, which is overexpressed by several cancers such as lung, breast, prostate, and colorectal. Along with the antigen, the vaccine consisted of monophosphoryl lipid A as the adjuvant. The preclinical and early clinical studies provided promising results; however, the recent phase 3 results evaluating the application of the vaccine against non-small-cell lung cancer were unable to meet the primary outcomes of the study. Further evaluation of the vaccine candidate is under discussion and may need a new direction.

Other significant advancements with liposomal vaccine delivery include use of pegylated liposomes to enhance the circulation time of these delivery systems for an improved immune response. Cationic liposomes and temperature- and pH-sensitive liposomes have been explored to augment the immune response generated by such liposomal cancer vaccines. Thus, combination of liposomal vaccine delivery systems along with the adjuvants and surface modifications are some of the key developments in this area of research.

Virus-like Particles and Virosomes

Both virus-like particles (VLPs) and virosomes provide a unique particulate structure similar to viruses, thus making them inherently immunogenic as a vaccine carrier. The viral genome is missing in these structures, rendering them noninfectious and safe for prophylactic applications.

VLPs have been successfully used to administer human papilloma virus (HPV) vaccine. These vaccines contain VLPs made of

only the major HPV coat or capsid protein L1. Cervarix and Gardisil are two of such VLP-based cancer vaccines that have been developed and commercialized. Cervarix is a bivalent and Gardisil is a quadrivalent vaccine containing either two (HPV 16/18) or four (HPV 16/18/6/11) L1 recombinant proteins. Findings from the clinical trials and use of these vaccines in the past few years have established their safety and efficacy in protection against cervical cancer caused by HPV. Both these vaccines are used prophylactically and function by generation of neutralizing antibodies against the viral strains. Thus, the application of such VLP-based cancer vaccines is limited to cancers caused by viruses and vaccines intended for prophylactic applications.

Similarly, virosomes have gained significance as a vaccine carrier for infectious diseases such as influenza (Inflexal) and hepatitis A (Epaxal). Virosomes are unilamellar lipid viral vesicles comprised of phospholipids and glycoproteins acting as antigens for induction of immune response. These characteristic features allow them to mimic the infectious disease agents and generate a strong cellular immune response. Immunopotentiating reconstituted influenza virosomes (IRIVs) are the most commonly used virosomes against infectious diseases. The IRIVs have been evaluated in preclinical studies as a cancer vaccine delivery system against melanoma and breast cancer as well. Further, using a similar approach, another class of virosomes—the Sendai virosomes—has been developed using the Sendai viruses. The viral envelope of Sendai viruses, used for these virosomes, hosts two types of glycoproteins, hemagglutinin-neuraminidase and the fusion protein. These two glycoproteins assist the virosomes to bind to the host-cell surface and eventually fuse into their cell membranes. These properties make Sendai virosomes a suitable nanocarrier for delivery of bioactive molecules for cancer immunotherapy.

Polymeric Particles

Micro- and nanoparticulate delivery systems have been formulated using both natural and synthetic polymers. Such polymeric particles have been used as drug delivery systems as they are capable of targeting and reaching biological sites, which are otherwise inaccessible. Such particulate systems have been successfully used to deliver chemotherapeutic drugs. Abraxane (human albumin-bound paclitaxel nanoparticle) is a good example of such a Food and Drug Administration (FDA) approved commercial nanoparticulate delivery system used for the treatment of breast, non-small-cell lung, ovarian, and pancreatic cancer. Researchers have attempted to translate these albumin-incorporating nanoparticulate delivery systems to prepare a particulate cancer vaccine.[4,7,10]

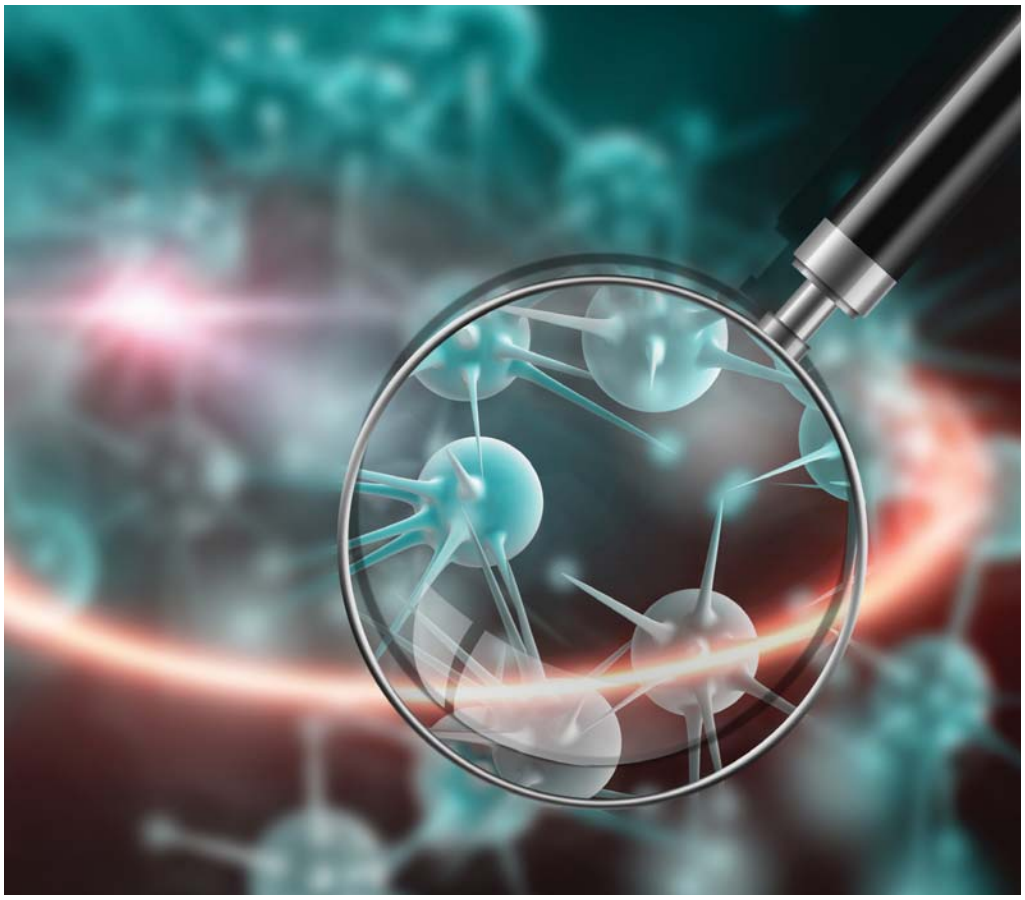
A variety of polymeric particles have been formulated and evaluated for antigen delivery and potential use as a cancer vaccine. Some of the most commonly studied polymers for this purpose include PLGA, polylactide (PLA), albumin, gelatin, chitosan, and cyclodextrin. The polymers are preferentially biodegradable, nontoxic, and nonimmunogenic to serve as a carrier. Further, these polymeric particles provide the following advantages:

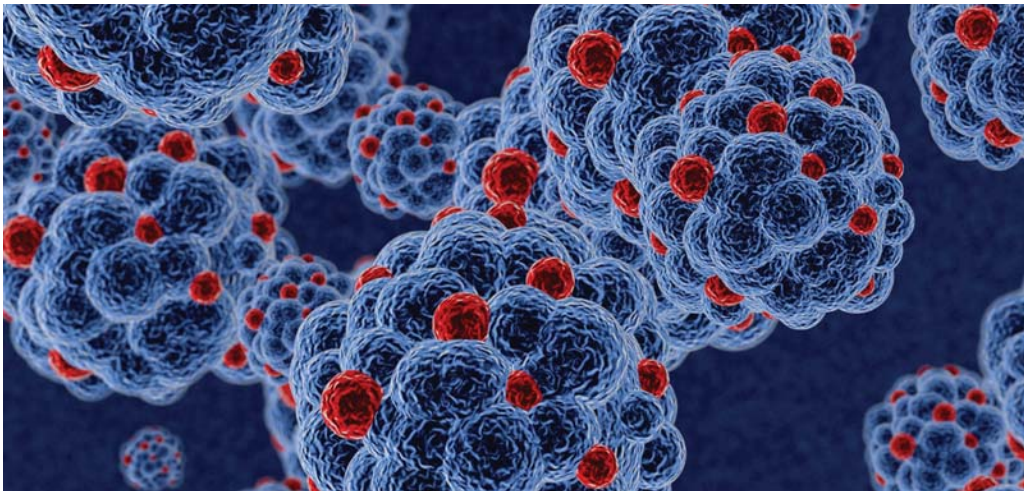
1) Protection of the cancer antigens (often susceptible to systemic degradation)

2) Enhanced uptake and presentation of the antigens via the MHC pathway

3) Sustained release of the antigens to obtain a prolonged effect of the vaccine

PLGA is one of the extensively studied polymers for immunotherapy and is also approved by FDA for drug delivery in humans. Research findings suggest that PLGA particles are well recognized by the dendritic cells and lead to effective antigen presentation and upregulation of inflammatory cytokines responsible for induction of cellular immune response. These particles also possess adjuvant-like properties, boosting the immune response further. However, the formulation of PLGA particles involves use of organic solvents and higher temperatures, which may not be suitable for all the





antigens and pose a limitation on scalability of these vaccine carriers.

As an alternative to synthetic polymers (PLGA and PLA), natural polymers like albumin and gelatin have been explored due to their inherent biocompatibility and safety profiles. The techniques required to formulate particles using these polymers do not involve any organic solvents and can be easily scaled up. Additionally, these techniques do not pose any risk to the biological structure of the antigens being loaded in the carrier. Research findings have established that particles made of these natural polymers are capable of protecting and delivering their cargo to result in an immune response against the variety of in vivo cancer models. Furthermore, there is a potential of crosslinking these polymers (albumin cross linked with glutaraldehyde) to obtain a robust sustained-release delivery vehicle, which can be administered via oral, transdermal, or subcutaneous route of delivery. The preclinical results from these particulate delivery systems confirm the potential of their use as carriers for cancer vaccines.

LESSONS LEARNED AND VISION FOR THE FUTURE

The preclinical and clinical outcomes of micro/nanoparticulate cancer vaccines are encouraging, yet additional studies are required in this direction to establish their scalability, safety, and efficacy. Some of the major challenges associated with micro/nanoparticulate vaccines are attributed to their biodegradability, stability, and toxicity. The choice of polymers, proteins, and adjuvants is vital to ensure these ingredients can be cleared from the system and do not pose any toxicity issues once digested at the cellular level. Stability profiles of these antigen-containing micro/nanoparticles has also been a concern, and techniques like lyophilization and spray drying are evaluated to stabilize these antigen formulations. Furthermore, it is imperative to be able to scale up these preparations, if additional clinical studies are warranted. As this approach is still in its infancy, researchers are exploring ways to optimize such cancer vaccines to induce stronger and longer-lasting immune responses that are capable of generating a memory response to avoid potential relapse of the cancer. Considering the scope of this approach to achieve a competent therapeutic cancer vaccine, it is promising to look forward to the advances researchers will be making in this direction in the upcoming decade.

DISCUSSION POINT

We want to know your opinion!

Please discuss the following question with your colleagues via the *AAPS Blog*. To find the blog entry associated with this article, visit http://aapsblog.aaps.org/tag/aaps-newsmagazine.

Which other alternative approaches can benefit formulation scientists to boost the immune response of such micro/nanoparticulate cancer vaccines?

Learn more about the AAPS Formulation Design and Development section; visit the section's webpage at www.aaps.org/FDD.

REFERENCES

1. Geary SM, Hu Q, Joshi VB, Bowden NB, Salem AK. Diaminosulfide based polymer microparticles as cancer vaccine delivery systems. *J Control Release*. 2015;220B:682–90. doi:10.1016/j.jconrel.2015.09.002.
2. Hanson MC, Bershteyn A, Crespo MP, Irvine DJ. Antigen delivery by lipid-enveloped PLGA microparticle vaccines mediated by in situ vesicle shedding. *Biomacromolecules*. 2014;15(7):2475–81. doi:10.1021/bm500337r.
3. Chablani L, Tawde SA, Akalkotkar A, D'Souza C, Selvaraj P, D'Souza MJ. Formulation and evaluation of a particulate oral breast cancer vaccine. *J Pharm Sci*. 2012;101(10):3661–71. doi:10.1002/jps.23275.
4. D'Souza B, Bhowmik T, Shashidharamurthy R, Oettinger C, Selvaraj P, D'Souza M. Oral microparticulate vaccine for melanoma using M-cell targeting. *J Drug Target*. 2012;20(2):166–73. doi:10.3109/1061186X.2011.622395.
5. Joshi VB, Geary SM, Gross BP, Wongrakpanich A, Norian LA, Salem AK. Tumor lysate-loaded biodegradable microparticles as cancer vaccines. *Expert Rev Vaccines*. 2014;13(1):9–15. doi:10.1586/14760584.2014.851606.
6. Xia X, Mai J, Xu R et al. Porous silicon microparticle potentiates anti-tumor immunity by enhancing cross-presentation and inducing type I interferon response. *Cell Rep*. 2015;11(6):957–66. doi:10.1016/j.celrep.2015.04.009.
7. Tawde SA, Chablani L, Akalkotkar A, et al. Formulation and evaluation of oral microparticulate ovarian cancer vaccines. *Vaccine*. 2012;30(38):5675–81. doi:10.1016/j.vaccine.2012.05.073.
8. Zhang H, Huang B. Tumor cell-derived microparticles: a new form of cancer vaccine. *Oncoimmunology*. 2015;4(8):e1017704.
9. Krishnamachari Y, Geary SM, Lemke CD, Salem AK. Nanoparticle delivery systems in cancer vaccines. *Pharm Res*. 2011 Feb;28(2):215–36. doi:10.1007/s11095-010-0241-4.
10. Akalkotkar A, Chablani L, Tawde SA, D'Souza MJ. Development of a microparticulate prostate cancer vaccine and evaluating the effect of route of administration on its efficacy via the skin. *J Microencapsul*. 2015;32(3):281–9. doi:10.3109/02652048.2015.1017615.