Review article

CLINICAL AND EXPERIMENTAL VACCINE RESEARCH

Application of radiation technology in vaccines development

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One of the earliest methods used in the manufacture of stable and safe vaccines is the use of chemical and physical treatments to produce inactivated forms of pathogens. Although these types of vaccines have been successful in eliciting specific humoral immune responses to pathogen-associated immunogens, there is a large demand for the development of fast, safe, and effective vaccine manufacturing strategies. Radiation sterilization has been used to develop a variety of vaccine types, because it can eradicate chemical contaminants and penetrate pathogens to destroy nucleic acids without damaging the pathogen surface antigens. Nevertheless, irradiated vaccines have not widely been used at an industrial level because of difficulties obtaining the necessary equipment. Recent successful clinical trials of irradiated vaccines against pathogens and tumors have led to a reevaluation of radiation technology as an alternative method to produce vaccines. In the present article, we review the challenges associated with creating irradiated vaccines and discuss potential strategies for developing vaccines using radiation technology.

Keywords: Gamma-radiation, Irradiated vaccine, GVAX, Killed vaccine

Radiation and Vaccines

Radiation is the emission or transmission of energy in the form of waves (ionizing radiation) or electron particles (non-ionizing radiation) [1]. The use of ionizing radiation, including X-rays and gamma rays, has increased substantially over the last 30 years in both medicine and industry [2-5]. In addition to its initial applications in the diagnosis and treatment of disease, radiation technology has expanded into other areas such as crop breeding, sterilization of food, polymer processing, and processing of environmental pollutants [6-9]. Radiation technology has also been used in the development of human and animal vaccines, especially in the sterilization and generation of random mutations.

Many vaccines used today rely on technologies developed over 100 years ago, and involve some form of attenuation (i.e., the use of an alternative or mutant strain of pathogenic organism with reduced virulence that maintains its immunogenicity, or inactivation, where chemical or physical methods are used to kill virulent pathogenic strains) [10-13]. These vaccines have been extremely successful in protecting against animal and human diseases caused by viruses and bacteria. Smallpox and Rinderpest have now been successfully eradicated throughout the world since the introduction of vac-

cines [14-17]. Nevertheless, the aim remains to maximize the effectiveness and quality of currently available or new vaccines, because current methods of vaccine manufacture are not cost-effective, are susceptible to chemical contamination, are difficult to match to current circulating strains, and are susceptible to other manufacturing issues.

Radiation technology is of interest to vaccine manufacturers, because it can remove chemical contaminants and penetrate pathogens to damage the DNA [18,19]. However, the development of irradiated vaccines has not been pursued avidly over the past 20 years for two main reasons. First, the development of new radiation techniques has been considered impractical or difficult due to issues accessing the radiation equipment. Second, it has been thought that modern subunit vaccines would provide a solution, as they can be developed more easily [20]. However, there are several reasons to reevaluate the use of radiated inactivation and attenuation for the production of vaccines [20,21]. The recent successful development of irradiated vaccines for human malaria and influenza have demonstrated the feasibility and practicality of this technique, and have shown that technical problems can be overcome using existing expertise, without needing to resort to sophisticated technology [22,23]. Moreover, this technology has been used to produce an anti-cancer vaccine by inactivating cancer cells [24-26]. In the present review, we discuss several promising candidates for irradiated vaccines that have undergone clinical trials, and assess recent advances in radiation vaccine technologies.

Vaccines Based on Inactivated Microorganisms and Tumor Cells

Inactivated vaccines are produced by killing the pathogens with chemicals, heat, or radiation. These vaccines are more stable and safer than live vaccines, as they can be stored and transported in a freeze-dried form that makes them accessible to people in developing countries [27,28]. Formaldehyde, the most common chemical used in vaccine production, was first tested in a vaccine by Madsen [29], and was later shown to be successful in preventing several infectious diseases such as typhoid, cholera, poliovirus, hepatitis A, Japanese encephalitis, and tick-borne encephalitis virus [28]. However, it can cause irreversible modifications by cross-linking antigens that can damage key antigenic epitopes, leading to reduced immunogenicity or even exacerbated disease following a microbial infection [30].

β-Propiolactone (BPL) is also a common inactivation method that was first described in 1955 [20]. It is used in the production of influenza and rabies vaccines, and is also used in vaccines currently under development, such as those for *Streptococcus pneumoniae*. The advantages of this chemical are that it is rapidly neutralized into a nontoxic, noncarcinogenic product by the addition of thiosulphate, and it interacts directly with nucleic acids by inducing DNA double helix cross-linking [31]. However, BPL may also interact with amino acids, which contain nucleophilic moieties that induce conformational changes on surface antigens [32]. Thus, inactivation of pathogens with BPL may also trigger adverse immune reactions, including the induction of allergic responses through chemical modification of the vaccine components [33,34]. Therefore, there is an increasing demand to identify safe and effective strategies to produce inactivated vaccines, which are crucial for the future of vaccine development.

Radiation inactivation of pathogens has potential applications in sterilization and the manufacture of biological reagents and laboratory supplies [35]. Since the 1940s, when ionizing radiation (e.g., gamma rays and X-rays) was introduced for the sterilization of pathogens, vaccine development using irradiation has been extensively investigated [36,37]. The major advantages of ionizing radiation in vaccine development compared to ultraviolet light or chemical agents are its ability to penetrate through most biological materials, and the fact that it targets both double and single stranded nucleic acids while causing less damage to surface antigenic proteins. Moreover, there is no need to remove any chemical residue after inactivation. Although the argument remains that irradiated vaccines elicit different immune responses than those generated by heat-killed or chemically killed methods, the demand for the application of gamma radiation is increasing for the development safe vaccines [38-40].

Irradiated Viral Vaccines

Trivalent inactivated or live attenuated influenza vaccines are commonly used worldwide [11,41-43]. Inactivated influenza vaccine is prepared by treating the virus with a chemical agent that contains either the whole inactivated virus or the active part (split or subunit vaccines) [11,43,44]. The live attenuated influenza virus has the reduced ability to replicate in human cells, but can still stimulate immune responses [45,46]. Each year, predictions are made of three potential influenza strains for the coming season, which are based on a continuous world-

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wide surveillance program by the World Health Organization [42]. However, most influenza vaccines against any predicted seasonal flu provide only modest protection for the given strains, and have little efficacy in the elderly [47-50]. This is because the influenza vaccine is highly dependent on how well the vaccine strain matches the newly emerging virus. Research is underway to develop a universal vaccine that has a broad coverage of influenza antigenic drift that will not require annual modification [42,43].

Over the last 60 years, the development of new methods of virus inactivation has been explored [51-59]. Gamma radiation inactivation has been suggested as an alternative method for inactivation of virus reproduction, primarily by damaging the nucleic acid while preserving immunogenicity. Mullbacher et al. [60] first demonstrated a high cross-protective immune response of irradiated influenza A virus against other influenza A strains. Gamma irradiated influenza vaccine was more effective at priming cross reactive cytotoxic T cells, and protected mice against a heterologous influenza virus [60-62]. Alsharifi and Mullbacher [63] showed that a single dose of nonadjuvanted intranasal gamma-irradiated influenza A vaccine (GammaFlu) provided robust protection in mice, which was mainly mediated by cytotoxic T cells. Unlike the chemical inactivation method, gamma irradiation preserved the functional domains of the viral proteins, which facilitated uptake and presentation on major histocompatibility complex class I (MHC-I) of antigen presenting cells. This approach has been tested in pre-clinical studies by Gamma Vaccines Pty (Manuka, ACT, Australia), and is now moving towards a full clinical trial.

A vaccine against the human immunodeficiency virus (HIV) would be highly effective for preventing acquired immunodeficiency syndrome. Because HIV was identified in 1983, significant progress has been made in the development of an HIV vaccine worldwide. However, to date, no vaccine has been fully successful. Initially, subunit vaccines, GP120 (VaxGen Inc., San Francisco, CA, USA) and Ad5 (Merck & Co., Kenilworth, NJ, USA), were approved in clinical trials, but have not shown sufficient efficacy in human subjects. In a recent clinical trial conducted by Sanofi Pasteur (Lyon, France), GP120 carrying the Canarypox virus vaccine showed ~25% improved protection compared to a non-vaccinated group of 60,000 human subjects; however, it was dropped at clinical phase III. Over the past 15 years, several groups have initiated the pre-clinical development of inactivated HIV or simian immunodeficiency virus (SIV) vaccines, which conferred potent serological responses against host cell components incorporated into HIV/SIV virions. Currently, the most promising result has come from two gamma-irradiated whole-killed attenuated HIV vaccines, SAV0001 and Remune [64,65].

Remune, invented by Jonas Salk in 1987 in collaboration with Dr. Dennis Carlo of the Immune Response Corporation (http://www.immuneresponsebiopharma.com) [66,67], was the first to go to large national trials to ascertain whether it could assist current antivirals by enabling the immune system to control HIV more effectively. This vaccine, derived from an intersubtype recombinant of clade A envelope and clade G Cag, is inactivated through the sequential application of BPL and gamma irradiation. The HIV envelope gp120 glycoprotein is depleted during preparation and inactivation [68]. Although a large-scale multi-center phase III trial with HIV patients on antiretroviral therapy showed no significant differences in the incidence of opportunistic infections or death, a statistically significant decline in viral load, increased CD4+ T cell counts, and enhanced HIV-1 specific antibody responses were observed in the subjects treated with Remune.

SAV001 was developed by Dr. Chil-Yong Kang at Western University, Canada [69]. This represents the first and only preventive HIV vaccine tested in clinical trials, and is based on a genetically modified killed whole-virus. The *nef* and *vpu* genes were deleted in the HIV-1 strain to make the attenuated strain, and the env signal peptide was replaced with the honey bee antimicrobial peptide melittin to enhance viral replication and production. Thus, this genetically modified HIV-1 strain is non-pathogenic, and can be produced in large quantities in a cell culture-based system. It is manufactured as a killed vaccine, by harvesting HIV-1 that is completely inactivated by aldrithiol-2 and sequential gamma irradiation [70,71]. The phase I clinical trial (ClinicalTrials.gov Identifier: NCT-01546818) was completed in 2013, and resulted in significant increases in the levels of gp120-specific and P24-specific antibodies, whereas no adverse effects were observed. Phase II/III large multi-center clinical trials on higher risk HIV patients will be conducted shortly.

Irradiated Bacterial Whole Cell Vaccines

Since the typhoid vaccine was first introduced as an inactivated bacterial vaccine at the end of the 19th century, the administration of inactivated whole cell bacterial vaccines is one of the most well-studied methods of vaccination against bacterial infections [20]. This approach offers several advan-

tages. First, they are naturally occurring microparticles that can carry multiple antigens that can be important in providing protection. Second, this approach is relatively quick and inexpensive to manufacture. Although pertussis and anthrax vaccines are the only current licensed inactivated bacterial vaccine used for immunization of the general public, the demand to develop new inactivated bacterial vaccines for emerging pathogens is increasing [72-74]. Irradiated bacterial vaccines, which prevent replication but retain their metabolic activity, generate higher humoral immune responses and protection against extracellular and intracellular bacteria, including human and animal pathogens (Table 1) [75-86]. Inactivated bacterial vaccines were originally intended for intranasal or oral administration to activate a mucosal immune response [87-90]. Intranasal immunization with inactivated bacterial vaccines elicits serotype independent humoral as well as cellular immune responses [91,92].

Leprosy and tuberculosis are the most common mycobacterial diseases representing a major cause of death worldwide [93-95]. The most effective strategy for treating tuberculosis (TB) is vaccination. Live bacille Calmette–Guerin (BCG), which was introduced in 1921, is the only available vaccine against both diseases [96]. Although it provides immunization protection for infants and young adults, it has had inconsistent and unpredictable results in adults, sometimes causing severe allergenic reactions in the skin, and offers less durable protection that often requires a second boosting immunization [96,97]. More than 10 TB vaccines are in the early development stages [98-100]. The most effective clinical results have being obtained using the heat-killed inactivated *Mycobacterium obuense* vaccine, DAR-901 (ClinicalTrials.gov Identifier: NCT02063555) [101-103]. A trial in Tanzania of >2,000 HIV positive subjects showed it to be both safe and effective. Irradiated killed TB vaccines were first reported by Olson et al. in 1947 [104] and Paterson et al. in 1949 [105]. Although the irradiated-killed TB vaccine gave a similar degree of protection as the live BCG vaccine in animal models, the allergenic effect was markedly reduced. Because many previous studies have shown that irradiated TB elicits the robust production of antibodies and protection against the challenge of infectious TB, this strategy should be considered an alternative inactivation method for TB whole-cell vaccines (WCVs).

Streptococcus pneumoniae is a causative agent in children and older adults. Currently the available polysaccharide conjugate vaccine (PCV) generates serotype-specific antibody responses [106,107]. Phase IV surveillance studies in many countries found an increasing number of non-vaccine serotypes and the appearance of new serotypes [108]. Thus, alternative approaches are being considered, such as protein-based vaccines and WCVs. An inactivated whole-cell pneumococcal vaccine has been sought since the beginning of the 20th century. More recently, a collaboration between PATH Vac-

Table 1. Summary of irradiated bacterial vaccines

Bacteria	Vaccine efficacy	References
Gram negative bacteria		
Mycobacteria lepae	Reduce *M. leprae* by boosting vaccination with irradiated *M. leprae* vaccine in a human study	[75,76]
M. avium subsp. *paratuberculosis*	Irradiated mycobacteria vaccine elicits a similar level of protection with live *M. bovis* BCG vaccine in both mouse and human models	
Bacillus Calmette-Guerin	Elicits both CD4- and CD8-specific immune responses in mouse, cow, and chicken models	
Salmonella typhimurium	Induce antigen-specific antibody and T-cell responses	[40,77]
Brucella neotomae	Protect *B. abortus, B. melitensis*, and *B. suis* infections in a mouse model	
Brucella melitensis	Induces mucosal immune response without adjuvant	[78-80]
Vibrio cholera	Prevents *V. cholera* colonization and toxin neutralization	
Gram positive bacteria		
Streptococcus agalactiae	Does not cause inflammation of the mammary glands of mice	[81-83]
	Elicits GBS antibody response in mice	
	Partial protection against Nile Tilapia	
Staphylococcus aureus	Elicits anti-*S. aureus* specific antibodies	[84-86]
	Reduces *S. aureus* colonization in the kidneys and skin	
Listeria monocytogens	Provides higher protection against a *Listeria* challenge model than heat and chemically killed vaccine	[39]
	Elicits an effective and specific CD8+ T-cell response	

BCG, bacille Calmette–Guerin; GBS, Group B Streptococci.

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cine Solutions (Seattle, WA, USA) and Malley's group [109,110] showed successful results using a chemically killed non-encapsulated pneumococcal vaccine in an animal model and in a clinical phase I trial (ClinicalTrials.gov Identifier: NCT-01537185) [93,111]. No serious adverse events were reported in these clinical trials, and currently, participants are being recruited to test its safety and tolerability with intramuscular administration in healthy Kenyan adults and toddlers who have been primed with PCV vaccines. We also investigated the possibility of creating an irradiated pneumococcal vaccine using non-encapsulated pneumococci. Irradiated killed pneumococci showed non-toxic effects *in vitro*, whereas chemically killed pneumonia did (unpublished data). In addition, irradiated WCVs elicited a significantly higher level of antibody responses in mice with intranasal and intramuscular vaccinations (Fig. 1). This shows that irradiation is one possible method to manufacture a whole killed pneumococcal vaccine.

Salmonella enterica causes a variety of infectious diseases in animals and humans [112,113]. Live attenuated vaccines generally confer better protection than killed vaccines; however, they are limited by their toxicity and cause mild diarrhea. Previte [114] first described the use of radiation to increase immunogenicity and decrease the toxicity of Salmonella compared to acetone-, alcohol-, and heat-killed vaccines in mouse and bovine salmonellosis models [115,116]. *Brucella* species (*B. melitensis*, *B. abortus*, *B. suis*) are the causative agents of brucellosis, a chronic bacterial infection in animals and humans. Some live attenuated vaccines (S19, RB51, Rev1) are licensed for use in animals, but not in humans. However, the live vaccine has been implicated in several accidental infections in humans and animals [117,118]. Several inactivated *Brucella* vaccines have been tested as alternative, safer vaccines. In a mouse model, irradiated *B. abortus* RB51 and *B. neotomae* induced protection against systemic and mucosal challenge with *Brucella* spp. Oral vaccination in mice also elicited the activation of CD4 and CD8+ T lymphocytes specific to infectious *Brucella* species [40,77,119-124].

Irradiated Malaria Vaccines

Human malaria is primarily due to infection with one of five *Plasmodium* species: *Plasmodium falciparum*, *Plasmodium vivax*, *Plasmodium malariae*, *Plasmodium ovale*, and *Plasmodium knowlesi*. Of these, *P. falciparum* is responsible for 92% of deaths that mostly occur in children living in sub-Saharan Africa [125]. In 2012 there was an estimated 220 million clinical cases and 0.63 million deaths worldwide from malaria infection [23]. An effective vaccine for *P. falciparum* is needed for use in malaria-endemic populations, but no licensed malaria vaccines and candidates have consistently produced a protective level of efficacy [126].

Based on the life cycle of the malaria parasite and the process of infection, malaria vaccines are divided into four potential target groups; interruption of human to mosquito transmission (parasite sexual and mosquito stages), inhibition of clinical consequences (asexual blood stage), prevention of mosquito to human transmission, and pre-erythrocytic in-

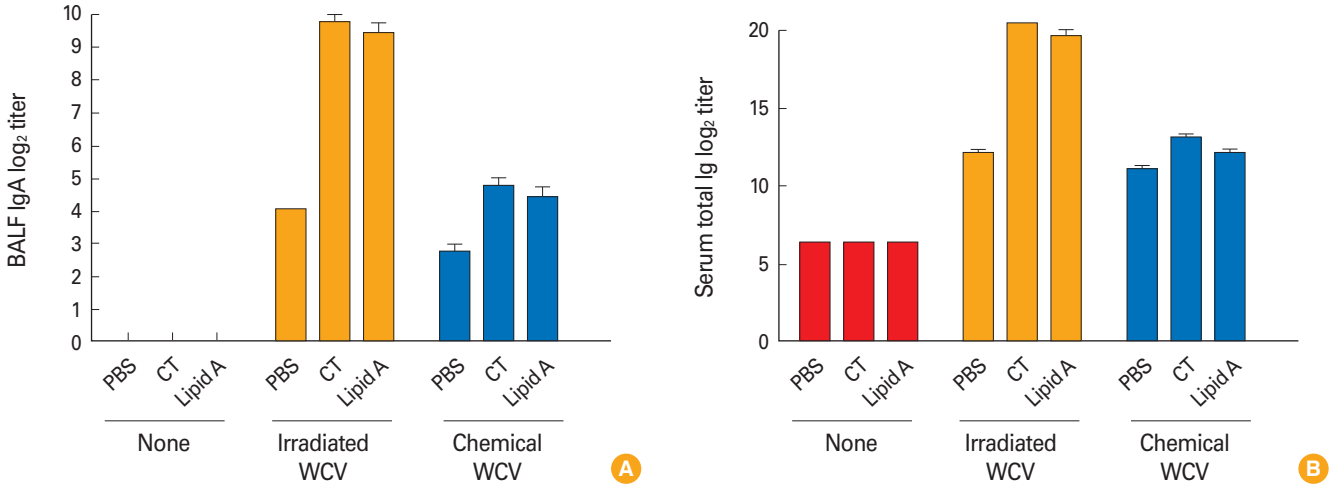

Fig. 1. Irradiated pneumococcal vaccine induced antibody titers. Irradiated or formalin-treated vaccines were administered intranasally to CD-1 mice (n = 5) with phosphate buffered saline (PBS), cholera toxin (CT), or lipid A at days 0 and 14. Pneumococcal specific immunoglobulin A in bronchoalveolar lavage fluid (BALF) (A) and total immunoglobulin (B) were measured at 5 days after final immunization.

fections (sporozoite [SPZ]/liver stages) [127]. Among them, only vaccines targeting pre-erythrocytic infections showed any significant levels of protection in human clinical trials [128]. The RTS,S/AS01 subunit vaccine consisting of the *P. falciparum* circumsporozoite protein (CSP) fused with the hepatitis B surface antigen (HBsAg) is one of most advanced anti-malaria vaccines at a clinical level. Although this vaccine did not appear to elicit a CD8+ T cell response, CSP-HBsAg induced a specific CD4+ T cell response targeting the whole SPZ. A large-scale clinical phase III trial (ClinicalTrials.gov Identifier: NCT00866619) in African infants aged 6-8 weeks showed that RTS,S/A01 vaccines provided modest protection (26.0%-36.6% of vaccine efficacy) with no serious adverse effects [129].

A recent landmark finding that set the standards for immunological protection against malaria infection was established by immunization with irradiated SPZ [130,131]. Because the parasite undergoes morphological changes and displays antigenic variation at each stage of infection, whole parasite vaccines have an advantage [132-134]. In the early 1940s, Russell and Mohan [130] first demonstrated that inactivated *P. gallinaceum* SPZ provided protection against challenge with infectious *P. gallinaceum*. In 1967, Nussenzweig et al. [131] reported that a killed *P. berghei* SPZ vaccine was unsuccessful, but that an X-ray irradiated SPZ vaccine provided significant protection in an SPZ-challenge mouse model.

In the 1970s, researchers showed that immunizing human volunteers with bites from irradiated mosquitoes carrying *P. falciparum* SPZ (PfSPZ) or *P. vivax* SPZ (PvSPZ) provided protection against challenges with infectious SPZ [135-139]. Because infected mosquitoes cannot be used for immunizing large numbers of individuals, a team at the Vaccine Research Center (VRC) of the National Institutes of Health (NIH) and Sanaria Inc. (Rockville, MD, USA), developed an injectable and cryopreserved irradiated PfSPZ vaccine that met the vaccine regulatory standards [18]. Sanaria Inc. succeeded in raising mosquitoes on an industrial scale to good manufacturing practice (GMP) levels and harvested large amounts of PfSPZ from the mosquito salivary glands. In the phase 1 clinical trials of irradiated PfSPZ (ClinicalTrials.gov Identifier: NCT01001650), all of the subjects administered five or six doses intravenously showed complete resistance to challenges by bites from infected mosquitoes at 3 weeks after their final immunization, whereas five of six unvaccinated controls developed malaria [136,139]. Additional clinical trials of intravenous administration of the PfSPZ vaccine are planned in multiple locations (ClinicalTrials.gov Identifier: NCT02132299, NCT02215707, NCT0215091, NCT02115 516, NCT02418962).

Irradiated Cancer Cell-Based Vaccine Therapy

Cancer, which is a major health concern worldwide, is a leading cause of morbidity and mortality in developed and developing countries. There were ~14 million new cases and 8.2 million cancer deaths in 2012 [140]. Currently, traditional therapeutic treatments for cancer control and cure include radiotherapy and chemotherapy, which are commonly used worldwide and are considered the most effective ways to prevent tumor growth [3,141,142]. Chemotherapy targets the cells that grow and divide quickly, one of the major properties of tumor cells, but causes serious adverse effects as it also targets healthy, fast-dividing cells, such as blood cells and those lining the mouth, stomach, intestines and hair follicles. Hence, myelosuppression (decreased production of blood cells), mucositis (inflammation of the lining of the digestive tract), and alopecia (hair loss) commonly occur after chemotherapy [143-147]. Radiation therapy using ionizing radiation works by damaging the DNA of tumor cells, leading to cell death. Compared to surgery, radiotherapy is less painful, but the severity and longevity of side effects depend on the dose and duration of radiation administered. The symptoms vary from patient to patient and depend on the concurrent treatment, such as chemotherapy. Common acute side effects include nausea, vomiting, epithelial damage, stomach sores, intestinal discomfort and even infertility [148-150]. The side effects are caused by damage to the blood vessels and connective tissues, which can lead to fibrosis, lymphedema, and heart disease, which represent hidden future threats. Thus, new and better prophylactic treatments are needed.

A recently developed therapeutic treatment in cancer prevention is a cancer vaccine. Numerous different kinds of cancer vaccines have been tested in clinical trials, but the clinical benefits for the majority of cancer patients still need to be evaluated and confirmed. Cancer vaccines are designed to prevent or cure cancers using the patient's own whole tumor cells or part of the tumor-specific cancer antigens as the source of the vaccines. Unlike chemotherapies and radiotherapies, these vaccines would not cause serious side effects, which would offer an alternative treatment for patients in cases where traditional treatments are not effective. Furthermore, the combination of a cancer vaccine with other cancer therapies could enhance the efficacy of any treatment.

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Table 2. Clinical trials of the GVAX vaccine

Trial code	Status	Sponsor	Conditions	Features	Combination
NCT00140400	Phase III	Cell Genesys Inc.	Metastatic hormone-refractory prostate cancer	Non-patient specific	Docetaxel and Prednisone
NCT00133224	Phase III	Cell Genesys Inc.	Metastatic hormone-refractory prostate cancer with pain	Non-patient specific	Docetaxel and Prednisone
NCT00089726	Phase II	Cell Genesys Inc.	Advanced stage non-small-cell lung cancer	Patient-specific	Cyclophosphamide
NCT00084383	Phase II	Sidney Kimmel Comprehensive Cancer Center	Resectable pancreatic cancer	Non-patient specific	Nil
NCT00116467	Phase II	Cell Genesys Inc.	Acute myelogenous leukemia	Patient-specific	Nil
NCT00116441	Phase II	Cell Genesys Inc.	Multiple myeloma	Patient-specific	Nil
NCT00656123	Phase I	Sidney Kimmel Comprehensive Cancer Center	Colorectal cancer	Non-patient specific	Cyclophosphamide
NCT00836407	Phase I	Sidney Kimmel Comprehensive Cancer Center	Pancreatic cancer	Non-patient specific	Ipilimumab
NCT01510288	Phase I	VU University Medical Center	Prostate cancer	Non-patient specific	Ipilimumab

Cancer treatment vaccines are made with the patient's own blood dendrite cells stimulated with cancer antigen(s). These can effectively inhibit or stop tumor cell growth and prevent re-occurrence of cancer after chemotherapy and radiotherapy [151]. In 2010, the Food and Drug Administration (FDA) approved the first cancer treatment vaccine, Provenge (Dendreon, Seattle, WA, USA), which is a patient-customized vaccine targeting metastatic prostate cancer [152]. This vaccine is made from cultured dendritic cells taken from the patient and stimulated with prostatic acid phosphatase (PAP) antigen with granulocyte-macrophage colony-stimulating factor (GM-CSF). Re-infusion of the stimulated dendritic cells into the patient effectively stimulates T-cell dependent immunity, which kills the tumor cells expressing PAP [153]. Results from a clinical trial showed that Provenge reduced the risk of death by 22% and increased survival by 4.1 months compared to the placebo group [154]. However, the cost of current patient-customized therapeutic cancer vaccines is extremely high and the time interval of treatment is long. Both these factors will prevent the vaccine from being widely used worldwide. Provenge treatment consists of three infusions at ~two-week intervals for one month, and the cost for a complete course of treatment is $93,000 [140]. Because of these limitations, new types of cell-based cancer vaccines are being developed.

One of most promising therapeutic cell-based cancer vaccines is GVAX (GM-CSF gene-transduced irradiated cancer vaccine cells) which is one of the vaccines furthest along the process in pre-clinical and clinical trials [155]. Unlike patient-specific cancer vaccines, this vaccine has been developed using patient-specific cancer cells genetically modified to secrete GM-CSF. This makes it easy to manufacture vaccines for various tumor types, such as melanoma, renal, lung, prostate, and pancreatic tumors [156]. Irradiated tumor cells can involve the apoptotic bodies, which would be accepted by the dendritic cells. When the dendritic cells interact with the antigens expressed by irradiated tumor cells, they become mature and present the antigens. In addition, allogenic tumor cell secreting recombinant GM-CSF chemotactically attract immature dendritic cells to induce maturation. The dendritic cells presenting the antigen expressed by the irradiated tumor cells then activate CD4 and CD8 lymphocytes directly [157]. To date, there are many types of GVAX vaccine in clinical trials, either alone or in combination with other therapies, to improve treatment options (Table 2). For example, a GVAX vaccine for prostate cancer (GVAX-PCa) with a co-treatment of Ipilimumab, which is a humanized monoclonal antibody and functions as a CTLA-4 blocker, was first approved by the FDA for the treatment of advanced melanoma in 2011 [158]. Clinically, GVAX immunotherapy combined with Ipilimumab leads to the development of specific antibodies against the tumor cells which prolonged patient survival in cases of pancreatic and prostate cancers in clinical trials (ClinicalTrials.gov Identifier: NCT00836407 and NCT01510288) [25].

Conclusion

Gamma radiation is not a new technique, and has been extensively utilized in the past to sterilize foods and create inactivated vaccines. Whether gamma radiation is superior to conventional inactivation methods, such as heat and chemical treatments, remains a controversial issue. However, due to its ability to effectively penetrate pathogens and cancer cells and specifically target nucleic acids whilst causing less damage to surface antigenic proteins, demands for the use of gamma ra-

diation are increasing to develop safe and simple vaccines. In addition, gamma radiation has several advantages over the use of inactive forms of vaccines, such as the ability to inactivate large volumes, they can be stored in closed containers, and there is no requirement to remove chemical compounds after inactivation. Despite these advantages, no irradiated vaccines have been licensed to date. Here, we reviewed and summarized the current situation regarding irradiated vaccines in pre-clinical and clinical studies. Some irradiated vaccines showed no surprising results compared to live attenuated- or chemically inactivated vaccines, but most of the pre-clinical studies suggested that irradiated vaccines provide more potential immunogenicity than other inactivation methods. Moreover, the metabolically active form of irradiated vaccines were able to activate cytotoxic T cells, which are important immune cells for treating intracellular pathogens and cancers. Therefore, radiation inactivation might provide a feasible, broad-spectrum, simple, and effective technique for the development of novel vaccines.

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