Cancer Vaccination and Challenges

Volume 2

Delivery Strategies for Cancer Vaccine and Immunotherapy in the Management of Various Carcinomas

Rishabha Malviya
Bhupendra Prajapati
Sonali Sundram
Editors

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Delivery Strategies for Cancer Vaccine and Immunotherapy in the Management of Various Carcinomas

Edited by

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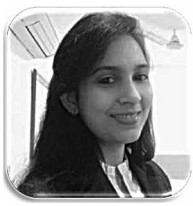

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Foreword

Cancer remains the second leading cause of death in developed and developing countries. This is despite the fact that some cancers are preventable and treatable if detected early. Surgery, chemotherapy, and radiation are still major approaches in cancer treatment. Developing effective immune protection through vaccination has proven to be technologically challenging due to various technical reasons. The book, which consists of 11 chapters, serves as a valuable resource in this field. The theme of the work is "Cancer Vaccination and Challenges," covering every aspect of cancer immunology and vaccine development. Each chapter is carefully curated to provide readers with a comprehensive understanding of the rapidly growing literature in this field. The compilation explores the most successful and promising approaches by incorporating the latest studies on preventive and therapeutic cancer vaccination. The information in this book serves as a comprehensive guide to cancer vaccine research.

The book offers up-to-date information on various topics, including tumor antigens for cancer vaccines, strategies, approaches, advancements, difficulties, and challenges in vaccine development for cancer treatment. Recent advances in DNA and RNA-based vaccines for cancer prevention and treatment are thoroughly discussed in this volume. This compendium enables researchers and practitioners to easily access the comprehensive concept of cancer vaccines and their implications in healthcare.

I commend the editors and contributors for their thorough and in-depth treatment of these complex topics in this book. In my opinion, this compendium will prove to be an essential resource in disseminating the vast and expanding literature on cancer immunization and vaccine development.

—Prof. Harish Padh
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About the Book

The development of potent therapeutic cancer vaccines that can activate both effector and memory T cells to respond specifically to tumor antigens is one of the main objectives of cancer immunotherapy. The customization of cancer vaccinations has received a lot of interest lately. Nevertheless, despite the numerous vaccine techniques targeting various tumors that have been studied recently, the performance of therapeutic cancer vaccines remains poor. Clinical trials have not yet produced satisfactory data despite positive findings typically seen in preclinical data. The main cause of such failures is the difficulty in finding certain target tumor antigens that should be distinct from or overexpressed only by tumor cells in comparison to normal cells. Most tumor antigens used in cancer vaccines are non-mutated, overexpressed self-antigens that primarily activate T cells with weak T cell receptors (TCR) that are unable to effectively mediate an anti-tumor response.

This book provides a summary of the most prevalent cancer vaccination, including the role of tumor antigens in cancer vaccine development, strategies, advancements, and challenges in the development of cancer vaccines, combined therapy of cancer vaccines and chemotherapeutic agents, delivery and prevention of cancer vaccines, cancer adjuvants in cancer management, and cancer vaccination for different diseases.

This book offers in-depth knowledge of cancer vaccination and its challenges in a concise and novel manner. It will assist graduates, researchers, and clinicians in advancing cancer vaccination.

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Preface

The second volume of *Cancer Vaccination and Challenges* explores delivery strategies for cancer vaccines and provides an understanding of managing various carcinomas using vaccines. In recent years, novel and efficient drug delivery methods have been extensively investigated to enhance the success rate of cancer immunotherapy, along with a deeper understanding of biomaterials and cancer immuno-oncology. The use of delivery technologies that increase accessibility to immune organs or tumor regions and decrease unwanted adverse effects has made cancer therapy easier.

Cancer Vaccination and Challenges, Volume 2: Delivery Strategies for Cancer Vaccine and Immunotherapy in Management of Various Carcinomas examines the prospects of delivering immuno-oncology therapies, focusing specifically on effective drug delivery strategies for the treatment of lung, prostate, and pancreatic carcinoma. This book provides an overview of current vaccine systems, adjuvants, and delivery methods being explored or approved. We also explore the possibility of using immune checkpoint inhibitors in conjunction with cancer vaccines to increase their efficiency and combat the immunosuppressive tumor microenvironment.

The book focuses on therapeutic cancer vaccines that could prevent the spread of advanced malignancies resistant to standard treatments such as surgery, radiation therapy, and chemotherapy. It offers extensive information to educate the scientific and medical community, as well as those working in immunology, cancer biology, and therapeutics, providing possible solutions from the drug delivery perspective.

As the lead editor of these two volumes, I am very thankful to all the authors who have worked diligently and enthusiastically. I would also like to express my gratitude to the prestigious Apple Academic Press. It has been a great experience completing these two sets of books with AAP.

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CHAPTER 1

Delivery System for Cancer Vaccine

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ABSTRACT

The most valuable and cost-effective health measure to combat the rise and spread of diseases is vaccination. Cancer vaccines (CV) serve as highly promising and beneficial tools for clinical oncologists. Numerous tumor-associated antigens offer excellent targets for immunotherapy and vaccination. To ensure the sustained release of antigens to the immune system, carriers are being utilized for vaccine administration. Researchers have recently focused on vaccine delivery systems that can be specifically targeted to tumor tissue, avoiding the challenges associated with systemic effects. This chapter discusses studies that aim to develop more precise vaccine delivery systems for neoplasm treatment. Antigen-loaded carriers have shown promise in targeted therapy by activating the immune system in a way that triggers a unique immune response to the disease's antigen. These carriers can be actively taken up by antigen-presenting cells (APCs). Various carrier systems are available, including physical approaches and non-physical approaches such as virus-like particles (VLP)/virosomes, polymeric micro- and nanoparticles (NPs), liposomes, archaeal lipid liposomes (archaeosomes), immune-stimulating complexes, and cell-penetrating peptides (CPP). The chapter will discuss their potential for future CV development as well as their therapeutic potential.

Cancer Vaccination and Challenges, Volume 2: Delivery Strategies for Cancer Vaccine and Immunotherapy in the Management of Various Carcinomas. Rishabha Malviya, Bhupendra Prajapati, and Sonali Sundram (Eds.)

1.1 INTRODUCTION

1.1.1 INTRODUCTION TO CANCER

For several decades, cancer has been a leading cause of mortality. However, developing a successful therapeutic approach for cancer treatment is quite challenging [1]. Understanding the etiopathology of malignancies and the processes involved in the spread of cancer cells is crucial. A wide range of malignant diseases characterized by the uncontrolled proliferation of abnormal cells are collectively referred to as "cancer." It is recognized as the second most common cause of mortality worldwide, following cardiovascular diseases. In 2015, the World Health Organization (WHO) reported that cancer alone accounted for 7.6 million deaths out of 58 million globally [1]. Studies suggest that by 2030, there will be 15.4 million cancer-related deaths worldwide, representing an increase of 10 million cases per year.

Chemotherapy, radiation, and surgery are common and effective treatments for cancer. The effectiveness of these treatments varies depending on the type of cancer. One drawback of chemotherapy is that it targets rapidly dividing cells without discrimination, resulting in the destruction of both malignant and healthy cells. The main limitation of radiotherapy and surgery is that they do not eliminate metastases. Therefore, there is still a need for more effective and less harmful cancer therapies. Research indicates that cancer vaccines (CVs) may be more effective than traditional chemotherapy because they work with the patient's immune system to inhibit the growth of cancer cells [2].

1.1.2 INTRODUCTION TO VACCINES

Since the first testing of Edward Jenner's smallpox vaccine in 1798, vaccines have significantly reduced disease morbidity and mortality, potentially making them the most significant medical innovation ever [3].

While vaccinations have undeniably proven beneficial, advancements in vector production, transportation, and usability would be highly advantageous. Louis Pasteur's "three is" paradigm—isolate, inactivate, and inject—has historically served as the foundation for vaccine development [4, 5]. However, with our improved understanding of immunology, pathology, and microbiology, vaccine development is shifting towards a more "rational design" perspective [6, 7]. These rationally designed vaccines typically consist of minimalistic components (such as subunits or peptides), which

makes them less immunogenic but offers advantages in terms of safety and production costs [8]. It is envisioned that integrating and optimizing these approaches, along with the implementation of novel dosage and adjuvant strategies, can help bridge this efficacy gap.

1.1.3 VACCINES FOR CANCER

CVs function by activating the adaptive immune system, which ultimately eliminates cancer cells while also providing prophylactic defense by inducing an immune response against the tumor. This approach reduces the likelihood of tumor recurrence in cancer cells originating from different organs or organ systems within the body [9–11]. This is attributed to the innate ability of the immune system to recognize tumor cells in the body. The antigens act as immune system stimulators, priming the body to combat cancer cells.

Numerous investigations have identified a wide range of antigens associated with cancer, some of which are currently utilized as CVs in both clinical trials and research [9]. Modern technology has made significant advancements in identifying antigens that are specifically recognized by T cells in tumors. Tumor antigens (TAs) can be categorized into two main groups: tumor-specific common antigens and tumor-specific unique antigens. Shared antigens expressed by multiple types of tumor cells are sometimes referred to as tumor-associated antigens (TAAs). The most effective immunotherapy agents and/or delivery systems work in conjunction with the most suitable TAs, which are expressed to varying degrees in normal tissues, to achieve optimal treatment outcomes [9]. Therefore, selecting an appropriate vaccine delivery mechanism is crucial for the development of immunological strategies for cancer therapy.

1.1.4 THERAPEUTIC IMMUNOTHERAPY

The recognition of the necessity for therapeutic anticancer vaccines over preventive ones has been acknowledged by researchers. Therapeutic vaccines are administered after the onset of the illness and aim to either completely halt the growth of malignant cells or control metastases and disease relapse. The main agents involved in immunological activation against cancer are antigen-presenting cells (APCs), particularly dendritic cells (DCs). By targeting DCs in the tumor microenvironment (TME), it becomes possible to redirect inflammation that promotes tumor growth towards a tumor-killing mode. DC-specific antibodies and DC activators, in conjunction with

cancer-specific antigens, induce a robust antigen-specific CD4+ and CD8+ T-cell mediated immune response [12].

After triggering a cell-mediated immune response, cancer cells are destroyed by interferon-gamma (IFN-γ), tumor necrosis factor-alpha (TNF-α), chemokines, and contact-mediated cytotoxicity. Many nanoparticles (NP)/liposome formulations contain targeted molecules, such as antibodies and immune modulators, along with tumor-specific antigens, to facilitate the activation of APCs, primarily DCs. Due to their size and composition, nanoformulations are readily taken up by DCs, resulting in a T-cell and antibody response [13]. The majority of therapeutic vaccination techniques lead to tumor regression through DC-mediated antigen-specific cytotoxic T lymphocyte (CTL) responses [14]. The cargo (antigen/antibody/Toll-like receptor (TLR) ligand/cytokines, *see* Figure 1.1) that these formulations are designed to deliver is used to further categorize this section.

Over the past 20 years, research into tumor vaccines and immunology, which are alluring replacements or complements to current cancer treatments, has significantly increased [15]. These approaches aim to stimulate the patient's immune system to recognize and eliminate tumor cells. They offer several important advantages, including the ability to:

- Induce targeted tumor cell death with minimal harm to healthy, non-tumor cells;
- Target both primary and secondary metastases by systemically inducing anti-tumor immune responses; and
- Generate immunological memory that provides long-lasting protection against potential tumor recurrences in the future [16, 17].

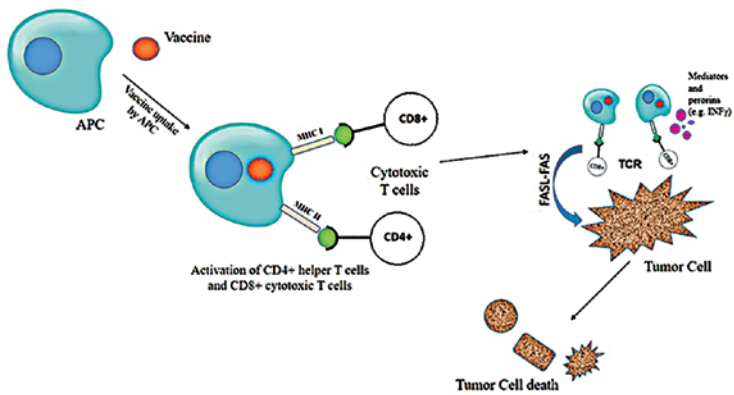

FIGURE 1.1 Mechanistic pathways involved in inducing the immunization by the cancer vaccines.

1.2 VACCINE DELIVERY SYSTEMS

1.2.1 PHYSICAL APPROACHES

The type of immunological response that a DNA vaccination induces can vary depending on its administration method. DNA can be administered intravenously (I.V.), subcutaneously (S.C.), intramuscularly (I.M.), or intranasally (I.N.) [18]. Several studies have shown that cutaneous administration has immunological advantages, such as stronger immune responses compared to conventional delivery methods. However, the outcomes of skin immunization have sometimes been unclear due to a lack of delivery systems that can consistently and accurately deliver vaccines to the skin [19]. The nasal route of vaccine administration is also receiving attention for its local and systemic effects. However, there is a lack of efficient vaccine formulations and safe delivery methods for intranasal administration. Current research is investigating particle systems based on polyacrylate polymers to enhance mucosal immune responses [20]. Standard vaccines typically use intradermal (I.D.) or subcutaneous (S.C.) inoculations. Intratumoral and/or intra-nodal vaccination has shown to be more effective than other methods in various animal models and clinical investigations. In a study reported in "Advances in Cancer Research," the sequential administration of primary immunization S.C. followed by booster vaccination I.V. resulted in more potent anti-tumor effects compared to either administration method alone [21].

The injection route may be influenced by several factors. Recent discoveries have shown that utilizing biolistic approaches, such as the Gene cannon or Biojector 2000, can increase efficiency. Studies conducted on mice have revealed that 100 times less DNA is required to elicit an antibody reaction compared to needle injections [18]. Biolistic and needle injections can result in different immunological reactions. Gene guns often induce T helper type 2 (Th2) responses with DNA vaccines, while needle injections typically elicit a Th1 response. This discrepancy may be attributed to the use of higher injectable doses. It is important to note that this result is not commonly observed [18]. Previous research has demonstrated that gene gun delivery using naked DNA vaccines without any coating caused a Th1-biased immune response, indicating that Au particles used in gene gun bombardment can alter the immune response generated [22].

Furthermore, regardless of the route, certain antigens can influence the immune responses [18]. Some techniques to increase the quantity of antigen expressing DCs include I.D. injection before laser therapy, I.M. injection before EP, and I.M. injection with microencapsulated vaccination. The

following discussion addresses strategies for enhancing gene-based immunization through physical delivery. These approaches are also depicted in Figure 1.2.

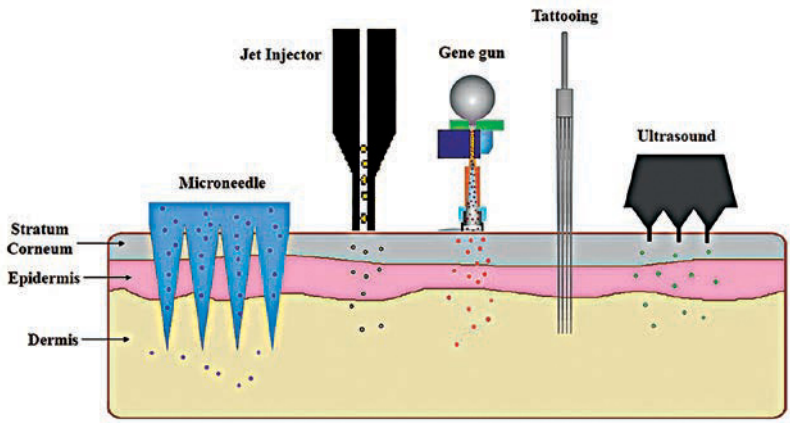

FIGURE 1.2 Different approaches for cancer vaccine delivery by physical methods.

1.2.1.1 TATTOOING

Recently, tattooing has been suggested as a method of physically injecting deoxyribonucleic acid (DNA) into epidermal cells. This approach shares similarities with the effective strategy used for smallpox vaccination, as it appears to expedite the development of robust host defense and effective immunity. The rapid and sporadic synthesis of antigens following immunization may account for this effect. Studies have shown that gene expression after DNA tattooing is higher compared to intradermal injection and gene gun delivery [23]. DNA transfer through tattooing seems to result in distinct patterns of gene expression when compared to intramuscular injection. In one experiment, injecting 100 g of DNA intramuscularly (I.M.) resulted in gene expression peaks at least 10 times higher than tattooing 20 g of DNA. Gene expression increased immediately after getting a tattoo and then gradually decreased over the next four days. In contrast, elevated levels of gene expression were observed after I.M. injection of DNA, with a peak after seven days that may have persisted for up to 30 days. Despite the lower DNA concentration and reduced gene expression, tattooed DNA induced more potent cellular and humoral immune responses to antigens compared to I.M. DNA injection [24].

Additionally, the effectiveness of DNA vaccination administered either by tattoo or intramuscular needle injection has been investigated in relation to the effects of two additives: cardiotoxin and plasmid DNA (pDNA) containing the mouse GM-CSF. The human papillomavirus type 16 (HPV16) gene, encoding the L1 main capsid protein, was employed as a model antigen in this investigation. The results showed that intramuscular administration of molecular adjuvants significantly increases the efficacy of the HPV16 L1 DNA vaccination. Furthermore, tattoo device administration of the HPV16 L1 DNA generated substantially stronger and quicker humoral and cellular immune responses compared to intramuscular needle delivery paired with molecular adjuvants. However, when more immediate and powerful immune responses are required, tattoo delivery of DNA is a practical and affordable technique that can be applied in the laboratory [23].

Getting a tattoo result in several minor mechanical injuries, followed by bleeding, necrosis, inflammation, and skin regeneration, which unintentionally boosts the immune system. As a result, tattooing "only" partially replaces the role of adjuvants [24].

1.2.1.2 GENE GUN

A gene gun is a biolistic device that allows DNA to directly enter cells by hitting the target DNA using a nearby chamber. Through *ex vivo* or *in vitro* gene gun technology, various somatic cell types, including primary cultures and well-known cell lines, have been transplanted as suspension or adherent cultures [25]. These events lead to the expansion and migration of DCs to nearby lymphoid tissue, where they prime T cells to respond to the antigen in a targeted manner [26]. Recently, skin vaccination for melanoma has been conducted using TAA, human gpl00, and reporter gene assays as experimental platforms [25].

The EGFR protein has been found to be highly expressed in different types of cancer, including lung, breast, bladder, and colon carcinomas. In a mouse experiment, immune and anticarcinogenic responses were evaluated by delivering pDNA encoding the human EGFR extracellular domain using three different techniques: intramuscular needle injection (I.M.), gene gun with DNA coated in Au, and gene gun with uncoated DNA. Non-coating pDNA gene gun injection showed the greatest anti-tumor effects and CTL activity in an animal lung cancer study. These findings may guide the development of a DNA vaccine for use in future human clinical trials. The creation

of an EGFR DNA vaccine to protect against malignancies that exhibit the gene may require DNA immunization [22].

Additionally, it has been discovered that the CpG motif, consisting of cytosine-phosphate-guanine, can modify the Th2-type cytokine environment that is established in the draining lymph nodes (LN) as a result of gene-gun bombardment. The findings indicate that the insertion of the CpG motif can elevate the levels of Interleukin (IL)-12 mRNA in the local LN, regardless of whether it is administered through intradermal injection, intramuscular injection, or gene-gun bombardment. These results suggest that CpG motif injection induces a T helper type 1 (Th1)-biased environment in the localized LN. The combination of DNA vaccination and CpG motif serves to activate the Th1 immune response and functions as a "warning signal" [26].

In contrast to regular intramuscular (I.M.) injection, intradermal (I.D.) administration using a gene gun has been found to be the most effective method for administering the human papillomavirus (HPV) DNA vaccine. Recently, it has been reported that uncarrier bare DNA can be delivered using a gene pistol under low pressure. Compared to Au particle-coated DNA immunization, the non-carrier unprotected prophylactic HPV DNA vaccination effectively reduces local skin injury. By comparing this method to the Au particle layered HPV DNA vaccine, researchers have determined that it is also capable of enhancing T cell immunity to the HPV antigen and improving anti-tumor effects [27].

Recent clinical investigations have utilized a Sig/E7detox/HSP70 DNA vaccine, known as an HPV16 DNA vaccine, which encodes a signal peptide linked to an inhibited variant of HPV16 E7 (E7 detox) and joined to HSP70. In a previous study, the pNGVL4a-Sig/E7 (detox)/HSP70 vaccine was administered using three distinct delivery methods: needle intramuscular injection, biojector, and gene gun. The purpose was to evaluate the antigenic and anti-tumor responses. The gene gun method of DNA immunization resulted in the highest number of E7-specific CD8+ T cells compared to needle intramuscular injection and biojector administrations [28].

1.2.1.3 ULTRASOUND

To facilitate the fusion of DNA in cells, cell membranes can be temporarily disrupted by ultrasound (US) [29, 30]. Furthermore, the combination of therapeutic US and microbubble echo contrast agents may enhance the efficiency of gene transfection [31]. This process enables direct and successful

transfer of DNA into the cytoplasm. While proteins have been delivered into cells using this technique [32], antigens have not yet been introduced into DCs for cancer immunotherapy. *In vitro* and *in vivo* studies have demonstrated that the use of US technology can facilitate the introduction of naked pDNA into colon cancer cells. Additionally, intra-tumoral injection of naked pDNA prior to US treatment enhanced DNA transport and gene expression in a mouse model of squamous cell cancer.

Currently, US is being investigated in a clinical setting. A CV developed by Memgen was repeatedly administered intravenously (I.V.) to patients with chronic lymphocytic leukemia/small lymphocytic lymphoma (CLL/SLL) in a phase II study (University of California, San Diego; ID: NCT00849524).

1.2.1.4 ELECTROPORATION

In this method, electrical pulses are applied to the skin, causing temporary pores to form, and facilitating DNA cellular entrance. The skin regains its structure once electrical energy is removed and holds the immunogenic substance due to pore closure.

Historically, milli- and microsecond pulses have been utilized in EP. Recent investigations have explored direct DNA transfer to the nucleus using extremely strong nanosecond electrical impulses (10–300 ns) at extremely high intensities (10–300 kV/cm) [33].

EP can enhance immune responses by increasing protein expression, releasing inflammatory chemokines and cytokines, and attracting APCs like macrophages and DCs to the EP site [34]. Compared to levels achieved by I.M. DNA injection alone, EP-mediated transport of pDNA increases antigen-specific humoral and cellular immune responses. It has been demonstrated that *in vivo*, EP reliably enhances immune responses in both small and large animals, suggesting its potential application in humans [34, 35]. Subsequent comparisons between EP and US have shown the effectiveness of transfecting skeletal muscle with exposed pDNA using EP [36].

In recent studies, it has been shown that administering pDNA by EP in mice alone has proven to be successful as a boosting immunization. This success may be attributed to the high level of antigen generation achieved by the EP-booster vaccine. It is noteworthy that this approach has shown greater success compared to using two DNA with EP doses [37]. This strategy is particularly appealing as it eliminates the need for two distinct vaccine types. For example, research has revealed that the dose and volume of the

vaccination significantly impact the efficiency of priming and tumor protection in mice when utilizing a DNA vaccine expressing the CTL epitope AH1 from colon cancer CT26 [38].

Animal models have been utilized to investigate an EP-driven DNA vaccine strategy for the treatment of prostate cancer. To effectively stimulate anti-tumor immune responses against tumors expressing prostate antigen, a plasmid encoding the phPSA was administered *in vivo* via I.M. mediated EP [39]. The results demonstrated that the phPSA vaccination therapy significantly delayed the progression of malignancies and prolonged animal lifespan.

The researchers have employed a variety of strategies to develop the optimal HPV DNA vaccine, including fusion of the E6 and E7 tumor antigens (E6/E7), tissue plasminogen activator (tpa) signal sequence, inclusion of CD40 ligand (CD40L), and Fms-like tyrosine kinase-3 ligand (Flt3L). E6/E7 antigen-specific CD8(+) T cell responses were decreased when E6 and E7 were fused (E6/E7 (Co)), but the preventative antitumor impact was increased. In contrast to CD40L-linked HPV DNA vaccination, the Flt3L-fused HPV DNA vaccine demonstrated improved therapeutic anti-tumor outcomes and increased E6- and E7-specific CD8+ T cell responses [40].

In phase I/II clinical investigations at Karolinska University Hospital, Chron Vac-C, a curative DNA vaccine administered to patients previously infected with the virus to hasten their recovery by boosting immune response, showed good efficacy when delivered by EP [41]. This clinical trial was conducted at the Gastroenterology and Infectious Disease Clinic at Sweden's Hospital. It was one of the earliest DNA vaccines against infectious diseases to be administered to people using an EP technique.

To enhance antigen processing and delivery through the MHC class I pathway and enhance the production of cytotoxic CD8+ T lymphocytes, it is necessary to localize TAs subcellularly through their interaction with immunostimulatory molecules such as calreticulin (CRT). Even with current advancements, finding a reliable mechanism for DNA delivery remains crucial to ensure the effectiveness of immunotherapeutic methods. In a study comparing three vaccination techniques – conventional intramuscular (I.M.) injection, I.M. delivery mediated by electroporation (EP), and epidermal gene gun-mediated particle delivery – the pNGVL4a-CRT/E7 (detox) DNA vaccine was used. The results demonstrated that EP was the most efficient method for immunizing against E7, as it generated the highest quantity of E7-specific cytotoxic CD8+ T cells [42].

Recently, several HPV DNA vaccines have been successfully administered to rhesus macaques and mice model organisms using EP. One such vaccine is VGX-3100, which contains plasmids targeting the E6 and E7 proteins of HPV subtypes 16 and 18. Currently, it is undergoing Phase I clinical trials. It is recommended that individuals with a history of CIN 2 or 3 and those who have undergone surgery receive this vaccine [43].

Cyto Pulse is more efficient than previous I.M. EP techniques in specifically targeting skin cells. Cyto Pulse has developed two clinical vaccine delivery devices, DermaVax and Easy Vax. Easy VaxTM focuses on the epidermal layer of the skin and is widely used for prophylactic viral vaccination. On the other hand, Derma VaxTM primarily targets the dermis layer of the skin. This method is suitable for situations where strong immune responses and high doses are desired, such as in gene therapy and CVs. Derma Vax will be utilized in ongoing or scheduled clinical investigations. One such study, with the ID NCT01064375, aimed to create a DNA vaccine for treating colorectal cancer using I.D. EP (El-porCEA). The study evaluated the immunogenicity and safety of a CEA DNA immunization technique in individuals with colorectal cancer.

1.2.1.5 LASER

Studies conducted *in vitro* have demonstrated that a laser beam can impart energy (for the first time as high as 20 mega eV) to a target cell, changing the membrane permeability through a local thermal impact. For medicinal uses, additional energy (up to 250 mega eV) is needed [44]. Recently, this cutting-edge approach has been characterized as an efficient way to increase the transfection effectiveness of plasmids injected I.D. and elicit an immune response that is antigen-specific in both T cells and humoral immunity. This novel approach was only used in a few trials to show that there is great promise for developing a therapeutic HPV DNA vaccination [26].

1.2.1.6 JET INJECTORS

Jet injectors are used to drive nebulized medicinal solution or suspension into the skin, using a spring mechanism or pressurized gases (usually carbon dioxide, nitrogen, or helium) contained in a small cartridge or large canister. This method allows the medication to reach the muscles directly or penetrate deeper into the subcutaneous or intradermal layers.

1.2.1.6.1 Liquid Jet Injectors

Liquid jet injectors are being developed as both single-use and multi-use components. For chronic conditions like diabetes, reusable devices are utilized to administer a daily dose for sustained effect. Replaceable devices, pre-filled with medication, are used for office-based immunizations, mass vaccinations, and emergency cases such as treating allergies and migraine attacks.

These devices pressurize the liquid without the need for a needle, which creates pores in the skin. Compressed gas or a spring can be used as the power source. This results in a lower pressure profile, pushing the remaining liquid into the skin. There is no need to reformulate the medication as it is already in liquid form.

Walther et al. [45] have demonstrated that syringe, limited jet injection of small amounts of exposed pDNA for galactosidase (LacZ), and green fluorescent protein target gene paradigms can successfully and safely carry out non-viral gene transfection in various preclinical cancer models.

An analysis of jet-injected tumors, both qualitatively and quantitatively, revealed efficient gene transcription, increased intratumoral dispersion, and deep DNA penetration. A phase I gene exchange experiment was conducted using jet injection to deliver trace levels of pDNA intratumorally, based on these intriguing preclinical data. The efficiency of gene transfer was assessed through quantitative and qualitative examination of LacZ expression at the mRNA and protein levels. The outcomes of this experiment demonstrate the effectiveness of this method in treating locally accessible metastases from solid tumors such as melanoma, breast neoplasm, and other types of malignancies. Preclinical research on the use of jet injectors to deliver small interfering RNA has also shown potential for additional cancer gene therapy applications, including DNA vaccination, immunogene therapy, and gene suppression techniques. Furthermore, the therapeutic *in vivo* jet-injection delivery of human TNF-α as a suicide gene for CD significantly decreased tumor progression.

However, liquid jet injectors have some drawbacks. They require precise power source control to administer vaccines accurately and reliably to distinct skin types or different areas of the same person’s skin. The high velocity of the jet impacts on blood vessels and nerves, causing bleeding and discomfort that may reduce patient compliance.

1.2.1.6.2 Solid Dose Injectors

The stability of vaccines delivered in solid form, whether medicinal or allergenic, eliminates the need for cold chain storage. This allows for the administration of both the prime dose and booster doses simultaneously, enhancing patient compliance.

One method for delivering solid formulations is the Powderject method [46], which utilizes helium-powered devices to propel medication into the outer layers of the skin at supersonic speeds. The drug particles are accelerated by the pressurized gas in the helium microcylinder, enabling them to penetrate the skin upon activation. The device is applied directly to the skin during use. Corgentech and Pfizer are both exploring this technology for the development of local anesthetics and the delivery of DNA vaccines on Au carrier particles, respectively.

Another approach is the glide solid dosage injector method, where a hand actuator pushes the medication. The actuator consists of a small solid rod containing both the medicine and excipients [47]. When the predetermined spring force is reached, the actuator automatically initiates and administers the medication. The pushing motion is crucial, as it ensures consistent and regulated delivery of the drug to the deep layers of the skin, regardless of the skin type or location. This distinguishes it from the Powderject method, where establishing a consistent and effective treatment rate may be challenging. In cases where multiple doses need to be administered, the actuator can be designed as a reusable device, with preloaded medication cassettes for convenience.

1.2.1.7 MICRONEEDLES (MN)

A microneedle (MN) is composed of multiple microstructured projections coated with medication or vaccines. It is applied to the skin to deliver active substances intradermally, bypassing the stratum corneum. Unlike transdermal administration systems that rely on diffusion, MNs manually disrupt the epidermis temporarily, facilitating the entry of the medication or vaccine to its target site. Due to their small size (1 μm in diameter and 1 to 100 μm in length), MNs differ from regular needles. They can be made from various materials such as metals, silicon, silicon dioxide, polymers, glass, and others. MNs can be designed to be short enough to avoid nerve endings while still reaching the stratum corneum, reducing the risk of discomfort, infection, or injury.

Hollow MNs, solid MNs, MNs coated with pharmaceuticals, and dissolving MNs are among the different designs available [48–50]. These designs enable the administration of medications through various mechanisms, allowing for the investigation of therapeutic timing control [51].

Solid MNs are often utilized for tissue pre-treatment in order to enhance the transport of therapeutic medicines to the selected tissue [52, 53]. Here, the introduction of tiny needles and their subsequent removal create pores in the tissue that are only a few nanometers wide, allowing the application of hydrogels or medication solutions later on to reach deeper tissue layers [52, 54]. On the other hand, the MNs may have a layered structure, with the lower levels transporting the therapeutic chemicals and the upper levels providing mechanical support for penetration into the target tissue [55, 56]. In this way, after the MNs are applied, the distinct MN layers will disintegrate, allowing the therapeutic molecules to flow into the tissue [57, 58].

Cancer immune-based immunizations aim to mobilize the host's innate immunity to destroy the tumor tissue [59]. Such methods often focus on the delivery of vaccines to the skin, the largest immune organ of the body, which is abundant in APCs, such as macrophages, Langerhans cells, and DCs [60]. These APCs can activate T and B cells, thereby triggering a broader immune response against tumors [61, 62].

In order to achieve this objective, researchers are investigating the use of MN patches containing immunostimulatory adjuvants and/or antigens. By combining methyl vinyl ether and maleic anhydride MNs with OVA-loaded PLGA NPs, Zaric et al. [63] demonstrated a method to stimulate the immune system against B16 melanoma tumors expressing OVA. The authors utilized a water-in-oil double emulsion technique to generate OVA-loaded PLGA NPs for the MNs in this procedure, followed by the addition of NPs to a solution of methyl vinyl ether and maleic anhydride (19 by 19 array). These researchers observed that the MNs were able to penetrate the mouse skin and reach the dermal layer and nearby DCs in the *ex vivo* tests. The researchers also observed that the transfected DCs were able to migrate to the proximal LN, where they could activate CD8+ T cells and induce the production of cytokines such as IFN-γ. Furthermore, this immune system activation, triggered by the release of NPs from the MNs, resulted in a 13-day delay in the growth of B16 melanoma tumors expressing OVA. Similarly, Kim and colleagues developed an MN patch using Pluronic F127 and polyethylene glycol (PEG) to co-deliver resiquimod (R848) and TAs simultaneously [64].

Human TLR7 and TLR8 serve as ligands for R848 and are expressed on immune cells such as macrophages, dendritic cells, and B cells. The

interaction between R848 and TLR7/8 leads to the production of IL-12, IFN-γ, and TNF, which subsequently triggers humoral and Th1 immune responses specific to the antigen [65, 66]. In this study, the use of lymphoma cells expressing OVA allowed for the concomitant administration of OVA to focus the immune response on tumor xenografts composed of E.G7-OVA.

A polydimethylsiloxane (PDMS) mold was utilized to deposit the combination of Pluronic F127/R848 and PEG/OVA, which was then cured at room temperature under vacuum before being cut into an MN array consisting of 49 pyramid-shaped needles. It was discovered that the MNs could penetrate the skin of mice and release their payload into nearby cells. The authors also observed that the dissolved MNs formed nano-micelles, potentially facilitating the distribution of R848 and OVA to RAW264.7 cells, thereby inducing macrophage maturation and cytokine release. *In vivo* experiments demonstrated that the R848 and OVA-loaded MNs could migrate to lymph nodes and activate cutaneous APCs.

On the other hand, MNs can be designed to deliver immune therapies based on antibodies that aim to overcome or bypass the immune suppressive signals sent by tumor cells. Ye and colleagues developed an MN array composed of hyaluronic acid (HA) to deliver 1-MT and anti-PD1 antibodies to B16F10 melanoma tumors [67]. By specifically targeting the PD-1 receptors expressed by T cells, aPD1 antibodies can evade the inhibitory signaling from cancer cells, allowing T cells to activate [68].

1.2.2 VIRAL AND NON-VIRAL DELIVERY SYSTEMS (NONPHYSICAL)

1.2.2.1 VIRUS VECTORS

1.2.2.1.1 Viral Vector

Viruses can have their genetic material altered to deliver transgenes into infected cells, as they are inherently immunogenic. However, the effectiveness of using viral vectors in clinical trials has not yielded similar results as infectious disease research. Recombinant viruses can express transgenes in immune cells, particularly antigen-presenting cells (APCs) such as dendritic cells (DCs) [69]. This leads to increased presentation of tumor antigens (TAs) to cytotoxic T lymphocytes (CTLs), resulting in more frequent and intense attacks on tumor cells. The antigens are expressed by the vaccination vector [70].

Viruses can naturally infect human cells and elicit T cell and humoral responses, making them suitable as vaccine delivery methods [71–73]. Certain viruses can be modified to specifically target tumor cells or possess inherent oncolytic properties. Oncolytic viral therapy (OVT) is widely used for cancer treatment, as it can selectively lyse tumor cells and induce an immune response against viral and tumor antigens, leading to the generation of long-lived memory T cells [74]. Instead of directly lysing cells, oncolytic viruses can be genetically modified to serve as delivery vehicles for vaccination therapy. Adenovirus, Herpes simplex virus, Paramyxovirus, Rhabdovirus, Vaccinia, and other viral strains are viable options for delivery mechanisms [75].

The adenovirus has been extensively researched as an oncolytic viral platform for treating breast cancer. This is due to the higher expression of E2F-1 in breast cancer cells compared to healthy tissue. The adenovirus genome is strategically located downstream of the E2F-1 promoter, allowing for selective virotherapy through the integration of the immunologically regulatory element IL-15 [76, 77]. Yan et al. demonstrated this replication selective virotherapy by creating a recombinant adenovirus vector that integrates the E2F-1 promoter and IL-15 [78]. Another recombinant adenovirus, developed by Zhu et al. controls the adenoviral E1A gene through the hTERT promoter and the adenoviral E1B gene through the hypoxia response element (HRE) promoter. This recombinant adenovirus also includes the IL-24 gene, which induces tumor-specific apoptosis and inhibits tumor cell growth [79].

Retroviruses, specifically RNA viruses, can be utilized as DNA delivery vectors for managing malignancies when the viral proteins gag, pol, and env are removed. Recombinant retroviral vectors have the ability to express transgenes that are unique to tumor cells [80]. GDEPT (Gene-Directed Enzyme Prodrug Therapy) employs recombinant retroviruses to convert inactive medications into active, toxic metabolites specifically within tumor cells [81]. The MetXia-P450 retroviral vector has shown promising results in reducing the growth of malignant tissue in the MDA-MB-231 breast tumor xenograft model and sensitizing T47D breast cancer cells to cyclophosphamide (CTX) [82, 83]. Rexin-G, a separate vaccination regimen, is used to treat various solid cancers, including breast cancer [84]. It is based on recombinant replication-incompetent retroviral vectors and expresses a synthetic human cyclin G1 transgene that promotes apoptosis and inhibits angiogenesis [84, 85].

TLRs 3 and 9 can be utilized by parvovirus-H1 to stimulate the human immune system, which triggers an NF-κB-dependent adaptive immunological

response [86, 87]. Primary breast tumor cells isolated from breast cancer patients' tumors demonstrate a higher affinity for H1PV compared to normal cells. Recombinant HIPV has also been employed in cancer immunotherapy. Genetically modified HIPV with increased inflammatory cytokine (IFN-γ and TNF-α) transgenes have shown therapeutic benefits [88].

The viral vector-DC vaccine and virus vector-CAR T are two crucial methods of virus-mediated vaccine delivery. Targeting DCs with transgenic technology has proven to be an effective strategy for directing the immune response towards tolerance or immunity [89]. The key advantage of using viral vectors to genetically modify DCs is the enhancement of DC maturation achieved through this approach [90]. In an *ex vivo* study by Chen et al. transmission of DCs with an Ad5 expressing the ErbB-2/neu gene resulted in curative and protective immunity against a breast cancer cell line that overexpressed this gene [91].

Recent studies on cancer therapy have focused a lot of attention on using CAR-modified T cells together with oncolytic viruses to target and direct solid tumors [92]. The potential to deliver gene-based vaccinations or therapeutic transgenes to the tumor microenvironment (TME) of solid malignant cells has been explored. The interaction of viral vectors with tumor-specific T lymphocytes has been found to enhance their effector capabilities. Patients with hematological malignancies have shown significant benefits from CAR-modified T cells [93]. According to findings by Bajgain et al. co-expression of the inverted cytokine receptor (4/7ICR), which connects the IL4 receptor exodomain and the IL7 receptor endodomain, enhanced the anti-cancer activity of CAR T cells in a breast cancer model *in vivo*. This was achieved by transforming the suppressive IL4 signal [94].

1.2.2.1.2 Bacterial Vectors

Busch and Fehleisen were the first to establish a connection between cancer and bacteria (*Streptococcus pyogenes*). They found that erysipelas infection by this organism in a cancer patient inhibited tumor growth [95]. In the 19th century, William Coley utilized an attenuated combination of *Serratia marcescens* and *Streptococcus* to treat bone and soft tissue sarcomas. This concoction was named Coley's poison [96]. This discovery motivated researchers to focus on locating and utilizing bacterial strains or their products for the treatment of different cancers [97]. In fact, certain bacterial constituents, including exotoxins, have been found to initiate direct anticancer actions on

tumor cells, rather than having an indirect effect. Using bacteria as a delivery system for transgenes with therapeutic value offers numerous benefits. In the anaerobic conditions of malignant cells, specific bacterial species like Clostridium are highly active and motile [98]. Due to their ability to reach tumors in hard-to-access areas and the high metabolic activity of tumor cells, which makes them resistant to chemotherapy, bacteria are considered potential options for delivering anticancer drugs or gene-based vaccinations to treat malignancies [99].

The most common species of bacteria used for breast cancer vaccination are *Salmonella typhimurium* [100], *Listeria monocytogenes* [101], *Clostridium novyi* [102], *Clostridium acetobutylicum*, *Bifidobacterium longum, Bifidobacterium adolescentis,* and *Escherichia coli.*

Salmonella and Clostridium have reportedly been utilized as oncolytic vectors [85, 103]. After the introduction of suppressed *S. Typhimurium* and *Bifidobacterium longum* along with vectors for the synthesis of molecules like TRAIL, which markedly slowed the growth of the neoplasm, it was shown that the transcription of apoptotic genes (Suicide genes) in the host genome increased [104, 105]. By disrupting the cell cycle and triggering cell death [106] through the production of p53 and Bax, the Azurin protein generated by *Pseudomonas aeruginosa* destroys malignant cells and stops the growth of breast cancer and melanomas.

For the purpose of testing the effectiveness of the cytokine LIGHT (also known as TNFSF14 or HVEM-L) in the D2F2 breast cancer model in Balb/c mice, Loeffler et al. created a chimeric S. Typhimurium VNP20009 strain [107]. Breast cancer growth and metastasis were prevented by *S. typhimurium* RE88 in combination with DNA vaccines expressing Fra-1 and IL-18, which activated immune cells (T cells, Natural killer (NK) cells, and DCs) and blocked angiogenesis [108, 109]. The progression of 4 T1 breast cancer is slowed down *in vivo* by oral administration of transgenic *S. typhimurium* SL3261 and human granulocyte/macrophage colony-stimulating factor (hGM-CSF) [109].

Salmonella strains that have undergone attenuation are commonly used as live vectors in both human and veterinary medicine. It has been demonstrated that humans respond favorably to attenuated forms of Salmonella (*S. typhimurium* and *S. typhi*) used for vaccine administration [110].

Gram-positive bacteria *Listeria monocytogenes* can escape from the phagosome and enter the cell cytosol by utilizing pore-forming enzymes such as hemolysin, listeriolysin O, and phospholipase. This provides a versatile vector for vaccine delivery and enables more efficient gene transfer.

Numerous studies have shown the effectiveness of *L. monocytogenes* as a delivery system for cancer treatment [111]. In a study by Wood et al., a mouse model of breast cancer was treated using listeria-based vaccinations targeting CD105 (Lm-LLOCD105A and Lm-LLOCD105B) [112].

The PROSTVAC vaccine, also known as PSA-TRICOM, includes booster shots of recombinant fowl pox-PSA-TRICOM and a priming shot of recombinant vaccinia-PSA-TRICOM (prostate-specific antigen plus a TRIad of co-stimulatory molecules). In phase II clinical trials, individuals with metastatic, hormone-refractory prostate cancer received PSA-TRICOM [113].

1.2.2.1.3 Virus-Like Particles (VLPs)

The highly structured structures known as VLPs, which are extremely repetitive, are packed with viral capsid proteins. This high density of capsid proteins provides numerous conformational viral epitopes that can induce potent immunological reactions [114]. Importantly, VLPs are self-assembled by viral capsid proteins without any viral pathogenic nucleic acids. Due to their inability to replicate, they offer a potentially safer alternative to the attenuated viruses commonly used for immunization.

The first VLP-based vaccination targeted the Hepatitis B virus (HBV) [115]. In the late 1960s, Dr. Baruch Blumberg serendipitously discovered the distinctive Australia antigen in the serum of patients with acute and chronic hepatitis B. He also provided the initial concrete evidence of VLP production [116]. In the subsequent years, electron microscopy was employed to study the virus morphology [117–119], and in the early 1980s, HBV VLPs were assembled for the first time using a yeast expression system [115, 120].

Gamma herpes virus EBV, the first oncogenic virus discovered in humans, attacks epithelial and B cells. EBV has been associated with various malignancies, including lymphomas, gastric carcinoma, and nasopharyngeal carcinoma, which can occur in both immune-competent and immune-compromised individuals. EBV is also the primary cause of mononucleosis [121]. To develop another model of an EBV vaccine, the EBV gp350/220 was fused with the fusion F protein of the novel disease virus, allowing the gp350/220 ectodomain to be presented in VLP particle form [122]. Chinese hamster ovary cells were utilized to produce the EBV-VLPs, and BALB/c mice were immunized with them. This resulted in a persistent and targeted antibody response that could inhibit EBV infection *in vitro*, even without the use of adjuvants [123]. In order to create a polyvalent vaccine, the glycoproteins

gH/gL or GB, which are essential for EBV entry, the EBV nuclear antigen 1, and latent membrane protein 2 (LMP2), expressed on the surfaces of all EBV-infected cells, were also incorporated into VLPs. The gH/gL-EBNA1 and GB-LMP2 VLPs were successfully produced in CHO cells without the use of adjuvants, and it was demonstrated that they induced high neutralizing antibody titers or EBV-specific T cell responses in BALB/c mice. Therefore, these EBV-VLPs can serve as an effective therapeutic vaccine for treating EBV-associated malignancies and as a preventive vaccine against EBV infection [124]. Some examples of VLPs as CV delivery agents are shown in Table 1.1.

TABLE 1.1 List of VLP's as a CV

Cancer Type	VLP	Antigen Used	Adjuvant	References
Melanoma	Polyomavirus	OVA (model antigen), TRP2	With or without quila-saponin adjuvant	[125]
Melanoma	*E. coli*	gp100	–	[126]
Melanoma	CuMVT	LCMV-TT830–843	Microcrystalline tyrosine (MCT)	[127]
Melanoma	Bacteriophage Qβ	Melan-A	IFA (Montanide), topical imiquimod +/– IFA	[128]
Pancreatic cancer	Baculovirus (SHIV)	mMSLN	–	[129]
Cervical intraepithelial neoplasia	Chimaeric HPV16-VLPs	E7 and 16L1	–	[130]
Breast cancer	Human parvovirus B19	IGF-1R	–	[131]
Pancreatic cancer	SIV	Trop2	Alone or with gemcitabine	[129]
Hepatocellular carcinoma	*E. coli*	CLDN18.2	–	[132]

1.2.2.1.4 Virosomes

These extremely small, circular, unilamellar lipid membrane vesicles (150 nm) lack the nucleocapsids that typically contain the genetic material of the original virus. However, they are packed with viral membrane proteins such as hemagglutinin and neuraminidase, which are found in influenza. These proteins enable virosome membranes to directly deliver their contents, which

are specific antigens, to target cells by fusing with immune system cells. This process triggers a specific immune response, even in the presence of weakly immunogenic pathogens. Once the antigens have been delivered, the virosomes within the cells are completely eliminated. Unlike other liposomal and proteo-liposomal carrier systems, the immunological characteristics of virosomes are strongly influenced by a viral protein that is sandwiched between phospholipid bilayers. This intercalation not only provides structural stability and uniformity to virosomal formulations, but also determines whether the antigen's epitopes are located on the surface of the virosome (PeviPROTM) or inside it, thereby influencing the type of immune response induced by virosome formulations (PeviTERTM) [133]. The use of PeviPROTM triggers an immunological humoral response, resulting in the presentation of an MHC II antigen due to the breakdown of the antigen in the cell's endosomes. In addition to eliciting a CD4+ and CD8+ positive response *in vivo*, antigens produced by PeviTERTM can also generate a strong CTL response. The virosomal encapsulation ensures correct antigen presentation via the MHC I pathway, as the antigen is naturally delivered into the cytoplasm of the antigen-presenting cell.

1.2.2.2 NON-VIRAL VECTORS

1.2.2.2.1 Cationic and Biodegradable Polymers

Pharmaceutical research and industry have recently utilized the capabilities of cationic and other carbohydrate polymers to regulate the distribution of drugs, proteins, antibiotics, peptides, DNA, or vaccines [134]. Various systems, such as poly-lysine and its conjugates, chitosan, polyethyleneimine (PEI), lipopolyamines, diethylaminoethyl-dextran (DEAE-dextran), polyamidoamine (PAMAM) dendrimers, dextranspermine polycations, and dextranspermine polycations, have been developed in recent years [134]. Numerous cationic polymers, including dendrimers (highly branched PAMAM) and chitosans (a biodegradable linear amino polysaccharide), have been examined for their gene transfer abilities [135].

Furthermore, chitosan can form complexes through electrostatic interactions with negatively charged DNA (polyplexes). Chitosan and chitosan/DNA nanospheres have recently been produced using an osmosis-based method [134]. PEI and poly(L-lysine) (PLL) have been extensively studied as polymers for gene therapy. It is possible to attach targeting ligands and/or PEG, which provide steric stabilization to gene carriers, to the surfaces of

PEI polymers due to their properties [135]. When pegylated PEI polyplexes were conjugated with a tumor-specific ligand and administered intravenously, the transfection effectiveness increased fivefold compared to pegylated (transferrin-free) PEI polyplexes [136]. Researchers investigated the effects of cationic polyelectrolytes on malignant cells *in vitro* and in animals transplanted with Ehrlich carcinoma or murine leukemia that causes ascites (L5178Y). Administration of high-molecular-weight PEI, polyvinylamine, and PPI at neutral pH, as late as 5 days after tumor transplantation, resulted in a 10 to 40% reduction in tumor growth in solid Ehrlich carcinoma-bearing mice. The only substance that extended the lifespan of mice with ascites generated from leukemia cells was PPI. Therefore, cationic polymers may be useful by directly electrostatically interacting with tumor cells and inducing a generalized immunological response in the host [137].

In 2013, an oncolytic Ad (Ad-DB7-U6shIL8) (oAd/PNLG) was coated with a polymer N-(2-aminoethyl)-2-aminoethyl methoxy poly(ethylene glycol)-b-poly-N-L-glutamate (PNLG) polymer [138].

Another cationic polymer was created in 2014, this time with arginine moieties specifically designed to promote lower levels and higher levels of CAR expression [139]. mPEG-PEI-g-Arg-S-S-Arg-gPEI-mPEG (PPSA) has a bio-reducible disulfide link to reduce cytotoxicity and several arginine functional moieties to increase transgene expression. The oncolytic Ad (DWP418) was delivered intratumorally to CAR-negative MCF7 xenograft mice after being complexed with PPSA (DWP418/PPSA). The outcome showed that, compared to bare DWP418, the DWP418/PPSA offered more potent anti-tumor effects.

1.2.2.2.2 Microparticles (MPs) as Vaccine Delivery Carrier

Micro-scale particulates or capsules, as a vaccine delivery vehicle, offer advantages over soluble antigens. By utilizing MPs, which can also provide sustained antigen release, prolonged antigen presentation, and simultaneous transfer of the antigen and adjuvant to the same APC, large quantities of antigens can be delivered to APCs. Adjuvants and antigens can be co-encapsulated in a single particle, spaced apart, or collectively attached to its surface. The optimal size range for vaccine distribution that can enhance and prolong an immune response remains ambiguous, as indicated by the findings of the referenced studies [140].

The literature contains numerous preclinical studies that employ various biodegradable particulate formulations as CVs. These particles for CVs have

been created using well-known poly (a-hydroxy acid)-based polymers, such as polylactic acid (PLA) and PLGA, as well as more experimental polymers like polyanhydrides. MPs derived from polyanhydrides have been reported to possess inherent immune adjuvant properties. Examples of such polyanhydrides include 2,6-dioxaoctane and 1,8-bis(p-carboxyphenoxy)-3,6-dioxaoctane (p-carboxyphenoxy) hexane [141, 142]. Synthetic adjuvants CpG and OVA were co-loaded onto polyanhydride particles (1–3 μm in diameter) using a double emulsion solvent evaporation technique [143]. These MPs provide additional prophylactic protection against an OVA-expressing tumor threat, as evidenced by slower tumor growth and improved mice mortality compared to mice vaccinated with soluble OVA and CpG. Mice administered with these MPs also exhibit higher levels of OVA-specific CD8+ T cells than mice vaccinated with soluble OVA and CpG [144].

In the context of tumor lysates, CpG demonstrates beneficial effects in an alternative particle-based vaccination formulation. The crude TA preparation, resulting from repeated freezing and thawing of tumor cells, whether they are autologous or allogeneic, can be utilized as a vaccine in soluble form or encapsulated in particles. Potential cancer vaccines utilizing tumor lysates are currently under investigation. According to De Temmerman et al. mice were administered intraperitoneal injections of PLGA (75:25) MPs containing tumor lysate (from B16.F1 melanoma) and CpG [145]. Consequently, the number of T cells in the spleen significantly increased. The studies demonstrated that dendritic cells (DCs) in the peritoneal cavity engulfed the MPs and transported them to the spleen, where they triggered TH1 immunological reactions, as evidenced by elevated IFN-c production in isolated splenocytes.

Calcium carbonate or silica-based inorganic MPs are being explored as vaccine carriers [146, 147]. Human EGFR 2 antigen has been loaded onto porous silicon MPs, measuring 1 μm in diameter and 400 nm in height [148]. Immunization with antigen-loaded silicon MPs stimulated the development of a tumor-specific CTL response, extensive infiltration of CD11c+ DCs, and increased intratumor type I IFN and MHC II expression.

Additionally, the porous silica MP vector (MSV) enables the simultaneous administration of the B16-derived antigen Tyrosinase-related protein (TRP) 2 and the TLR agonists like CpG or MPLA into the same DC [149]. The immunization based on silica MPs may significantly increase the median survival of tumor-bearing mice by coordinating effective host immune responses. Inorganic $CaCO_3$ MPs have also been developed to encapsulate tumor lysates with the intention of using them for individualized anticancer

vaccination. Due to a higher rate of TLR agonist absorption by APCs, the TLR ligand-adsorbed and antigen-encapsulated $CaCO_3$ MPs outperform the soluble mixture of TLR agonists and antigens in terms of activating APCs and boosting the effectiveness of the CV.

1.2.2.2.3 NPs as Vaccine Delivery System

The utilization of nanoparticulate (NP) systems as a vaccine carrier has the potential to enhance immunological responses in immune cells by increasing the efficacy of antigen delivery to specific immune cells [150]. Currently, the use of orally delivered nanoparticulate systems for vaccines is considered the most promising approach. In fact, controlling the temporal release profile may not be as crucial for immunological stimulation as it is for generating a pharmacologic response at a higher dose [151]. Through chemical modification, the vaccination antigen can be either compressed within or conjugated on the surface of NPs [152, 153]. Antigens delivered using NPs have shown to be more effective compared to other methods, which could lead to quick antigen disintegration or a diminished immune response. Table 1.2 lists some NPs used for cancer delivery. Furthermore, in addition to the site-directed release of antigens, certain composite NPs also enable sustained release of antigens to enhance exposure to the immune system. Moreover, several NPs RNA interference treatments are currently undergoing human clinical studies to evaluate their effectiveness and safety. Figure 1.3 illustrates different NPs used for cardiovascular (CV) delivery.

1. **Polymeric NPs:** Chitosan, gamma polyglutamic acid (γ-PGA), HT, and other synthetic and natural polymers such as PEI, PLA, polypropylene sulfide, acrylic acid-based polymers, and PLGA are included. The utilization of polymers including PLGA, PEI, and chitosan has shown great potential in pre-clinical and clinical trials. Chitosan exhibited much lower toxicity compared to poly-l-lysine and PEI [175]. The primary benefits of PLGA/PLA particles for neoplasm vaccines and immunotherapies are their ability to act as a non-toxic, therapeutic vehicle for the concomitant delivery of antigens and adjuvants that can be dispersed over a long period of time. Chitosan NPs demonstrated tumor cell selectivity [176]. More studies are focusing on modifying chitosan to enhance the selectivity and bioavailability of chitosan NPs. Modified chitosan NPs exhibit characteristics such as targeting precision, pH sensitivity, and thermo-sensitivity [177]. *In*

TABLE 1.2 Summary of Nanoparticles as Carriers for Vaccine Delivery

Formulation	Antigen	Tumor Model	References
Polymeric NPs	Antigen-DC	Melanoma	[154]
	OVA, MPLA, CpG	Breast cancer	[155]
	OVA	E.G7-OVA	[156]
	TRP2	Melanoma	[157]
	OVA/Gp100/Trp1/Trp2Obsl1Kif18b/Def8/Reps1/Adpgk/Dpagt1	Melanoma/colon cancer	[158]
Inorganic NPs (Au)	OVA	EG7-OVA	[159]
	SIINFEKL peptide	None	[160]
Inorganic NPs (silica)	OVA	CpG reduced B16-OVA	[161]
	–	–	[162]
Inorganic NPs (aluminum)	OVA	Melanoma	[163]
Microneedles	OVA	Melanoma	[164]
Lipid NPs	Synthetic long peptides	None	[165]
	OVA	E.G7-OVA	[166]
	MART-1	Melanoma	[167]
	gp100 and TRP2	Melanoma	[168]
	NY-ESO-1, MAGE-A3, tyrosinase, and TPTE	Melanoma	[169]
	L-BLP25, MUC1	Breast cancer	[170]
	OVA	None	[171]
Virus-like particles	PSA	Prostate	[172]
Virus vaccine	HPV	Cervical	[173]
Peptide or protein conjugated	E7 peptide/Trp2	TC-1 tumors/melanomas	[174]

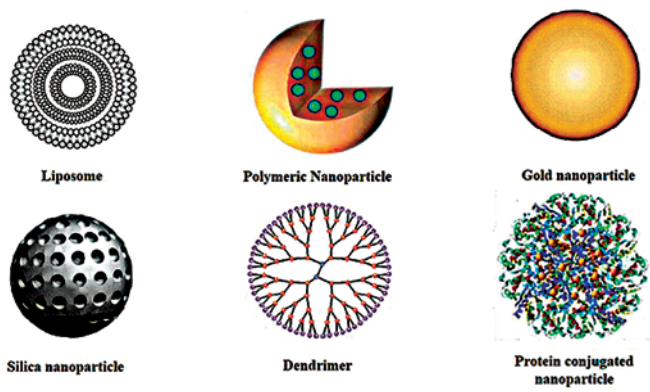

FIGURE 1.3 Different NPs used for cancer vaccine delivery.

vitro anti-tumor testing of chitosan NPs revealed that the 500 mg/L concentration inhibited cervical cancer Hela cells by 27%, liver SMMC-7721 cells by 23%, gastric cancer BGC-823 cells by 29%, and breast cancer MCF-7 cells by 55% [178].

When dendritic cells (DCs) were exposed to soluble antigens, PLGA-modified particles, or PLGA NPs with encapsulated antigens, Shen et al. investigated antigen absorption and CD8+ T cell activation [179]. Reddy et al. developed pluronic-stabilized polypropylene sulfide NPs that activated DCs by activating the complement cascade and generating an *in-situ* danger signal [180]. Liu et al. described a multi-adjuvant whole-cell tumor vaccination based on NPs for cancer treatment [181]. They also utilized PLGA NPs as a delivery system for whole-cell tumor vaccination and demonstrated effective suppression of tumor growth. To address these challenges, polymeric NP-encapsulated curcumin, or nano-curcumin, is currently being explored [182]. In mouse models, intravenous injection of PLGA particles carrying a TAA peptide (TRP-2) [180–188] and a TLR-4 ligand (7-acyl lipid A) has shown inhibition of tumor growth with two doses.

Bourquin and colleagues expanded on their earlier research by utilizing cationized gelatin NPs loaded with CpG [183, 184]. They demonstrated that mice immunized with OVA and these NPs were able to generate specialized and protective antitumor immune responses [184]. Nanoemulsion vaccination was employed to deliver tumor-specific antigens, resulting in the elicitation of strong tumor-targeted antibody and CTL responses in mice. This ultimately provided protection against tumor growth [185–188]. Wei and colleagues developed melanoma-targeted nanoemulsion vaccines encapsulating TAAs, MAGE-1, and/or MAGE-3. Additionally, their formulations included A SEA and HSP70, which are believed to enhance the development of tumor-specific immunity. Shi et al. created an immunostimulatory vaccination to prevent stomach cancer, delivering the CpG and MG7 antigens [185].

In a recent study, mice were vaccinated with γ-PGA NPs loaded with the TAA EphA2 to assess the level of protection induced against EphA2-expressing tumor cells [189]. Mice immunized with EphA2-PGA NPs exhibited increased EphA2-specific CD8+ T lymphocyte stimulation, target cell breakdown, and reduced overall liver growth, serving as indicators of tumor protection [190].

2. **Inorganic NPs:** Vaccines based on inorganic materials have received extensive research despite their poor biodegradability. Inorganic particles have been utilized as adjuvants and antigen delivery carriers to enhance immune response [191]. The four most commonly used inorganic particle types in vaccinations are aluminum, Ca3(PO4)2, silica, and Au.

   Studies have shown that aluminum particles can act as both carriers and adjuvants to stimulate the immune system, although they can securely bind to antigens and alter their structure, thereby reducing the effectiveness of vaccination [192, 193]. Li et al. used OVA and the Bacillus anthracis protective antigen protein as model antigens to develop aluminum hydroxide NPs that exhibited enhanced antigen-antibody responses.

   Calcium phosphate particles are an attractive choice for vaccination applications due to their bioresorbability, nontoxicity, adjuvant properties, and ease of antigen loading [194].

   Colloidal gold, a solution composed of gold nanoparticles (AuNPs), has been utilized as an immunodiagnostic marker and a therapeutic agent for cancer treatment. As an effective delivery approach for cancer vaccines (CVs), Ojeda and colleagues developed an AuNP system with self-assembled monolayers of carbohydrate antigens, known as glyconanoparticles [195]. According to Lee et al., AuNP-based CVs can enhance the immune response by activating Toll-like receptor 9 (TLR-9) on dendritic cells (DCs) [196]. Kang et al. investigated the impact of OVA-carrying AuNPs on the transport of OVA to lymph nodes (LNs) and the activation of CD8+ T-cell responses [167]. Anionic poly I:C and cationic antigen peptides were self-assembled on gold NP templates (iPEM-AuNPs), forming an immune-potentiating nanosystem without the need for solvents or additional structural components, which significantly increased the number of antigen-specific CD8+ T cells [168].

   Silica-based particles are a common type of inorganic particle used in vaccine development due to their biocompatibility and versatility in terms of size and shape [197]. It has been demonstrated that mesoporous silica particles function effectively as persistent antigen carriers *in vivo* [198]. Kim et al. developed mesoporous silica rods using the OVA model antigen and CSF adjuvant as a controlled

vaccine delivery system [199]. Mesoporous silica NPs have been studied for their interactions with various cell lines *in vitro,* including HeLa cells [200, 201], 3T3 endothelial cells [202], human mesenchymal stem cells [203], and human colon cancer cells [204]. The use of silica NPs as a vaccine delivery technology that enhances the generation of antigen-specific B and T cell responses, thereby inducing protective anticancer immunity, has been documented by Myungi and colleagues [205] in a mouse tumor model.

3. **Magnetic NPs:** Hai-Yan Xie, Wei, and their team reported a magnetic cancer nanovaccine that exhibits significantly improved lymph node retention under the influence of a magnetic field, leading to enhanced vaccine effectiveness against carcinoma [206]. When a magnetic field was applied, CD8+ DCs in the lymph nodes showed a significant increase in the uptake of anti-CD205-decorated magnetosomes [207].

4. **Dendrimers:** These are synthetic polymers that typically form spherical macromolecules and have a structure composed of chains that repeatedly branch. These dendrimers consist of three distinct domains of structure: terminal groups, recurrent branching units, and a central core that offers variable surface functionalities [208]. Among the various dendrimer classes, PPI-based dendrimers and PAMAM are widely utilized and have garnered significant attention [209]. Chahal et al. developed novel alkylated dendrimers by treating PAMAM G1 dendrimer with 2-tridecyl-oxirane, resulting in the production of nanoparticles encapsulating lipid-anchored PEG and mRNA. This technology facilitated the production of antibodies and CTLs that specifically recognize the antigen [210].

   By utilizing dendrimers, the G250 renal cell carcinoma specific antigen is fused to a GM-CSF chimeric molecule, creating an exceptionally effective vaccine that elicits an immune response specifically targeted against kidney cancers [211].

1.2.2.2.4 Liposomes and Lipid Nanoparticles (LNP)

The phospholipid bilayers that constitute the spherical nanovesicles are referred to as liposomes [174]. The head of the phospholipids is hydrophilic, while the tail is hydrophobic, enabling them to self-assemble and form liposomes

in aqueous environments [212]. Lipid-based technologies, such as liposomes, are commonly employed in human clinical trials, particularly in cancer gene therapy [37, 213]. By adjusting the lipid composition, charge, size, and surface characteristics of liposomes, a versatile NP vaccine delivery system can be created, offering a wide range of desirable properties [214–216]. Liposomes have been utilized to transport RNA, DNA, and antigens, enhancing the immune response to target antigens for cancer vaccines. Liposomal nanomedicines hold great promise for advanced therapies in areas such as cancer treatment, vaccine development, and cholesterol management [217].

In 1998, Longenecker's research team described a 24-mer human mucin 1 (MUC1) peptide liposomal vaccine [218]. This vaccine, which utilized MPLA as an adjuvant, elicited stronger Th1 responses and provided protection in mice compared to a KLH-conjugated vaccine. Furthermore, liposomal co-encapsulation of HER-2/neu63-71 with CpG resulted in significant CD8+ T cell responses and regression of existing tumors [219].

In comparison to PLGA NPs, ISA-51, and emulsions, liposomes have been found to be more effective in inducing functional antigen-specific T cells, as indicated by another research [220]. Stimuvax, also known as BLP25 liposome vaccine or L-BLP25, is a CV developed by Oncothyreon in collaboration with Merck KGaA. It targets the exogenous core peptide of MUC1 to stimulate the immune system. MUC1 is a type I membrane glycoprotein that is extensively expressed in various tumors, including prostate, breast, lung, and colorectal carcinoma [221]. VacciMax (VM) is another liposome-based technology used in cancer peptide vaccinations [222]. The AS01 liposomal formulation, which includes MPLA and QS-21, has the ability to generate CD8+ CTL and antigen-specific antibody responses [223]. A three-part vaccination that combines a short MUC1 epitope, a Th epitope, and a synthetic triacylated lipopeptide (Pam3CSK4) that activates TLR2 can be integrated into liposomes and result in high IgG antibody titers in mice, even without the use of an external adjuvant [224, 225].

Early trials against lung, liver, colon, and rectal cancers have shown that traditional liposomes with a muramyl dipeptide derivative (ImmTher®) enhance the cell destroying action of macrophage activity against Ewing's sarcoma [226]. A combination of non-UV active Sendai virus liposomes and replicative protein antigens was developed. These liposomes enable the addition of Sendai fusion protein to various types of mammalian cells, facilitating direct uptake into the cytoplasm [227]. Clinical tests comparing these vaccinations to traditional liposomes demonstrated a lower risk of cancer. Zhao et al. conducted experiments using protein antigen carriers to enhance

immune recognition in mouse models of malignancies using the DNA-liposome-polycation complex. Due to its high efficiency, it has recently been investigated as a vaccine adjuvant in experimental testing for delivering the antibiotic protein HPV16 E7, which is associated with cervical cancer [228].

To create pH-sensitive fusogenic dioleoylphosphatidylethanolamine/egg yolk phosphatidylcholine liposomes, Eiji Yuba and his colleagues utilized two different types of poly(glycidol) derivatives that are pH-sensitive and have either a linear (MGlu-LPG) structure or a hyperbranched (MGlu-HPG) structure [229]. LNPs, similar to liposomes, are a crucial platform for biocompatible and biodegradable drug administration [174]. Xu et al. developed calcium-phosphate-lipid NPs with an uneven lipid bilayer and a calcium phosphate core for use as a TRP2 peptide vaccine delivery approach to treat skin cancer. Lipid-calcium-phosphate vaccination stimulated a potent antigen-specific CTL response compared to free TRP2 peptide/CpG, resulting in a greater reduction in tumor growth in lung carcinoma models as well as B16F10 S.C. [159].

1.2.2.2.5 Immuno-Stimulating Complex (ISCOM)

The particulate antigen delivery vehicles, known as immuno-stimulating complex (ISCOM®) vaccines, are composed of phospholipids, cholesterol, immunogens, and saponins. In this study, we demonstrate the advantages of utilizing ISCOM® technology for developing vaccines with anti-cancer properties. Through the use of animal models and a model cancer antigen, it has been observed that ISCOM® vaccines can stimulate CD8 T cell responses independently of CD4+ T cells, provide protection in three different tumor models, and enhance Th1-biased immunity. Furthermore, it has been discovered that coupling the vaccine antigen to the ISCOM® structure significantly improves the first three stages of immune response. Notably, the activation of CD8 T cells by the ISCOM® vaccine remains unaffected by the presence of *in vivo* antibodies against the vaccination antigen. While immunization has proven effective against tumors expressing the vaccine antigen, no activity has been observed against neighboring tumor cells expressing the vaccine antigen negatively. This suggests that significant anti-cancer action is not achieved through determinant transmission or bystander activation.

As part of the third generation of anti-neoplastic vaccines, which have yet to undergo substantial human testing, various synthetic non-living adjuvants and delivery strategies are being developed. These include ISCOM® vaccines, VLPs, liposomes, MF59, and heat shock proteins (HREs).

A different category of immunostimulatory adjuvants consists of plant extracts rather than chemicals linked to bacterial or viral pathogens. The bark of *Quillaja saponaria* in South America yields saponins, which are soluble in water and are structurally diverse compounds with potent pro-inflammatory activities. The most commonly used RP-HPLC fraction of *Quillaja saponaria* extracts for vaccine development is QS21 [230]. The triterpene aldehyde group, assumed to be the active site of the adjuvant, has been found in preclinical studies to induce a strong mixed T-helper 1 (Th1), humoral, and CD8 T cell response. The ASC/NALP3 inflammasome pathway transforms pro-IL1 and pro-IL-18 into their bioactive forms, which QS21 has been shown to largely activate. This justifies its combination with a TLR4 ligand to trigger the upstream expression of the pro-forms. However, large amounts of QS21 can cause cell membrane lysis and death of antigen-presenting cells (APCs), suggesting that the intensity and significance of the resulting immunological response are not directly associated with the extent of inflammasome activation [231]. QS21 has undergone extensive testing in cancer immunotherapy vaccine formulations using ganglioside antigens (GD2, GD3, or GM2). Despite the frequently robust humoral response, there is no definitive evidence of cell-mediated immunity in individuals. QS21 and MPLA are combined in the adjuvant formulations AS01 and AS15, similar to the MAGE-A3 cancer vaccines (GSK).

1.2.2.2.6 Peptide and Protein Conjugated Delivery System

In comparison to other methods, peptide vaccines have several advantages. Firstly, since Merrifield's Nobel Prize-winning invention of solid-phase peptide production in 1984, the processes of peptide fabrication and purification have continuously evolved and become automated. Consequently, numerous contract manufacturing firms now offer high-quality synthetic peptides for research and therapeutic purposes, which are thoroughly described and reasonably priced.

Peptides are easier to synthesize and scale up compared to entire proteins or most other types of antigens. Their synthesis is straightforward, allowing for the creation of peptide vaccines tailored to specific antigen targets. This eliminates concerns about proper protein folding or the location of the target antigen, whether it is surface-bound or intracellular. On the other hand, CAR T cell therapy requires a focus on surface antigens.

Secondly, peptides represent the simplest and most fundamental biological component of a cancer epitope. By utilizing peptide vaccines, vaccine

strategies with clear mechanisms of action can be developed. Immune responses can precisely target the desired epitopes. In contrast, identifying the contents of vaccines such as tumor lysates, which contain a range of antigens, other proteins, and additional tumor components, can be challenging or even impossible.

However, the modest immunogenicity of peptide vaccines is likely their greatest drawback. Peptides are often combined with delivery mechanisms and/or adjuvants, as they are typically insufficient on their own to elicit adequate immune responses. Despite ongoing research on these systems, they still require improvement.

Tetanus toxoid is an additional carrier protein that has been used in the development of peptide conjugate vaccines (CVs). Kunz's research team described a synthetic vaccine consisting of tetanus toxoid and a peptide derived from MUC1. This vaccination significantly increased the level of antigen-specific immune responses in mice [232]. Subsequently, researchers combined MUC1 glycoprotein with the Thomsen-Friedenreich antigen and a difluoro derivative of tetanus toxoid to generate MUC1-specific antibodies that could bind to the MCF-7 breast cancer cell line [233]. According to Tsuruma et al., a phase I clinical trial of a survivin-derived peptide vaccine involved patients with advanced or recurrent colon and rectal cancer [234]. However, this vaccination alone, which contained a peptide derived from survivin, did not show any discernible clinical effects. According to research by Jaeger et al. the tumor vaccine utilized tyrosinase, melanoma-associated peptides from Melan A/MART-1, gp100/Pme117, and influenza matrix peptide [235].

Peptide sequences or modifications in self-assembling peptide delivery systems can induce supramolecular assembly. For example, amphiphilic peptides can be engineered to form micelles, nanoparticles, worm-like micelles, fibrillary structures, or hydrogel networks [236]. Liu et al. developed amphiphilic peptide vaccines using TRP2 from melanoma or E7 peptides derived from HPV-16. These vaccines, when combined with the adjuvant CpG, effectively inhibited the progression of melanoma tumors, and induced sustained regression of large TC-1 tumors, respectively [237]. Compared to their parent compounds, these vaccines are less harmful to the body and exhibit enhanced efficacy in combating tumors.

Zhu et al. generated albumin/vaccine nanocomplexes *in situ* by utilizing endogenous albumin and self-assembling extrinsic vaccine components (CpG and SIINFEKL). These nanocomplexes significantly enhanced the accumulation of CpG and antigens in the lymph nodes [238]. In their

research, Wang et al. concluded that albumin improved the stability of antigens in acidic intracellular compartments and facilitated their internalization by antigen-presenting cells (APCs). Additionally, albumin facilitated the accumulation of antigens in draining lymph nodes [161].

1.3 CHALLENGES AND DEVELOPMENTS

Despite numerous advancements and remarkable clinical outcomes, there are still several challenges that need to be addressed. These include low antitumor efficacy, costly procedures, and adverse effects [239, 240]. Controlled vehicles (CVs) must possess qualities of being secure, efficient, and cost-effective. Nanoparticles (NPs) significantly contribute to achieving these objectives due to their enhanced immunogenicity, ability to target dendritic cells (DCs), safety, improved antigen absorption, and increased bioavailability. Various physical and chemical characteristics, such as size, targeting ligand, particle rigidity, zeta potential, and intrinsic immunogenicity, have been shown to have a significant impact on antigen presentation, cellular absorption mechanisms, antigen distribution, and the types and strength of immune responses. Considering these factors is crucial to maximize antitumor responses [241].

However, tumor growth involves a delicate balance between the host immune system's induction of an anti-tumor reaction and the immune system's repressive microenvironment [242]. CVs and treatments that reduce the cancer microenvironment (CME) can be combined to enhance the effectiveness of immunotherapeutic outcomes [243]. Both preclinical and clinical studies have been conducted in this regard. Treatment options include modest kinase inhibitors, antibodies, or RNA interference that regulate the suppressive immunological milieu, as well as the addition of chemotherapy drugs such as doxorubicin, cisplatin, and docetaxel.

We have described various polymer-based systems in this chapter that aim to enhance the overall effectiveness of cancer immunotherapies mentioned earlier. These systems can address the aforementioned limitations of immunotherapies by utilizing suitable polymers, which can further improve therapeutic efficacy, biocompatibility, and specificity. Polymeric systems offer a unique delivery strategy for conjugating immune ligands and loading immunotherapeutic drugs, providing advantages such as low toxicity, high biodegradability, and customizable surface and size. It is worth noting that polymeric systems can protect bioactive molecules from

undesirable immune responses or promote favorable ones that benefit the body's condition. However, there are still challenges to overcome before these polymeric system-mediated immunotherapy delivery approaches can be implemented in clinical settings, including low therapeutic effects, immunotherapy resistance, patient safety concerns, and high treatment costs. Therefore, further research is needed to enhance current distribution strategies. Additionally, more studies are required to explore the therapeutic potential of nanomedicine, considering their biocompatibility and the effects of different polymeric systems on various body parts [244].

Additionally, the future class of polymeric nanoparticle-based immunotherapy should be exceptionally multifunctional, possessing the capability to target, exhibit intelligent responsiveness, and facilitate easy application. Future research trends will primarily focus on and hold a particularly optimistic outlook on personalized immune therapy utilizing polymer nanoparticles.

Peptide-based cancer vaccines can be produced safely, inexpensively, and effectively. They have demonstrated potential efficacy in targeting both tumor-specific and tumor-associated antigens. Peptides are particularly attractive due to their ability to be utilized in multiplexing techniques that target multiple epitopes and convey the minimum number of biochemical signals necessary to generate antigen-specific T cells. Addressing the issue of low potency is one of the primary concerns that peptide drug carriers can tackle to enhance immune response stimulation. The majority of preclinical investigations on advanced peptide delivery systems have employed a significant subset of well-characterized peptide epitopes and cancer cell models. In clinical and preclinical studies, efforts to standardize peptide dosage may prove beneficial in facilitating direct comparisons [245].

Fresh peptide variants that exhibit enhanced TCR binding, and MHC binding can also be synthesized. Utilizing peptide blends that elicit multiple CD8+ T cell responses in addition to CD4+ responses can enhance T cell immunity. In terms of MHC restriction, it is conceivable to identify larger peptide sequences or pools of peptides that can accommodate a broader range of MHC reactivity, potentially benefiting a larger patient population. In the near future, we anticipate the persistence of certain patterns in cancer peptide vaccinations. The development of novel peptide vaccine delivery systems can be facilitated by the advancements in checkpoint blockade techniques and neoantigen targeting. Currently, there are several phase I/II clinical trials underway, investigating the use of a peptide jab in combination with checkpoint blockade inhibition [246].

Advancements in vaccine delivery systems have led to the development of NPs, self-assembling peptides, and needle-free delivery methods. In our phase 1 clinical trial, SCIB1 was administered using EP, as has been done with previous cancer and infectious disease vaccines. These more recent delivery technologies offer superior alternatives, although their implementation has been limited by the discomfort associated with EP administration and the need for a specific vaccine administration system.

Due to their flexibility, high safety profile, and ability to store minuscule drug molecules or RNA or peptide antigens, liposomes are being utilized as delivery vehicles. Optimizing liposomes is necessary to achieve the correct charge and particle size for effective integration as carriers and transport across the cell membrane. Cancer antigens can be targeted through various methods such as adding a peptide to an adjuvant, encapsulating the antigen in NPs, or modifying genetic vectors to enhance the local immune response and increase traffic to LN.

Another crucial technical challenge is the large-scale production of nanocarriers for antigenic vaccine delivery [247]. Currently, most nanocarriers are produced in laboratories in small batches before being scaled up to larger industrial capacities. Given the high cost of the scale-up process, it is essential to closely evaluate scale-dependent variables that directly impact treatment effectiveness. Additionally, the economic viability of the scale-up process is carefully assessed to prevent any quality crisis [248, 249].

In addition to formulation challenges, significant regulatory barriers hinder the use of genetic vaccinations for cancer treatment. To obtain regulatory approval, it is crucial to address the main formulation concerns related to the excipients used in generating nanosystems for administering genetic vaccines and their toxicity profile [249].

1.4 CONCLUSION

The primary objective of gene therapy is the development of efficient and safe gene carriers capable of delivering exogenous genetic components into specific cell types, particularly tumor tissue. The development and evaluation of vectors for delivering therapeutic genes into cancer cells include both viral and non-viral approaches. Various viruses, such as retroviruses, poxviruses, herpes simplex viruses, adenoviruses, and adeno-associated viruses, have been modified to eliminate toxicity while maintaining their strong gene transfer capabilities.

Non-viral vectors have gained attention as an alternative to viral vectors due to the limitations associated with the latter. Biodegradable and cationic polymers like PEI, PLL, MPs, NPs, peptides, and liposomes are examples of non-viral vectors.

To enhance transfection efficiency, several innovative transport technologies and improvements to existing delivery systems have been developed. The administration method of vaccination can also influence the interaction between the vaccine and different antigen-presenting cells at the injection site, thereby impacting the development of the immune system response.

KEYWORDS

- **cancer vaccine delivery systems**
- **cell-penetrating peptides**
- **immune-stimulating complexes**
- **liposomes**
- **polymeric microparticles**
- **polymeric nanoparticles**
- **tumor targeting**
- **virus-like particles**

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CHAPTER 2

Current Knowledge and Prospects of Various Vaccine Delivery Systems for Lung Cancer Treatment

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ABSTRACT

A typical occurrence of minor intragenic mutations is responsible for the diverse characteristics and prognosis of the molecular mechanism underlying lung cancer. In this review, we highlight the pulmonary route as a promising method of medication delivery for the treatment of pulmonary cancer. Tumors exhibit all the hallmarks of neoplastic growth and are typically distinguished from other types of growth by their autonomy in developed indicators, insensitivity to antigrowth signaling, evasion of apoptosis, genomic instability, ability to invade surrounding tissue and metastasize, sustained angiogenesis, and involvement of surrounding stroma. Systemic delivery is required to reach all lung tissues and the entire circulation for drug access to both localized and distant metastatic lung cancer. The majority of individuals who develop lung cancer today are either smokers or have had prolonged exposure to occupational or environmental factors that disrupt the organization of the epithelial cells in the lungs. Clinical oncologists possess extensive experience in working with cancer vaccines (CVs). The advancement of innovative techniques for vaccine administration holds the potential to enhance the success of clinical trials. Cell-penetrating peptides, also referred to as CPP, present promising candidates for utilization as carriers in medication administration, gene transfer, and DNA vaccination. DNA

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delivery serves as an effective method for studying gene structure, parameters, and functions. We assessed CVs based on cells, peptides, viruses, and nucleic acids. Local drug delivery strategies aimed at tumor targeting have the capacity to improve patient endurance and quality of life, enhance drug accumulation at the targeted site, and reduce the likelihood of systemic side effects. Currently, it is common practice to consider pulmonary administration of systemic medications for patients with respiratory and non-respiratory conditions. Nanoparticles (NPs) that have been PEGylated, peptide- or receptor ligands-conjugated can selectively deliver drugs to specific tissues at lower concentrations and with reduced cytotoxicity compared to topical drug administration. Investigation into local drug administration for lung cancer.

2.1 INTRODUCTION

Since the first use of immunizations, medical professionals have discovered a variety of innovative methods to prevent and treat infectious diseases. In 1796, Edward Jenner made the groundbreaking discovery that the cowpox vaccination provided protection against smallpox infection [1]. This discovery is often considered the starting point of the modern era of vaccinations. The success of vaccines in preventing diseases that were previously difficult or impossible to treat led to their application in combating cancer. When people hear the term "vaccine," they often think of anti-infective vaccines used against viruses and bacteria. These vaccines have historically prevented epidemics and saved numerous lives [1]. When administered to a healthy individual, vaccines stimulate the immune system to develop an artificial response against the pathogen. This is achieved by immunizing the body with weakened or inactivated infectious organisms. Lung tumors are currently one of the most frequently treated cancers and a significant contributor to cancer-related deaths globally [2]. It is one of the most commonly detected cancers, and unfortunately, the mortality rate remains high despite recent advances in therapy. The vaccine window for lung cancer is strategically positioned during the lengthy clinical latency phase to halt carcinoma development and establish long-lasting anti-carcinoma immune surveillance in high-risk patients [3]. Our specialized vaccination management can be utilized to improve pulmonary cancer outcomes and prevent the disease. In the past decade, significant progress has been made in understanding cancer histology, utilizing prognostic biomarkers, and developing more effective

treatments for lung cancer patients [4]. Despite the increasing number of non-smokers, lung cancer still accounts for approximately 1.8 million deaths and 3 million new cases each year [4].

2.1.1 TYPES OF LUNG CANCER

Based on histopathologial features, lung cancer can be classified into two:

i. Non-small cell lung cancer (NSCLC); and
ii. Small-cell lung cancer (SCLC).

Lung cancer patients comprise 85%–90% of all cases, with non-small cell lung cancer (NSCLC) accounting for over 90% of cases. Carcinoma scientists prioritize NSCLC due to its higher prevalence. NSCLC is typically treated with radiotherapy, monoclonal antibodies (mAbs), and surgery. However, NSCLC often exhibits resistance to chemotherapy and radiotherapy, resulting in poor anticancer integration in distinct tissues and undesirable side effects. To address this, simple drug carrier methods for targeted tumor exposure have been explored to improve lifespan, quality of life (QoL), local medicine concentration, and minimize systemic side effects.

Nanoparticles (NPs) have emerged as a promising nanostructure for anticancer drug delivery. Their high surface-to-volume ratio and ability to encapsulate a variable amount of drugs make them ideal for targeting specific tissues. In the case of lung cancer, delivering NPs via inhalation has shown significant advancements. However, conventional inhaled NPs have certain limitations, which can potentially be overcome by using systemic polymeric NPs with customized properties. These NPs can be delivered to lung cancer cells through clathrin-treated endocytosis. Biomaterials, both natural and synthetic, can be utilized to develop NP-based medication delivery systems.

In addition to NP-based delivery systems, cancer vaccines (CVs) have shown promise as immune system modulators [5, 6]. This report focuses on advancements in lung CV delivery technology. Specifically, we will discuss new vaccine delivery technologies, molecular adjuvants, and immunomodulatory agents [7].

2.2 MECHANISM OF LUNG CANCER ANTIGEN BEHAVING

Mechanism of lung cancer antigen behaviors is represented in Figure 2.1.

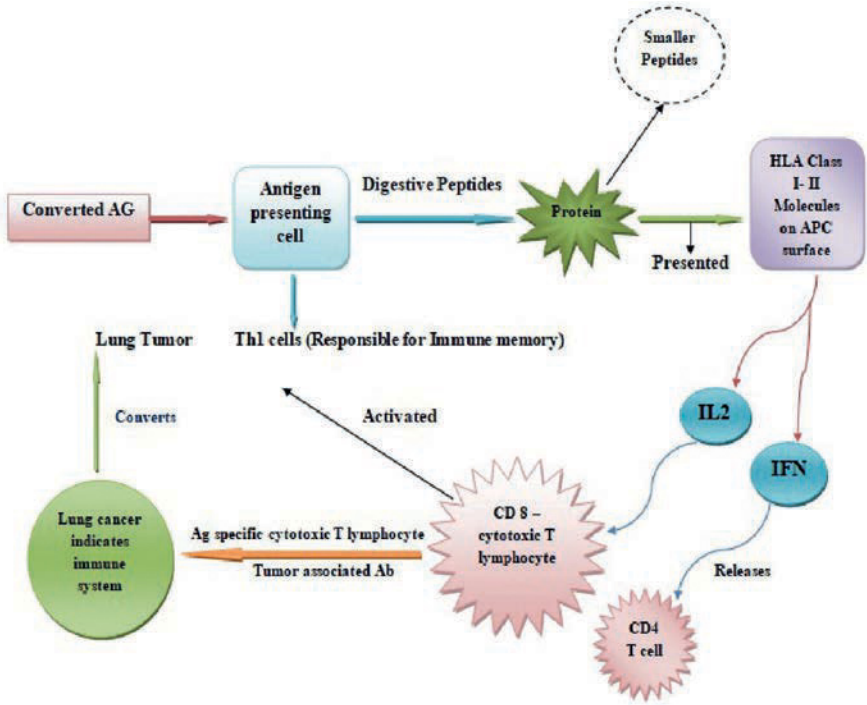

FIGURE 2.1 Mechanism of lung cancer antigen behavior.

2.3 CONVENTIONAL CHEMOTHERAPY IN LUNG CANCER

Conventional chemotherapy is divided into two types [8, 9]:

i. Tumor associated antigen (TAA) built carcinoma vaccine; and
ii. Non-mutant peptide-built carcinoma vaccine.

2.3.1 TUMOR ASSOCIATED ANTIGEN (TAA) BUILT CARCINOMA VACCINE

Curative immunization of lung tumor patients with tumor-associated antigens (TAA) such as cancer testis antigens, transformed antigens, and viral antigens is a crucial aspect of modern carcinoma vaccines. The goal is to activate tumor-specific T cells. Transformed antigens, also known as tumor-specific antigens (TSA) or neo-antigens, are expressed exclusively in cancer cells. They result from gene alterations that lead to the production of unique, abnormal proteins. Tumor antigens can also be found in certain normal

organs. The most effective curative vaccines are synthetic long peptide (SLP) derivatives of antigens, delivered with a carrier, as well as DNA and RNA vaccines targeting MHC-I and MHC-II molecules on specialized antigen-presenting cells (APC), such as dendritic cells (DC). This approach stimulates both CD8 and CD4 T-cell responses in affected individuals. The advent of next-generation sequencing (NGS) technology and *in silico* epitope prediction has ushered in a new era of therapeutic vaccinations. This allows for the identification of patient-specific tumor antigens (TAs) resulting from somatic alterations in specific malignancies.

2.3.2 NON-MUTANT PEPTIDE BUILT CARCINOMA VACCINE

The major antigenic peptide derivatives originate from TAAs and are recognized by CD8 T lymphocytes, which are generated from cleavage in the proteasome of intracellular proteins. Suppression of major histocompatibility complex-I (MHC-I) molecules has been observed in 17 to 60% of initial lesions from various forms of cancer in individuals. Furthermore, between 40% and 89% of human cancerous nodules were found to be deficient in histocompatibility complex I, including 74% of pulmonary carcinoma, with a complete absence of group one molecules in 39% and a locus failure. Previous studies have focused on the suppression of tumor-associated antigen protein 1 and/or tumor-associated antigen protein 2 in pulmonary cancer cells, resulting in resistance to T cell receptor-dependent lysis. Tumor-associated antigen protein deficiencies have been analyzed in a wide range of carcinomas, with up to 80% of non-small cell lung cancer expressing lower levels of tumor-associated antigen protein 1 and/or tumor-associated antigen protein 2 and are associated with uncontrolled growth and resistance to immune system regulation. Therefore, the development of alternative approaches for TAA processing may enhance anti-tumor T-cell responses and T-cell-based immunotherapy strategies [10].

2.4 TARGETED THERAPY [11]

Dependence on inadequate treatment leads to unfavorable outcomes in advanced stages of lung cancer. An analysis of treatment options that combine clinical responses to chemotherapy and radiation is necessary. Additionally, complementary treatments that target residual viruses after surgical removal show promising results. New treatments should aim for improved outcomes

while having a more favorable side effect profile compared to traditional chemotherapy.

Selective treatment can be categorized into three types:

- Pharmacological agents, including enzyme suppressors and dominant-negative oligonucleotides;
- Gene transfer; and
- Immunotherapy (passive and active).

2.4.1 PHARMACOLOGICAL AGENT AND ENZYME INHIBITORS [11, 12]

A significant number of pharmacological agents used to date are gene-active drugs, which have limited efficacy and significant toxicity. Pharmacological agents that target various pathways responsible for mitogenesis, survival, migration, invasion, and angiogenesis (the cancerous phenotype) have been developed; some may also inhibit malignant transformation. Pharmacological tyrosine kinase inhibitors (TKIs) have shown remarkable potential based on current medical research, such as the molecular agents gefitinib and erlotinib. The cancer cell cycle suppressor pemetrexed and the proteasome inhibitor bortezomib are both approved for use in other malignancies and show some promise in the treatment of lung cancer [13]. Clinical trials are evaluating numerous other molecular agents that target one or more pathways essential for cancer development and progression.

2.4.2 GENE THERAPY

Gene therapy is more of a novel carrier system than an actual therapy itself. The ability of DNA delivery vectors to deliver a therapeutic gene to an organ, region, or specific cell type (e.g., benign tumor) can be applied to various different structures and targeted therapeutic approaches. A key aspect of DNA-based therapy is the high production of a beneficial protein intracellularly or its release into the local environment, thereby sparing non-targeted tissues from the effects of the expressed protein. As the term "DNA therapy" can be confusing, it is important to differentiate between DNA delivery vectors and DNA-based therapeutic proteins [14].

2.4.3 IMMUNE CELL THERAPY

Immune cell therapy is a concept based on the understanding that the immune system can differentiate between tumor cells and normal cells. It

can be categorized as either passive or active. Passive immune cell therapy involves the use of biologically active agents administered externally, without relying on the body's own machinery. In contrast, active immune cell therapy utilizes the host's immune cells and requires a fully functioning immune system. Anti-inhibitor treatment is a specific type of immune cell therapy used to counteract factors in both the host and the tumor that provide a survival advantage in lung cancer.

2.5 CV PLATFORMS [15]

Cell-based vaccines, peptide-based vaccines, viral-based vaccines, and nucleic acid-based vaccines, including DNA vaccines and mRNA vaccines, are different types of cancer vaccines.

2.5.1 INITIAL FORM OF CANCER-BASED VACCINE

Cell-based CVs are created using whole cells or cell waste. These vaccines carry malignant antigens and elicit a strong immune response [16].

2.5.2 PEPTIDE-BASED CV

Peptide-based vaccines involve the chemical and biosynthetic formulation of specific tumor antigens (TAs). They induce a robust immune response against the targeted TAs. When combined with adjuvants, peptide-based vaccines can effectively enhance immune reactions, making them suitable for preventing and treating infectious diseases.

2.5.3 VIRUS-BASED CV

Virus-based CVs utilize viruses that are known to be major contributors to tumor development. These vaccines can include virus antiserum that lyses cancer cells or virus vector antiserum. Approximately 13% of cancers are associated with viral infections, with human gamma herpes virus, HBV, Hepatitis C virus, and Human papillomavirus being the most common cancer-related viruses [17]. Inactivated whole virus vaccines have demonstrated efficacy in treating diseases like COVID-19 or Ebola. It is reasonable

to expect similar efficacy in the treatment of virus-associated cancers. However, these vaccines are less frequently used in oncological diseases, possibly due to challenges in manufacturing and safety concerns.

2.5.4 NUCLEIC ACID VACCINES CARRIER

Nucleic acid vaccines utilize genetic information to encode cancer-specific antigens in the host, leading to the production of foreign particle proteins through common physiological processes. These engineered antigens can then elicit immune responses against cancer cells.

2.5.5 DNA VACCINES

DNA-based cancer vaccines are constructed using bacterial plasmids that carry instructions for one or multiple cancer-specific antigens. This approach aims to induce innate immune responses and modulate the immune reaction.

2.5.6 MRNA VACCINES

mRNA-based cancer vaccines represent a promising immunization strategy. They involve the delivery of synthetic mRNA into cells to facilitate antigen production. The expressed antigens are then presented on the surface of antigen-presenting cells via MHC molecules, stimulating anti-tumor immune responses [18] (Figure 2.2).

2.6 PHARMACEUTICAL TECHNOLOGIES FOR DIFFERENT PULMONARY DRUG DELIVERY [19]

Recent years have witnessed an increase in research on utilizing the pulmonary route for systemic medication delivery to treat both respiratory and extra-pulmonary disorders. Chemotherapy for lung cancer benefits from the potential of needleless release of smaller amounts of medication to specific lung tissues, as well as its self-activating capacity.

2.6.1 LIPOSOME

Liposomes are vesicles with a bilayer structure, typically composed of phospholipids as their building blocks. Depending on their size and composition, liposomes can be either large (45–95 nm) or small (25–45 nm) unilamellar

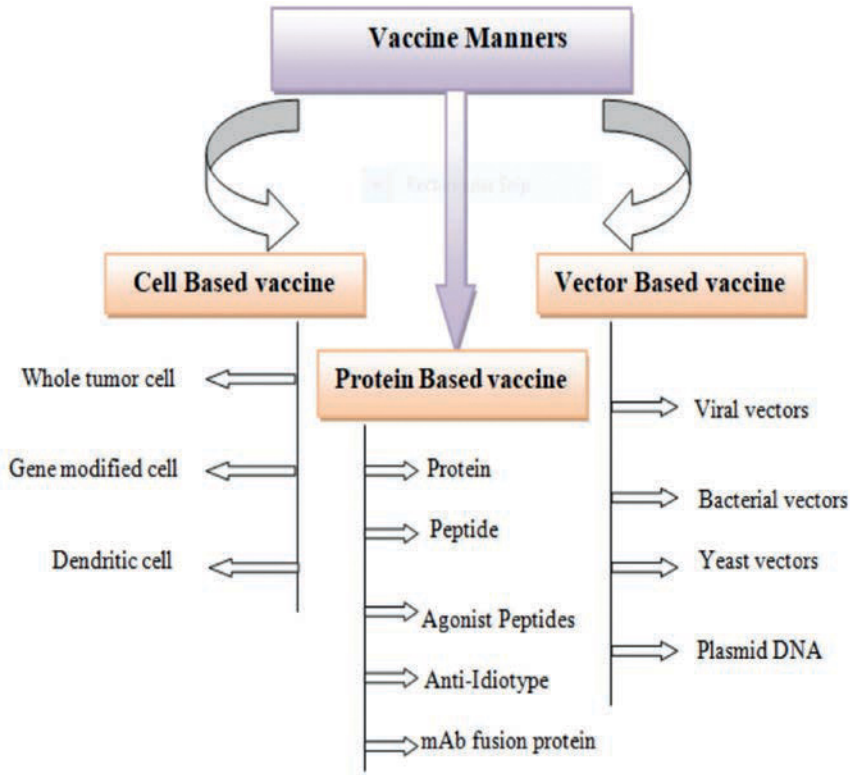

FIGURE 2.2 Schematic representation of CV platforms.

vesicles with a distinct bilayer. Multilamellar vesicles consist of multiple concentric bilayers of phospholipids (1.0–5.0 m), while unilamellar vesicles have a single bilayer. Liposomes can enhance the efficacy of chemotherapeutics due to their phospholipid bilayer, which enables the transport of both hydrophilic and hydrophobic drugs. Since phospholipids and cholesterol are naturally found in lung surfactants and plasma membranes, liposomal formulations composed of these two components are efficient and biocompatible for pulmonary medication administration [20].

2.6.2 MICELLES

Micelles are nano-scale structures that spontaneously form in aqueous environments due to hydrophobic interactions. They consist of an amphiphilic

core surrounded by a hydrophilic shell. Micelles are created by dissolving separate polymer chains in water at a concentration and temperature above the critical point. The choice of polymeric materials is crucial in determining micelle size, shape, stability, and drug retention. Micelles provide a unique platform for the co-encapsulation of two different anticancer drugs, thereby increasing the effectiveness of drug loading and resulting in a synergistic anticancer effect.

2.6.3 DENDRIMERS

Dendrimers, also known as dendritic polymers, are tree-like nanoparticles. The multifactorial exterior covering and hyper-branching tree-like core, along with vacancies, aid in the encapsulation of medicinal ingredients. Generation 4 (G4) dendrimers with NSCL cancer targeted peptides have the potential to enhance drug delivery [21].

2.6.4 POLYMERIC NPS

Therapeutic elements in polymeric nanoparticles can either be encapsulated within the polymeric matrix or located at the interface of the nanoparticles' surface, making them a type of colloidal system. In nanospheres, the drug is uniformly dispersed throughout the nanoparticle, while in nanocapsules, the drug is enclosed within a hollow sphere surrounded by a polymer shell. However, an effective drug delivery method requires a polymer that is – (i) biocompatible; (ii) nonimmunogenic; (iii) easily synthesized and affordable; (iv) solvent-free; and (v) environmentally friendly *in vivo* to minimize the risk of toxicity from accumulating unmetabolized polymeric particles.

2.6.5 PEPTIDE-BASED NPS

Peptide-based nanoparticles involve attaching small peptides, such as growth factors and medicinal drugs, to a soluble polymeric backbone through ecological or separated joints or spacers. Numerous bodily processes rely on neurotransmitters. The limited clinical application of these naturally occurring peptide fragments, which contain 20 amino acids, is due to their poor stability, short half-life in circulation caused by renal ultrafiltration and serum protein breakdown. However, peptides are promising therapeutic options as they are small molecules that can easily enter cells and release medications in response to changes in tissue pH.

2.7 AEROSOLES FOR LUNG CANCER TREATMENT

Direct delivery of anticancer medication via inhalation offers numerous advantages. However, complete drug release through a nano-delivery system proves to be more effective than predictable intravenous drug release for pulmonary cancer. The ability of aerosols to deliver anticancer agents directly to targeted cancer cells allows for increased therapeutic efficacy at lower drug concentrations [22].

2.8 VACCINES FOR LUNG CANCER [22]

Table 2.1 summarized the status of the various lung cancer vaccines, while Figure 2.3 present the mechanism of the lung cancer vaccine.

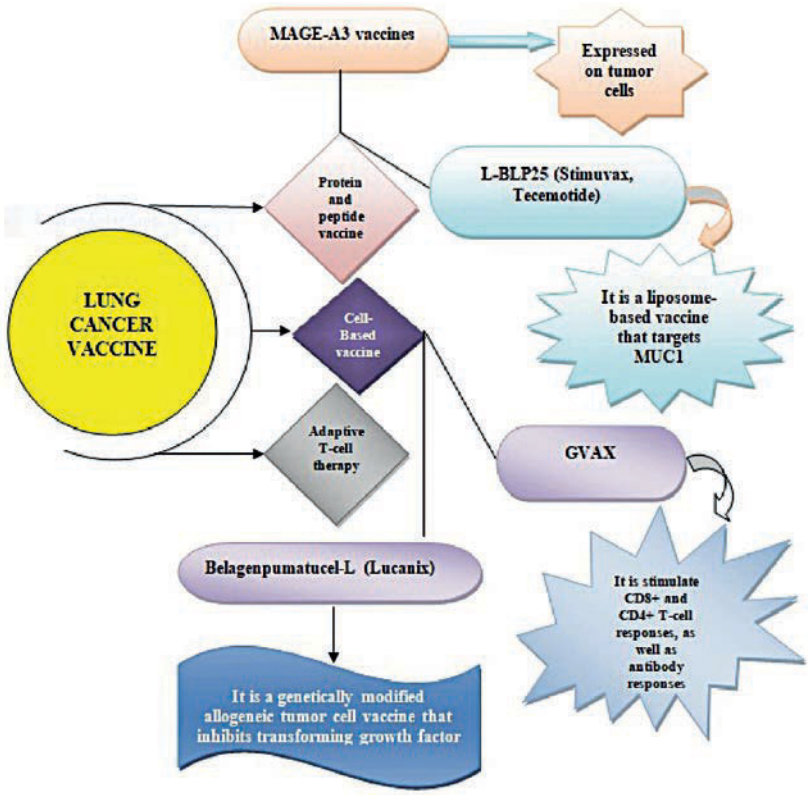

FIGURE 2.3 Different types and their mechanisms of vaccines for lung cancer treatment include MAGE A3 (melanoma-associated antigen-A3), L-BLP25 (liposome-based lipopeptide 25), MUC1 (mucin 1), GVAX (granulocyte-macrophage colony-stimulating factor), and CD8 (cluster of differentiation 8).

TABLE 2.1 Status of Lung CV

Types of Lung Cancers	Past Status of Cancer Vaccine	Current Status of Other Cancer Immunotherapy	Future Prospects of Cancer Vaccine	References
Lung nodule carcinoma	When managing the assessment of a patient with lung nodule carcinoma, particular attention should be given to the source of the cancer.	The increasing use of novel carrier and immunotherapeutic agents has led to the emergence of unique imaging patterns, such as pseudo-progression.	After vaccination, lung nodule carcinoma in the pulmonary area can occur in patients with gastrointestinal cancer, and the presence of duodenum cancer can be an indication of metastatic or stage IV disease.	[23]
NSCLC	Cluster of differentiation 4 (CD4) and cluster of differentiation 25 (CD25) are T cells that have been exposed to transforming growth factor-beta (TGF-β), which can inhibit immune responses. This is particularly important in immune disorders.	They did not express drug-limiting toxicity, with a limited response at the vaccination area.	Vaccine-focused lung cancer requires the transfer of immune stimulation and immune inhibition stability to support immune stimulation.	[24]
Small cell lung cancer	Focusing on the melanoma-associated antigen 3 and Mucin short variant S1 tumor antigen with a cell vaccine enhances tumor endurance.	The vaccine utilizes the immune system to specifically target and eliminate tumor cells.	This CV investigation specifically focuses on known and unknown tumor-related antigens.	[25]
Mesothelioma	In the early period, medicine did not have satisfactory screening modalities for the high-risk population.	VEGFR1 and VEGFR2 (vascular endothelial growth factor receptors) have been detected in the majority of mesothelioma cases, and anti-VEGF rabbit polyclonal antibodies inhibit the growth.	Continued research for novel agents and ongoing clinical trials are critical.	[26]

TABLE 2.1 *(Continued)*

Types of Lung Cancers	Past Status of Cancer Vaccine	Current Status of Other Cancer Immunotherapy	Future Prospects of Cancer Vaccine	References
Chest wall tumors	The patient specified a mutated peptide antigen assumed by a lineage series, which has only turned out to be liable to execute regularly in the particular patient.	There are numerous reports suggesting that a significant number of antitumor T-cell responses are directed towards somatically mutated peptides presented by human leukocyte antigen class I-II.	A future challenge concerns the best way to increase the potency of the chest wall tumor vaccine using a specific immune adjuvant.	[25]
Squamous cell carcinoma	Fast and spectacular deterioration of numerous pulmonary tumors following neo-peptide vaccination.	Focusing on numerous adapted peptide epitopes may have led to a broader immune response through vaccination.	Immune evasion within the tumor microenvironment also presents a significant limitation to any immune-based therapy.	[26]
Adenocarcinoma	The enzyme-linked immunospot assay indicated that the vaccinated patient exhibited robust antigen-specific interferon-γ-related immune responses.	The Kristen rat sarcoma vaccine significantly increased the number of intra-tumoral cluster of differentiation 4+ T cells compared to the adjuvant control.	There was no similarity observed in the cluster of differ-entiation 8+ tumor-infiltrating lymphocytes.	[26]

2.9 DELIVERY SYSTEMS FOR IMMUNOTHERAPY (LUNG CANCER)

2.9.1 ECOLOGICAL GENE CARRIER TECHNOLOGY

For the improvement of vaccines, the design of proficient direction and tumor gene treatment is an area of extensive research. Live vectors such as *vaccinia* virus, other of viruses, *retroviruses*, and bacillus Calmette-Guérin have been specifically developed to deliver genes into cells and are the most commonly used gene carrier tools in gene therapy [27, 28]. The major advantage of live vectors is that they produce the foreign protein in its native conformation, which is crucial for generating neutralizing antibodies and facilitating the entry of foreign proteins into the MHC class I antigen presentation pathway for CD8+ T cell priming.

The most effective vaccination strategy may involve combining one type of immunogen with another, making it stronger. This approach can be beneficial for several reasons:

- This method may be more efficient in priming naïve cells, while another technique may be more successful in stimulating memory responses.
- Both different arms of the immune system, such as CD4+ and CD8+ T cells, can be enhanced using two different techniques.
- Some of the most effective vaccination approaches, such as the use of recombinant *vaccinia* virus or adenoviruses, may have a limited duration of effectiveness due to host immune responses.

2.9.2 NON-ECOLOGICAL GENE CARRIER TECHNOLOGY

Non-infectious vectors must possess the ability to efficiently condense and protect DNA, target specific cell receptors, disrupt the endosomal membrane, and deliver the DNA cargo to the nucleus [29]. Non-infectious vectors generally include naked DNA, DNA-liposome complexes, and DNA-polymer complexes [30]. In other techniques, non-infectious molecular carriers used for gene delivery are categorized into microparticles (MPs), nanospheres, and liposomes [31]. Encapsulating recombinant DNA into micro- or nanospheres can provide protection from the environment prior to release and aid in targeting a specific cell type for efficient delivery.

2.9.3 PROTONATED LIPOIDS

Lipid-based formulations (e.g., liposomes) are commonly used in human clinical trials, particularly in anticancer gene therapy [32]. Protonated lipids are amphiphilic molecules composed of multiple fatty acid side chains, with the cationic portion being lipid-containing. In aqueous media, protonated lipids assemble into a lipid bilayer structure. The combination of microspheres/chromosomes is often referred to as a lipoplex. Negatively charged DNA can interact with protonated liposomes, resulting in aggregation and stable complex formation over time. Due to their reduced stability, lipoplexes are typically used immediately after their formation. The development of new liposomal formulations has led to the production of smaller, more stable, and uniform lipoplex molecules.

2.9.4 POLYSACCHARIDES AND PROTONATED GLYCANS

Protonated polyurethane and other related materials have recently been employed in pharmaceutical assessment and manufacturing facilities due to their ability to control the release of antibacterial medicines, amino acids, proteins, oligonucleotides, and drugs [33]. They are also extensively studied as non-viral DNA delivery systems for gene therapy.

2.9.5 MICRO/NANO-MOLECULE A FURTHER COME UP TO FOR DNA VACCINE

Carriers utilizing micro-particle-based automation can effectively target antigen-presenting cells [34]. Chromosome assembly or DNA encapsulation has led to the enhancement of cytotoxic T lymphocyte responses to engineered proteins [35]. Additionally, environmentally friendly and non-antigenic poly-lactide micro-particles serve as advantageous immunization carrier systems. These systems can elicit immune responses against foreign particles encoded in or conjugated onto the carrier.

2.9.6 PROTONATED PEPTIDES/CELL INCISIVE PEPTIDES

Protonated peptides and cell-penetrating peptides (CPPs) have also emerged as valuable tools in immunization strategies. Various natural or synthetic

CPPs have been identified as efficient agents for delivering therapeutic cargo into biological compartments. CPPs facilitate the entry of hydrophilic substances into cells by interacting with the cell membrane. This approach has been successfully applied to transport drugs, micelles, polysaccharides, lipoproteins, oligonucleotides, and fluorescent dyes into cells, showing potential for future immunization techniques [35].

2.9.7 VIRUSES RESEMBLING MOLECULE AS A PROFICIENT TRANSMISSION

Virus-like particles (VLPs) have gained increasing attention for their use as immunization agents due to their repetitive foreign particle arrangement, which is capable of efficiently stimulating the innate immune response. In addition to their use as immediate immunogens, VLPs have the potential to serve as carrier particles for the delivery of epitopes, DNA, and other small particles targeting different diseases [36].

2.9.8 CARRIER TECHNOLOGY IN DENDRITIC CELL-BASED SYSTEM

Dendritic cells (DCs) are highly efficient macrophages capable of initiating a primary immune response and have the ability to activate T cells and promote the proliferation and differentiation of β cells. DCs provide a direct link between the innate and adaptive immune responses and originate from bone marrow (BM) precursors that reside in lymphoid tissues, where they are capable of capturing antigens. DCs migrate from the lymphoid tissues to the adjacent lymph nodes (LN) and initiate a cellular immune response [37].

2.9.9 CARRIER TECHNOLOGY IN PROTEIN PEPTIDE IMMUNIZATION

Nucleic acid glycoprotein polysaccharides and other similar units are inadequately immunogenic and require auxiliary transport systems or carriers to enhance the natural immune response following vaccination [38]. For optimal presentation, antigen delivery vehicles must accurately mimic the function and immunological distribution of specific microorganisms. They must actively or passively target antigen-presenting cells such as dendritic cells, protect the antigenic protein from degradation, modulate the environment for subsequent immune reactions, and induce antigen-presenting cell

maturation by interacting with components of the nonspecific immunity such as Toll-like receptors (TLRs). Several strategies have been reported, including direct conjugation of TLR agonists as immunostimulatory agents [28, 40].

2.9.10 NON-ENVELOPING TECHNIQUE FOR MONITORING IN VIVO ANTIGEN CAPTURE AND DELIVERY [41]

The main consideration for preventing adverse reactions to immunization is the quantity of stimulated antigen-presenting cells that capture foreign particles and transport them to the draining lymph nodes. The utilization of magnetic resonance imaging (MRI) is an effective approach for these purposes.

2.10 CHALLENGES AND LIMITATIONS OF LUNG CANCER THERAPY

The main objective of recent therapeutic lung cancer vaccines is to create a long-lasting immunological memory in the body against tumor cells, leading to effective tumor regression while minimizing non-specific or adverse events. Currently, the clinical focus is on developing effective therapeutic vaccines that can stimulate a successful and sustained T cell response to specific tumor antigens. The main challenge in vaccine development is the identification of precise target antigens that are shared among multiple tumor types and are uniquely expressed or overexpressed by tumor cells [39].

2.11 CONCLUSION

In this review, we have studied various types of drug delivery systems using vaccines for lung (pulmonary) cancer, which pave the way for society as well as rural areas.

CONFLICT OF INTEREST

We do not have any conflict of interest.

KEYWORDS

- **cell penetrating peptide**
- **cytotoxicity**
- **dendrimers**
- **nanoparticles**
- **nanoshells**
- **neoplastic growth**
- **pulmonary route**
- **tumors**

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CHAPTER 3

Advance Drug Delivery System for Targeting Carcinomas

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ABSTRACT

The development of innovative vaccine delivery techniques, such as colloidal immunostimulatory delivery systems, is a multidisciplinary scientific field that is currently experiencing rapid progress through immunotherapy and gene therapy-based treatment. Due to the weak immunogenicity of tumor antigens (TAs), formulation strategies for cancer vaccines (CVs) play a crucial role. A colloidal vaccine delivery system can alter the kinetics, body distribution, uptake, and release of the vaccine. This chapter examines recent studies focusing on the development of colloidal vaccine delivery systems for more precise cancer therapies. Various carrier systems, including virus-like particles (VLPs), polymeric micro- and nanoparticles (NPs), liposomes, and archaeal lipid liposomes, are utilized. Additionally, several technologies for drug delivery have been developed to enhance lymphatic channels and lymph node uptake, resulting in a targeted immune response for cancer control. Numerous antigens associated with malignancies serve as excellent targets for immunotherapy and vaccine development. To achieve superior therapeutic outcomes, optimally developed CVs should combine the most effective TAs with the best immunotherapy drugs and delivery techniques. Alongside NPs, platforms for vaccine delivery are gaining increasing

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interest. Furthermore, NP-based vaccine distribution technology holds great potential for enhancing vaccine immune-gene city. Consequently, this chapter encompasses a variety of dosage forms currently used to administer chemotherapeutic drugs to patients with different types of carcinomas.

3.1 INTRODUCTION

A variety of illnesses are collectively referred to as cancer, which occurs when malignant cells proliferate uncontrollably and have the ability to invade or spread to other parts of the body. Cancer is one of the most dangerous diseases globally, causing millions of deaths each year [1]. Surgical intervention, radiation, chemotherapy, and targeted therapy are the most commonly employed treatment modalities for cancer [2, 3]. Surgery is often the primary approach for removing tumor masses, while radiation and chemotherapy, considered standard treatment options, effectively inhibit tumor growth. Following surgical removal of tumors, chemotherapy, and radiation are still commonly used to prolong life or provide temporary relief from symptoms [4]. However, both procedures can cause significant suffering for patients, and in some cases, the intensity of pain leads to treatment discontinuation. Additionally, contemporary chemotherapeutic drugs circulate throughout the body and affect both cancer cells and normal cells [5]. This lack of selectivity can result in undesired side effects in healthy tissues during treatment.

To selectively administer therapeutically effective concentrations of medicine to the tumor area, targeted drug delivery holds immense potential for enhancing cancer treatment. These strategies are based on the fundamental principle of selectively eliminating cancer cells while minimizing toxicity to normal cells. Despite recent advancements in cancer prevention, diagnosis, treatment methods, and public awareness, cancer remains the leading cause of death worldwide. Cancer is a complex disease that cannot be easily regulated or tracked. Its progression is not limited to a single node or subset of nodes in the network of the human body, but rather depends on the individual patient, necessitating personalized therapies. Several factors hinder effective cancer treatment and drug development, including inadequate delivery of therapeutic drugs to the tumor site, non-specific distribution of anticancer agents leading to severe side effects, and the development of acquired resistance by cancer cells during chemotherapy, resulting in cross-resistance to multiple drugs [6].

These multifactorial conditions require the development of meticulously planned strategies within the field of drug research. The conventional concept of a “magic bullet” drug, which targets a single drug target, should

be replaced by the formulation of a guided vehicle that can deliver the medicine at the precise time and location. This shift would alter the scientific foundation of medication development. Therefore, the term "targeted drug delivery" should be replaced with "navigated drug delivery," as it involves a vehicle that guides the route of the loaded medication to the intended site of action, in addition to the cytotoxic agent that targets a specific cellular area. To enhance the selective absorption of the cytotoxic agent by tumor cells and spare healthy cells, these drug-loaded and guided vehicles should have a multidimensional design. The current theme issue aims to address the challenges posed by the complexity of cancer by presenting a range of cutting-edge molecularly targeted cancer treatments and innovative drug delivery methods [6].

Innovative nanoparticles (NPs) are continuously being developed to address gaps in the delivery of therapeutic and imaging compounds for the diagnosis and treatment of cancer. NPs have been considered as potential carriers to provide an optimal platform for personalized approaches to cancer diagnosis and therapy in cancer management. When searching for new targets for cancer therapy, it is important to focus on epigenetics, as carcinogenesis involves various genetic and epigenetic changes that contribute to the transformation of normal cells into a malignant phenotype. Cancer is considered both a genetic and an epigenetic disorder. The immune system is designed to protect the body from microbial invasion and prevent diseases. Therefore, cancer vaccinations can enhance the immune system's ability to effectively combat cancer. One of the main goals is to create vaccines that elicit strong and targeted T-cell responses. This is achieved by exposing antigens to the cell surface molecules of dendritic cells (DC), which effectively activate T-cell responses.

3.2 DRUG DELIVERY PROCESS

To effectively deliver medications into solid tumors, nanocarriers must overcome various biological obstacles [7]. For instance, a cancer nano-drug system is introduced into the bloodstream through intravenous injection and subsequently distributed throughout the body [8]. The nano-drug then penetrates deeply into the malignant tissue by leveraging the Enhanced Permeability and Retention (EPR) effect, leading to the aggregation of nanoparticles around the tumor. The cancer cells ingest the medication, causing the nanocarriers to release the cancer nano-drugs. Two factors contribute to the difficulty of nano-drug penetration into tumor tissues:

1. **Nano-Drug Dimensions:** Nano-drugs can range in size from a few nanometers to over 100 nanometers. Larger nanoparticles exhibit significantly lower diffusion capacity compared to small molecules, as diffusion rate is inversely proportional to particle size [9].

2. **Pathological Properties of Tumor Tissue:** Tumor tissues possess exceptionally thick stromal tissue, high cell density, elevated interstitial pressure, and lack of a capillary network due to uncontrolled cell growth. These factors make it extremely challenging for nanomaterials to penetrate and disseminate within the tumor [10].

   Additionally, many tumors develop hypoxia due to their distance from the blood artery network. Hypoxic areas harbor tumor cells that exhibit high levels of treatment resistance and have the potential to metastasize. Even small amounts of small-molecule medications can be found in these areas. Understanding the mechanisms of penetration is crucial for improving the delivery of nano-drugs into tumor tissues. Different nano-drug delivery methods can penetrate tumor tissue through various means, such as paracellular or transcellular transport. The mode of penetration is determined by the characteristics of the nanocarrier, including its size, surface charge, cellular uptake of the drug, and modified targeting polypeptide. All these factors influence how the drug penetrates tumor tissues [11].

3.2.1 REMODELING THE MICROENVIRONMENT OF TUMOR TISSUE

The intercellular components of a cell's cytoplasm and the surrounding environment of neighboring cells constitute the cellular microenvironment [12]. Both the environment and the tumor exhibit adversarial characteristics and resist each other, but they are also interconnected and mutually reinforcing. This understanding is crucial for comprehending the origin, progression, and metastasis of malignancies, as well as their diagnosis, prevention, and patient prognosis. Unlike healthy tissues, tumor tissue demonstrates immunosuppressive conditions, hypoxia, a rigid extracellular matrix (ECM), abnormal vasculature, and a slightly acidic pH [13, 14]. Nano-drugs penetrate tumor cells through the microenvironment of the tumor tissue, which is of utmost importance. Within tumor microenvironments (TMEs), there is a significant presence of tumor-associated

fibroblasts (TAF). These fibroblasts secrete an ECM consisting of collagen, laminin, and fibrin [15].

Nano-drugs are required to pass through this "barrier" in order to enter tumor cells. Additionally, fibroblasts [16], immunological cells [17], vascular endothelial cells [18], stellate cells, and other cells impact the effectiveness of nano-drugs. Consequently, to enhance the anti-tumor effect, researchers design carriers that utilize the characteristics of the cancer tissue microenvironment. When nanomedicines exit the blood vessels within tumors, the first obstacle they encounter is the dense ECM. Nano-drugs do not deeply penetrate the tumor tissues due to the significantly higher density of the tumoral stroma compared to normal tissue. The excessive production of the tumor vascular endothelial growth factor (VEGF) stimulates a high rate of new blood vessels with larger vessel walls than normal blood vessels in the tumor tissue. Hypoxia, as indicated by the study, also hinders the growth and spread of blood vessels, resulting in increased drug resistance and the failure of radiation and photothermal therapy (PTT). Various methods, such as increasing blood flow, providing oxygen, generating oxygen on-site, and minimizing oxygen consumption, can be employed to alleviate hypoxia. When combined with oxygen carriers, PTT can enhance blood flow, reduce hypoxia, and address hypoxia-related tumor radiation resistance. Tumors are characterized by a mildly acidic pH, which facilitates their spread and migration. Moreover, tumor cells can secrete immunosuppressive compounds, regulate the activity of immune effector cells, and restrict the synthesis of regulatory cytokines. This shields the tumors from cytotoxic T lymphocytes (CTLs) [14], thereby promoting tumor growth.

3.2.2 NEED FOR TARGETED DRUG DELIVERY

The pursuit of therapeutic agent specificity is inherent in all treatment methodologies (Figure 3.1). Chemotherapeutic and radiotherapeutic alternatives have been developed to target and eliminate cancer cells, making the specificity of pharmaceutical action crucial in cancer treatment. These strategies are based on the fundamental principle of selectively eradicating cancer cells while minimizing toxicity to normal cells. Complete remission in individuals with disseminated disease requires the eradication of all cancer cells. This can be achieved either through direct pharmaceutical action or indirectly through the bystander impact of therapy [19].

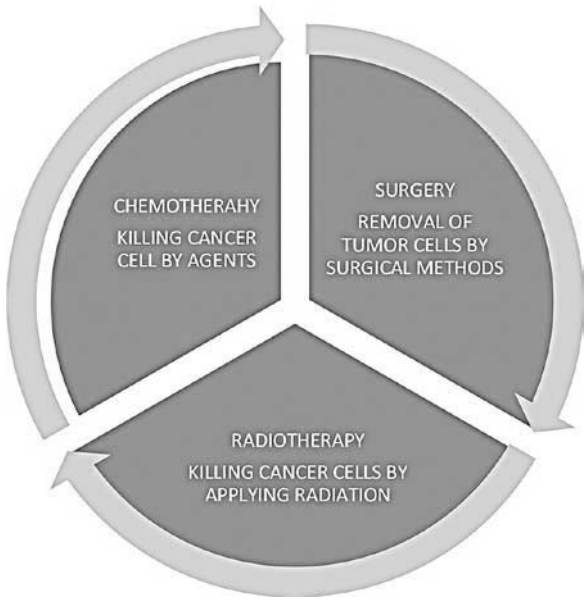

FIGURE 3.1 Different strategies for cancer treatment.

Chemotherapy regimens alone are ineffective in advanced carcinomas and may only result in temporary responses. A combination therapy involving high doses of radiation (60–70 Gy) and continuous infusions of chemotherapeutic drugs (such as paclitaxel) has been studied for the treatment of locally advanced cancers that cannot be surgically removed. Another significant challenge is achieving therapeutically effective drug concentrations in the tumor mass for a sufficient duration to allow the agent to work effectively, particularly in solid tumors. Even after prolonged treatment with these cytotoxic therapies, residual tumor cells remain due to the inability of these drugs to penetrate the physiologically diverse tumor mass [20].

The high-dosage treatment required to maintain complete remission produces unacceptable systemic side effects, leading many patients to discontinue the medication. These adverse effects have a significant negative impact on patients' quality of life (QoL). Due to the limited therapeutic indices of various treatment alternatives, there has been a quest for effective delivery methods for existing medications that can maximize therapeutic effectiveness while minimizing undesired effects. The utilization of specially designed drug delivery systems to target pharmaceuticals is a feasible approach to enhance therapeutic efficacy and reduce systemic toxicity in anti-cancer therapy. Therefore, the need to develop highly targeted drug

delivery systems (Figure 3.2) arises not only from a therapeutic standpoint but also to assist patients in eliminating cancer before it becomes fatal [21].

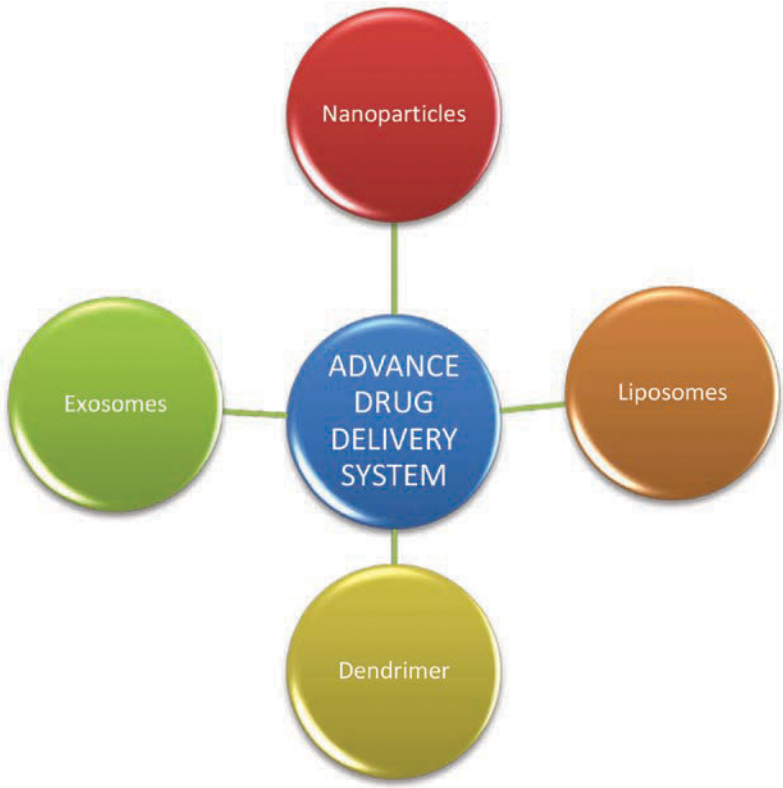

FIGURE 3.2 Types of drug delivery system.

3.2.3 NPS IN CANCER THERAPY

Since the efficacy of nano-drug delivery and subsequent therapeutic success is greatly influenced by the sizes, shapes, and surface features of NPs employed in medical therapy (Table 3.1), these three parameters are frequently considered in NPs [22]. NPs with a diameter of 10 to 100 nm are commonly used in cancer therapy because they can effectively transport drugs while also achieving enhanced permeability and retention (EPR). However, particles larger than 100 nm are likely to be removed from circulation by phagocytes. Smaller particles (less than 1–2 nm) can easily leak from normal vasculature and be filtered by kidneys (less than 10 nm in diameter) [23, 24]. The surface characteristics of NPs can also impact their bioavailability and half-life. For

example, NPs coated with hydrophilic substances like polyethylene glycol (PEG) reduce opsonization and thus evade immune system clearance [25]. Consequently, NPs are often modified to become hydrophilic, prolonging drug circulation and enhancing drug penetration and accumulation in tumors [26, 27]. The diverse characteristics of NPs influence their therapeutic effectiveness in cancer treatment.

TABLE 3.1 Different Nanoparticles and Their Applications

Types of Nanoparticles	Structure	Applications
Polymeric nanoparticles	Polymers such as chitosan, PLGA	Delivery system Tissue regeneration
Liposomes	Lipid bi-layer globules	Drug delivery for hydrophobic and hydrophilic drugs
Dendrimers	Highly branched ends and central core.	Delivery system, tissue engineering, antimicrobials.

3.2.4 LIPOSOMES

To carry both hydrophobic and hydrophilic drugs, liposomes are vesicles with one or more concentric phospholipid bilayers separated by water compartments. They have long been the subject of extensive research [1, 28]. Liposomes have been used to deliver anticancer drugs that do not cause toxic or allergic reactions, as they are physiologically inactive and biocompatible [29–31]. Additionally, specific liposomes can be created using appropriate ligands (peptides, antibodies, etc.) and utilize overexpressed receptors as docking sites for delivering anticancer medications [28]. Cancer cells that are EGFR-positive (epidermal growth factor receptor) absorb liposomes more effectively when treated with cetuximab-ILs. Combining an anti-EGFR antibody with a chemotherapeutic drug has revealed that an IL containing 5-FU modified by cetuximab can enhance the efficacy of Squamous cell cancer (SCC). In addition to increasing the penetration of 5-FU into SCCs, ionophoresis with ILs is a more efficient technique for reducing cell proliferation and invasion compared to subcutaneous administration. This makes it a more appealing therapeutic strategy for SCC.

3.2.5 EXOSOMES

Mammalian cells release extracellular vesicles known as exosomes. When observed under a transmission electron microscope, these exosomes appear

as cup-shaped nanosized structures (50–100 nm) composed of phospholipids [32–34]. The surface of exosomes contains various labeled proteins and ligand proteins, including ALIX, tetraspanins (CD9, CD63, and CD81), integrins, and cell adhesion molecules (CAM). These proteins indicate that the exosomes are internal endosomes and enable them to interact with target cells for cargo delivery.

Exosomes, depending on their properties and origin, can be utilized to target specific disease tissues and/or organs. They possess durability, biocompatibility, low immunogenicity, and safety during circulation [32]. Recently, exosome-biomimetic NPs have gained attention as a reliable drug delivery platform. These NPs are produced using exosomes as natural biomaterials and functionalized NPs. However, the repeated physical extrusion or freeze/thaw cycles involved in the production of exosome-biomimetic NPs may disrupt the protein integrity on exosome membranes and interfere with cancer therapy.

Luminescent PSiNPs (silicon NPs) have been employed to create biocompatible tumor cell-exocytosed exosome-biomimetic PSiNPs (doxorubicin silicon NPs) as a drug carrier for targeted cancer treatment. These PSiNPs exhibit excellent drug-loading capacity, high biocompatibility, and biodegradability. The use of doxorubicin silicon NPs as a delivery vehicle for DOX is possible. *In vitro*, doxorubicin silicon NPs can induce low expression of the multidrug-resistant protein P-glycoprotein, resulting in reduced cell membrane fluidity and improved intracellular retention. Additionally, the ability of doxorubicin silicon NPs to target tumor cells is regulated by CD54, leading to significant cellular absorption. When comparing doxorubicin silicon NPs to free doxorubicin, the latter exhibits greater cytotoxicity against cancer stem cells (CSS). Intravenous administration of doxorubicin silicon NPs results in enhanced tumor accumulation, tumor penetration, and cross-reactive cellular absorption by bulk cancer cells and CSCs. This leads to higher DOX enrichment in all tumor cells and side population cells, further enhancing anticancer activity [35].

3.2.6 DENDRIMER

Donald A. Tomalia's groundbreaking research was initially published in 1985 after he successfully created the first polyamidoamine (PAMAM) dendrimers in 1979 [36, 37]. Dendrimers are a relatively new type of dendritic polymers with a three-dimensional, branching, highly monodispersed, sequentially synthesized macromolecular nanoscopic (1–100 nm) architecture [38, 39].

Dendrimer architecture presents a novel strategy for solubilizing drugs that are insoluble in water, with three main sites for drug entrapment using various mechanisms, such as void spaces (through molecular entrapment), branching points (via hydrogen bonding), and outside surface groups (through charge-charge interactions). This offers a unique nanocontainer feature for drug delivery [27, 30]. It also provides a polyvalent platform for binding a range of biological targets, improving therapeutic effects, which has been utilized to develop a novel nanodrug for antiviral and anti-inflammatory therapy. Reports suggest that dendrimers can enhance transdermal permeability and target specific medications. As a transdermal permeation enhancer, dendrimers have successfully delivered indomethacin via transdermal routes, offering an exciting delivery method for potential anti-inflammatory treatment possibilities [26, 40]. The most widely used critical tool for treating cancer, however, has been dendrimer-based targeted delivery since it is highly biocompatible and has few side effects on healthy cells, immunological function, and blood medicines. TZ-grafted dendrimers were developed to efficiently distribute DTX to breast cancer cells that express the human epidermal growth factor receptor 2 (HER2), which is more selective and has higher anti-proliferation action than HER2-negative cells. TZ-conjugated dendrimers showed better targeting and cellular internalization than unconjugated dendrimers, as well as less hemolytic toxicity and a longer circulatory half-life. The levels of ROS, mitochondrial membrane potential, cell cycle distribution, and activation of caspases 3/7, 8, and 9 in PAMAM-DTX-TZ conjugates were also assessed and compared to free DTX. These findings could help improve DTX's therapy profile for HER2-positive breast cancer [41–43].

3.3 NPS USED IN BIOMEDICAL APPLICATIONS

3.3.1 EXTENDED-RELEASE NP-DELIVERY SYSTEMS

Extended-release medication delivery systems utilize steady-rate drug release or regulated release to provide a stable and increased therapeutic potential while minimizing adverse side effects [44]. Extended-release NPs can retain drugs either on their surface or adsorbed in a matrix to achieve continuous release when used in a therapeutic environment [41]. Currently, hydrophobic biodegradable polymeric NPs are commonly employed to continuously deliver therapeutic medicines to the tumor site. To enhance longer circulation, NPs can undergo specific cell-surface modifications. Nanomedicine

extensively utilizes PEGylation, which involves conjugating PEG to a nanopolymer. It has been demonstrated to increase the drug-hydrodynamic radius, prolong plasma retention time, reduce proteolysis, decrease renal excretion, and prevent immunodetection of antigenic determinants [45, 46].

3.3.2 HYDROGELS

In particular, polysaccharides can be physically or chemically crosslinked to form hydrogels, which are hydrophilic 3D porous networks. The remarkable properties of hydrogels, such as controlled degradation, sustained release of cargo, low toxicity, functionality, and high drug loading capacity with a reversible sol-gel transition, provide a platform for encapsulating various materials [47]. Hydrogels can generally be classified in various ways, including bioresponsive hydrogels, bioinspired hydrogels, static hydrogels, dynamic hydrogels, and hybrid hydrogels. Additionally, there are several therapeutic fields, such as ophthalmology, oral medicine, gastroenterology, cardiovascular disease, and cancer, where hydrogels are utilized, along with numerous delivery methods including peroral, rectal, vaginal, ocular, transdermal, and implantable. Thermosensitive poloxamer 407 hydrogels, which undergo a rapid phase transition from liquid at room temperature to solid at 37°C, enable prolonged release in body organs and reduce off-target toxicity [48]. Some of the various polysaccharides and their composites used to create hybrid hydrogels include alginate-cyclodextrin, alginate-chitosan, alginate-keratin composite, alginate-Polyamidoamine (PAMAM) hybrid nanogel, and alginate/liposome hydrogels. In this context, hydrogels composed of alginate Ca^{++} and sodium carboxy-methyl cellulose are crosslinked to construct complex hybrid dual-drug delivery systems (DDDs). By utilizing the different pH conditions of the small intestine and colorectum, this dual drug delivery system selectively transports aspirin and methotrexate-loaded $CaCO_3$ microspheres to their respective target organs. Free radical polymerization methods were used to produce dual bioresponsive pH/thermo-sensitive hydrogels loaded with DOX and curcumin due to the tumor microenvironment (TME) features of colorectal cancer (CRC), which include high temperatures and acidic pH values [49]. These hydrogels demonstrated improved loading capacity, effective drug release, and the ability to induce apoptosis in colon cancer cells. Hyaluronic acid (HA) and methylcellulose (MC) hydrogels were created as efficient drug delivery systems for rectal distribution [50], taking advantage of MC's thermosensitive feature and HA's inherent glycosaminoglycan composition.

3.3.3 BIONICS

Innovative cell-based carriers have gained increasing popularity as conventional targeted therapy's drawbacks, such as toxicity and short half-lives, have been exposed. These carriers possess fascinating properties, including sustained drug release, slow clearance rates, effective targeting, and biocompatibility. In addition to exosomes, biological carriers known as bionics, such as red blood cells (RBCs), macrophages, platelets (PLTs), neutrophils, microbes, and stem cells, are utilized for drug delivery. Among them, immune cells have the greatest applicability due to the surface membrane proteins that enable interactions with cancer cells [51]. To achieve this, the cell membrane needs to be separated, a core needs to be built, and a shell-core structure needs to be formed. This process involves several steps, such as cell membrane separation and fusion with core nanoparticles (NPs). The unique properties of cell membranes make bionics useful for targeted delivery and deep penetration into cells while evading immune surveillance. Furthermore, bionics can serve as vectors for controlled drug release. Cell membrane-disguised NPs have shown great promise in the treatment of resistant cancer types when combined with chemotherapy, photothermal therapy (PTT), photodynamic therapy (PDT), and immunotherapy [52].

3.4 DRUG DELIVERY SYSTEMS FOR RESISTANT COLORECTAL CANCER

3.4.1 DRUG RESISTANCE MECHANISMS IN CRC

Exosomes are vesicles with a plasma membrane that are released by cells and can be found in physiological fluids [53, 54]. These vesicles transport genetic resources and proteins to distant regions in cancer cells, resulting in tumor development, metastasis, and drug resistance. VEGF, fibroblast growth factor (FGF), platelet-derived growth factor (PDGF), basic (FGF, transforming growth factor (TGF), tumor necrosis factor (TNF), and interleukin-8 (IL-8) have all been reported to induce angiogenesis, a process that leads to the formation of new blood vessels. These molecules can all be carried by exosomes from tumors. The persistence of CSCs (cancer stem cells) after conventional therapy, which allows them to regain their capacity for renewal and dedifferentiation, is one of the most challenging problems in cancer recovery. Additionally, tumor dormancy allows tumor cells to remain dormant but alive until they resume proliferating in response

to the appropriate signals, leading to colorectal cancer recurrence [54]. The classification of dormancy includes four subtypes with multiple processes: basic cancer dormancy, metastatic dormancy, therapy-induced dormancy, and immunologic dormancy. Autophagy, by preserving the viability of cancer cells, promotes cancer dormancy and is necessary for tumor cells to progress into the proliferative stage. Several drugs used to treat cancer induce autophagy, resulting in resistance. There is a significant link between the presence of circulating tumor cells and tumor resistance with the capacity of EMT (epithelial-mesenchymal transition). Furthermore, these circulating tumor cells can acquire stem cell characteristics, leading to colorectal cancer recurrence and metastasis.

Studies utilizing whole genome sequencing have identified significant genetic variability in tumors, which can lead to treatment resistance, recurrence, and a poor prognosis [55]. Chromosomal instability (CIN) is the most common type of instability observed in malignancies, including colorectal cancer. CIN and subsequent cancer development can be triggered by various pathways, such as mutations in the TP53 gene, defects in DNA repair genes, overexpression of AURKA, and GINS1. Copy number alteration (CNA) investigations on organoid cultures have revealed the de novo formation of whole-chromosome and sub-chromosomal modifications during tumor growth, with errors in chromatin serving as the underlying causes [56]. For example, lagging chromatin leads to whole-chromosomal CNAs, while chromatin bridges result in sub-chromosomal CNAs. Aneuploidy is a characteristic feature of several malignancies.

3.4.2 ADVANCED STRATEGIES FOR CRC DRUG DELIVERY

Researchers have shown increasing interest in colon targeting due to the development of drug delivery systems as a new tool, the presence of many adverse effects and poor patient survival rates associated with conventional therapies, and the emergence of alternative therapies that utilize more focused targeted drug delivery systems [57]. When aiming for optimum delivery of colorectal cancer (CRC), factors such as the CRC microenvironment qualities, tumor heterogeneity profile, chemo-physical properties of medications, and colon transitory duration should be taken into consideration. Various vectors and techniques, such as neural tube defects (NTDs), can be employed [58].

The use of single-cell technology enables the creation of novel therapeutic strategies against resistant cancers, making it a promising technique in the fields of cancer therapy and precision medicine. It provides a profile of

heterogeneities in tumors and their environments. Due to the heterogeneity and variable response of the same malignancies to a given therapy, it is crucial to utilize single-cell analysis [59]. Several steps are involved in using single-cell RNA sequencing, starting with the preparation of tissue samples from cancerous tumors. The next step is to choose a platform, such as 10X Genomics Chromium, Nadia (Dolomite Bio), Illumina's Bio-Rad ddSEQ Single-Cell Isolator, BD Rhapsody Single-Cell Analysis System (BD), or Fluidigm C1, to sequence the RNA in those samples. In the end, scientists employ a variety of methods to assess their data, including cell cycle phase assignment, normalization, batch effect correction, and quality control. They also cluster cells and reconstruct the trajectory of each cell using pseudo time to determine whether any regulatory genes control gene expression patterns throughout the cell cycle phase [60, 61]. Finally, they search for enrichment patterns among genes and gene regulatory networks to infer which genes are involved in regulating specific aspects of cell function [62]. Additionally, single-cell analysis can unveil the phylogeny of a tumor and the evolution of clones. For example, single-cell sequencing forms the basis of Simulated Annealing Single-Cell Inference (SASC) [63].

3.5 CONCLUSION

Many different types of NPs, both organic and inorganic, have already been extensively utilized in the clinical treatment of various types of cancer. NP-based drug delivery systems offer superior pharmacokinetics, biocompatibility, tumor targeting, and stability compared to conventional medications. They also play a crucial role in reducing systemic toxicity and combating drug resistance. Due to these advantages, NP-based medications are commonly employed in gene therapy, radiation therapy, targeted therapy, hyperthermia, and chemotherapy. Furthermore, nanocarrier delivery technologies provide enhanced platforms for combination therapy, which helps overcome drug resistance mechanisms. The use of nanotechnology in cancer treatments has ushered in a new era characterized by the overexpression of efflux transporters, compromised apoptotic pathways, and hypoxic tumor microenvironments (TMEs). According to various multidrug resistance (MDR) pathways, NPs loaded with targeted agents in addition to cytotoxic agents can reverse drug resistance. With further investigation, many hybrid NPs have garnered interest and demonstrated improved delivery capabilities. More comprehensive research on the molecular characteristics of specific

tumors will lead to more targeted approaches for these medications. Further investigation is warranted regarding the precise targeting of cancer cells. It is important to note that complex interactions exist between NPs and the immune system. The size, shape, composition, and surface of the NPs all influence their interaction with the immune system. Despite nano-vaccines and synthetic APCs demonstrating greater success compared to conventional immunotherapy, the clinical effectiveness of this therapy remains unsatisfactory, necessitating further research to determine the safety and tolerability of these innovative techniques. The incorporation of immunomodulatory factors into NPs may also enhance the potency of immunotherapy vaccines. Therefore, a deeper understanding of the TME and additional research on the interaction between NP-based drug delivery systems and tumor immunity are necessary for the development of hybrid NPs that are more suitable for cancer therapy and the engineering of NPs that target.

KEYWORDS

- **cancer**
- **cancer therapy**
- **drug delivery system**
- **hydrogels**
- **hypoxic tumor microenvironments**
- **immune**
- **immune system**
- **immunomodulatory factors**
- **nanoparticle**
- **vaccine**

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CHAPTER 4

Correlation Between Cancer Vaccine and Immunity

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ABSTRACT

The human immune system is responsible for both recognizing and eliminating various antigenic molecules and compounds that possess both endogenous and exogenous characteristics. The onset of various forms of cancer has been known to be triggered by multifactorial antigens and antigenic mutations over several years. In recent years, there has been a significant increase in interest within the scientific community regarding cancer immunology, leading to major breakthroughs, primarily through the development of cancer vaccines (CVs). Cancer vaccines have been extensively studied worldwide and have been found to initiate various immunological responses within organisms, which have been mechanistically proven to suppress cancer growth. Despite the extensive research and development of such vaccines, the success and comprehensive understanding of their immunological effects and their fate within the human system remain limited. This chapter aims to provide insight into the factors linking immunology and cancer, focusing on the outcomes of CVs. Additionally, the chapter will discuss the mechanistic aspects of CV efficacy and their immunological effects in the treated host, providing a structural basis for future advancements in the field of cancer immunology.

Cancer Vaccination and Challenges, Volume 2: Delivery Strategies for Cancer Vaccine and Immunotherapy in the Management of Various Carcinomas. Rishabha Malviya, Bhupendra Prajapati, and Sonali Sundram (Eds.)

4.1 INTRODUCTION

Cancer is initiated by the proliferation of a clonal cell population within tissues. Carcinogenesis and the onset of malignancy can be analyzed and characterized in various ways. Cancer can result from chromosomal defects, which are induced by abnormalities in genes that regulate cellular functions, particularly cell proliferation and reproduction. One way to describe this process is by highlighting the defining factors of cancer tissues: the "hallmarks" of tumors [1]. Cancer progression requires the acquisition of six key features: self-sufficient growth, insensitivity to anti-proliferative signals, evasion of apoptosis, unlimited replicative potential, maintenance of vascularization, and, in the case of malignancies, invasion, and metastasis [2]. However, a single gene alteration is insufficient for the full development of cancer. Additional genetic mutations are necessary for the progression towards malignancy and invasion. Therefore, the likelihood of developing the disease depends not only on the initial mutations that initiate tumorigenesis but also on subsequent mutations that drive tumor growth [3].

Cancer can be conceptualized as a sequential step functionally divided into three stages: initiation, promotion, and development. The first stage in the two-stage model of cancer progression is initiation. Initiators are often rendered electrophilic by drug-metabolizing enzymes in the body and can then affect DNA if they are not already reactive with mutated DNA. Many initiators are specific to certain tissue types or species, as they require oxidative metabolism to become active. Once a cell is impacted by an initiator, it becomes susceptible to promotion until it dies. Initiators have irreversible effects. Since initiation is the result of a permanent genetic mutation, all daughter cells produced when the defective cell divides will always carry the mutation [4]. In contrast to initiators, promoters do not form a covalent bond with the cell's DNA or other biomolecules. To modify intracellular pathways that result in enhanced cellular proliferation, many molecules bind to the cell's surface receptors. Specific promoters, which interact with receptors on or in target cells of specified tissues, can be divided into two main categories: nonspecific promoters that influence gene expression without the involvement of an identified receptor. Promoters are frequently selected for a particular tissue or species due to their interactions with receptors that are present in many tissue types at varying levels [5]. Benign papillomas develop when the promoter is applied frequently to initiator-exposed skin. After therapy is terminated, a significant number of these papillomas regress, but some transform into cancer. Papillomas that progress into cancer have

a second, spontaneous mutation, according to the frequency of progression. Leslie Foulds' term "progression" describes the slow transition from a benign tumor to a neoplasm and then to cancer. Progression is linked to a karyotypic mutation (having the wrong number of chromosomes) since nearly all progressing cancers are aneuploid. Along with this karyotypic shift, the tumor also exhibits altered physiology, morphology, metastasis, and a faster pace of development [6].

The investigation of interactions between the immune response and malignancies or other abnormalities is known as cancer immunology. At the core of this field is the precise detection of malignant cells and the antigens they express. This concept is the basis of spontaneous immunosurveillance, which was proposed by Burnet and Thomas in 1957. According to their proposal, lymphocytes act as sentinels that detect and eliminate continuously emerging, immature altered cells. The stepwise immunosurveillance process begins with the recognition and removal of dysfunctional cells, and both innate and adaptive immune cells work together to detect and eradicate tumors in their early stages of development. It is conceivable to infer that the proliferation of cells and tissues is maintained in a balanced equilibrium state. However, over time, cellular escape from naturally defensive responses can occur, potentially leading to the development of cancer [7]. As a result of this process, tumor cells function as a constant source of novel and modified proteins that are recognized as antigens by the adaptive immune system. The recognition of tumor antigens (TAs) by the human body's immune system can trigger an inflammatory response, characterized by the production of various cytokines, among other factors. Tumor cells will be completely removed if the immune response is adequate. If this does not occur, a prolonged equilibrium will be established where tumor progression and immune surveillance will dynamically balance each other. Due to the significant genetic instability of cancer cells that resist immune-mediated destruction, malignant cell clones eventually develop into clinically evident malignancies. Antitumor effector cells play a crucial role in cancer immunology. Tumor-infiltrating lymphocytes (TILs) primarily consist of T-cells, characterized by the presence of the cluster of differentiation 3 (CD3) surface protein. They infiltrate various tumor types and are considered one of the most important specific antitumor effector cells. Within the TIL population, two subgroups of T-cells can be identified: CD8 þ and CD4 þ T-cells [8].

The significance of tumor cell immunotherapy has largely been the notion that vaccination can initiate an immune response to cancer. Cancer vaccines (CVs) may differ in several crucial ways from more "conventional"

vaccinations used to prevent infectious diseases, although they share a similar concept. CVs are considered therapeutic rather than preventive, as they are intended to be administered after the onset or detection of the disease [9]. CVs can be designed to induce cell-mediated immunity, such as cytotoxic T cells, rather than humoral responses, since many tumor antigens may be intracellular proteins rather than cell surface molecules. Lastly, unlike foreign antigens, self-molecules could be the targets of CVs [10].

The past several decades has witnessed remarkable advancements in immunotherapeutics, which complement the available treatment options. In contrast to other therapeutic approaches, immunotherapy primarily aims to enhance the quality of life (QoL) for those affected by the disease. The technique of immunotherapy involves boosting or supplementing the immune system using a wide range of substances, including antibodies, vaccines, *in vitro*-stimulated effector cells of the immune system, and lymphokines [11].

CVs are classified into two main categories: preventive and therapeutic CVs. Therapeutic CVs are designed to activate the patient's adaptive immune system against specific tumor antigens (TAs), with the aim of halting the progression of existing tumors, slowing tumor growth, and eliminating any remaining disease. The key elements necessary for effective therapeutic vaccination against malignancies include the delivery of substantial amounts of high-quality antigens to dendritic cells (DCs), optimal stimulation of DCs, generation of strong and long-lasting CD4+ T helper cell and cytotoxic T lymphocyte (CTL) responses, infiltration of the tumor microenvironment (TME), and durability of the immune responses.

In the fight against cancer, a preventive CV, especially one that targets multiple types of cancer, would be a significant milestone. The potential to use immunotherapies for cancer treatment has been demonstrated in recent times, although progress in developing cancer vaccines is still in its early stages. Scientists in both academia and industry are exploring various approaches to using vaccines for targeting cancers, but only a small fraction of these approaches have entered clinical trials [12].

4.2 UNDERSTANDING OF THE IMMUNE SYSTEM

Dynamic interactions between organs, tissues, cells, and molecules that constitute the immune system are crucial for protecting against infectious diseases. The immune system is composed of three main host defenses and can be considered a stratified system. External barriers include ciliated

epithelia, mucous, epidermis, and biochemical barriers such as stomach acids and destructive enzymes in secretions. Innate and adaptive immune responses are the second and third lines of defense, respectively [13]. The term "immune system" refers to a group of cells and proteins that work together to defend the epidermis, nasopharynx, gastrointestinal system, and other regions against external antigens such as pathogens (toxins, viruses, bacteria, fungi, and parasites). Innate immunity and adaptive immunity are the two lines of defense of the immune system. Innate immunity is the primary defense against bacterial invasion [14].

4.2.1 INNATE IMMUNE SYSTEM

The protection provided by physiological and anatomical barriers is enhanced by innate immunity. The innate immune system focuses on a constrained set of receptors to recognize invasive infections, but it compensates for this lack of invariant receptors by focusing on conserved microbial elements that are shared by several pathogen subgroups. One distinguishing feature of the innate immune system is its speed; minutes after exposure to a pathogen, the innate immune system begins producing a protective inflammatory response. Furthermore, the ensuing adaptive immune response is greatly influenced by innate immunity [15]. Because of the extensive breadth of innate immunity, it can occasionally be intimidating to distinguish between the innate immune system and the rest of the host. This is partly because innate immune systems change during evolution. The selection forces imposed by microorganisms on the host population shape it and help it survive. On occasion, infections are killed by proteins that were co-opted to execute an activity unrelated to host protection [16]. The fundamental components of the innate immune system are, for the most part, established in the tree of life, which has existed for hundreds of millions (and in some cases, billions) of years. As a result, they are widely distributed. The innate immune system has been honed for a longer amount of time than the body's immune response and is more flawless in practically every regard, even though it is frequently thought of as "primitive" or "crude" in comparison to adaptive immunity. The fundamental components and roles of the immune system:

- The ability to identify a wide variety of diseases;
- Destroying these pathogens when they have been identified;
- Preserving host tissues (i.e., self-tolerance is required).

Physiological and anatomical barriers, intracellular internalization processes, and inflammatory reactions that are quickly triggered by the presence of an antigen are all included in innate immunity. Innate immune systems block pathogen entry, halt the spread of disease, and eliminate microbial and host waste. While some innate defenses are completely antigen-neutral, others utilize broadly specialized pattern recognition molecules (PRMs) that assist in the elimination of a specific subset of pathogens. Some PRMs are soluble molecules that mark pathogens for removal, while others are pattern recognition receptors (PRRs) secreted on the surfaces of effector cells. Innate immunity either successfully eradicates the pathogen or keeps the infection under control until delayed, lymphocyte-mediated adaptive immune responses can take effect [17]. Recent research has provided new insights into the cellular and molecular mechanisms that directly interact with inflammation and cancer.

Inflammation and cancer are connected through two mechanisms. The activation of several classes of oncogenes in the intrinsic pathway drives the release of inflammation-related proteins, which in turn create an inflammatory environment. Inflammatory conditions also promote cancer development through the extrinsic route, such as intestinal cancer associated with colitis. Transcriptional factors like NF-kB, Stat3, and HIF, as well as cytokines like TNF and chemokines, play crucial roles at the intersection of the extrinsic and intrinsic pathways. Consequently, inflammation is a critical aspect of the tumor's microenvironment and can be targeted with medications [18].

4.2.1.1 IMPORTANT CELLULAR ELEMENTS IN INNATE IMMUNITY

Innate immunity is composed of a variety of cell types that serve as the body's "first line of defense" against microbes. While the functions of each cell type are distinct, there is overlap in the cellular machinery, including the production of PRRs and the inflammatory response to the detection of PAMPs/DAMPs.

4.2.1.2 DENDRITIC CELLS (DCS)

Antigen-presenting cells (APCs) belonging to the DC family play a crucial role in the development of antigen-specific immunity and resistance. The Major Histocompatibility Complex (MHC), a co-stimulatory molecule necessary for T cell activation, and CCR7 expression are both upregulated

by the diverse array of PRRs that DCs express in response to the detection of PAMPs and DAMPs. The latter is a crucial chemokine receptor that instructs migration into tumor-draining lymph nodes (TDLN). As a component of the cancer immunity cycle, DCs in TDLNs present tumor-associated antigens and produce CD8+ T cells that are specific for those antigens [19]. Common myeloid progenitor cells are the source of DCs, which may develop into four distinct kinds of DCs: conventional type I DCs (cDC1s), conventional type 2 DCs (cDC2s), plasmacytoid DCs (pDCs), and monocyte-derived DCs (MoDCs). Due to their skilled antigen manufacturing and cross-presentation, cDC1s—which exhibit BATF3 and IRF8 expression—are the most potent inducers of cell-mediated immunity. Intra-tumoral CD4+ T cell frequency has been observed to be enhanced by DC2s, supporting CD8+ T cell activity [20]. When subjected to viral stimuli, pDCs, a rare subtype of DCs, may create interferons (IFN) and activate innate immunity, earning them the name interferon-producing cells (IPCs). In addition to directly controlling T cell activity, pDCs also have a limited ability to prime naive T cells in comparison to other DC subtypes. According to a more recent study, pDCs may have a pro-tumor function because their infiltration into tumors is a bad predictor of prognosis in many malignancies. MoDCs have been found to help maintain immunity after chemo- or radiotherapy-induced cell death, albeit their function in anti-tumor immunity is less apparent [21].

4.2.1.3 MACROPHAGES

Macrophages, known as myeloid cells, are present in healthy tissues throughout the body and play a crucial role in coordinating innate immune responses and maintaining tissue homeostasis. TAMs (tumor-associated macrophages) exist on a spectrum of polarization states, ranging from protumorigenic M2 macrophages to antitumorigenic M1 macrophages, which correspond to distinct gene expression programs. In many cancer types, macrophages are directed towards an M2 functional program that promotes tumor development. M2 TAMs induce local immunosuppression through various mechanisms, including the synthesis of IL-10 and TGF-β, extracellular arginine depletion, enrichment of regulatory T cells, and regulation of T cell proliferation [22]. Hypoxia and inhibitory cytokines like IL-4, IL-10, and IL-13 drive the differentiation of monocyte precursors into M2 macrophages. On the other hand, antitumorigenic M1 macrophages contribute to tumor control through processes such as phagocytosis and the

release of pro-inflammatory cytokines (PICs) like IFN-α, IFN-β, and IFN-γ. Recent studies using single-cell RNA sequencing have identified multiple subclusters of TAMs in solid tumors, suggesting that macrophage function exists along a continuum of states and cannot be simply categorized [23]. For example, research on pancreatic cancer has revealed five subgroups of TAMs, each with unique gene expression patterns that correspond to different immunosuppressive or immune-stimulatory behaviors. Histological analysis of clinical specimens has shown that tumor-associated macrophages (TAMs) are associated with worse overall survival (OS). Depletion of TAMs has been shown to enhance the effectiveness of radiation, chemotherapy, and immune checkpoint inhibitors (ICI) in preclinical animal models [24].

4.2.1.4 NEUTROPHILS

The innate immune system's reaction to bacterial infection is carried out by neutrophils, which are circulating myeloid cells. Numerous solid tumors have been found to include large amounts of neutrophils in the immune infiltrate and increases in both tumor-associated neutrophils (TANs), as well as peripheral blood neutrophils, are linked and associated with decreased outcomes [25]. The pro-neoangiogenesis, pro-tumor migration and invasion, and pro-local immunosuppression pathways of TANs are among their tumorigenic properties. Contrarily, TANs have been found to have antitumorigenic effects through direct cytotoxicity of tumor cells, the formation of reactive oxygen species, and the release of PIC [26]. Due to the functional flexibility of TANs, protumorigenic N2 TANs and antitumorigenic N1 TANs have been classified in a bipolar manner, similar to TAMs. According to research, early tumor formation is governed by N1 TANs. TGF-, IL-10, and IL-6 signaling, however, encourage the differentiation of tumorigenic N2 TANs over time. Despite recent developments, the tumor immunobiology of TANs is still substantially a subject of current investigation [22].

4.2.1.5 MYELOID-DERIVED SUPPRESSOR CELLS (MDSCS)

A subset of myeloid cells known as MDSCs is distinguished from other types of myeloid cells by their predominantly immunosuppressive characteristics. The differentiation of myeloid cells into the MDSC phenotype is linked to chronic inflammation and brief exposure to tissue factors and inflammatory mediators necessary for proper development. MDSCs consist of two morphological subgroups, namely Mo-MDSC and Polymorphonuclear,

which are mononuclear or polymorphonuclear [27]. Compared to conventional neutrophils and monocytes, MDSCs express higher levels of immunosuppressive chemicals such as nitric oxide (NO), IL-10, and arginase-1, while also exhibiting poorer phagocytic capacities. MDSCs have been found to inhibit both the innate and adaptive immune systems in various types of solid tumors, contributing to a "cold" tumor microenvironment (TME). The infiltration of MDSCs into the tumor site is associated with a poorer prognosis, higher cancer stage, and increased tumor load. These characteristics underscore the significance of MDSCs in therapeutic strategies aimed at combating an immunosuppressive TME [22].

4.2.1.6 MAST CELLS

Mast cells, which are granulated innate immune cells residing in peripheral tissues, play a crucial role in muscle repair and local immunological responses by releasing various signaling chemicals. The presence of mast cells in the tumor stroma of different solid malignancies has been associated with a poor prognosis. However, in breast cancer, the presence of mast cells has been identified as a favorable predictive marker [28]. The precise location of mast cells within the tumor microenvironment (TME) may potentially impact tumor growth. Mast cell-secreted molecules, such as VEGF and matrix metalloproteases, have been found to enhance tumor development and metastatic potential by promoting angiogenesis, lymph angiogenesis, and altering the extracellular matrix (ECM). Mast cell tryptase, acting as an agonist for proteinase-activated receptor-2 (PAR-2), promotes endothelial cell proliferation and has been linked to tumor cell migration. Tumor-associated mast cells exhibit anticancer properties, including mediating tumor cell death through inflammatory cytokines and the peroxidase system. Ongoing studies aim to fully comprehend the mechanisms by which mast cells operate in the tumor microenvironment [22].

4.2.1.7 NATURAL KILLER (NK) CELLS

Innate lymphocytes known as natural killer (NK) cells have a shorter half-life compared to B and T lymphocytes and require continuous replenishment from bone marrow (BM) progenitors. NK cells undergo a linear differentiation process, transitioning from highly proliferating, functionally inferior, immature cells to a community of potent big granular effectors, and eventually reaching a non-neuronal, hypo-functional state. This process, which spans

several weeks, ensures a constant supply of circulating cytotoxic and inflammatory antitumor lymphocytes that are poised for spontaneous activation upon stimulation. Many characteristics of NK cells resemble those of CD8+ T cells, which are the target of immunological checkpoint blockade (ICB) therapies that are currently revolutionizing clinical cancer treatment [29].

4.2.2 ADAPTIVE IMMUNE SYSTEM

The adaptive immune system, often referred to as the acquired immune system, is a component of the immune system composed of systemic, specialized cells, and processes that destroy or suppress infections. Adaptive defenses have evolved to provide a broader and more precise repertoire of self- and non-self-antigen recognition. Adaptive immunity involves a well-controlled interaction between T and B lymphocytes and APC, which promote the development of immunologic effectors specific to pathogen pathways, immunologic memory formation, and the regulation of a healthy host immune system. The acquired immune system is one of the two fundamental types of immunity found in vertebrates [30]. After an initial response to a particular pathogen, adaptive immunity develops immunological memory, resulting in an enhanced response upon subsequent encounters with the same pathogen. Antibodies are a critical component of the adaptive immune system [31]. Adaptive immunity can provide long-lasting protection, potentially lasting a person's entire lifespan. However, in certain cases, such as with chickenpox, it does not confer permanent protection. For example, a person who survives measles is now protected from measles for the remainder of their life. This mechanism of adaptive immunity forms the basis of vaccination [32]. The lymphatic system consists of various lymphoid organs where lymphocytes mature and become activated. During development, genomic sequences are rearranged and assembled to generate the genes that encode the distinct antigen receptors of B and T cells. This rearrangement process generates an incredibly diverse array of receptor specificities capable of recognizing all possible components of pathogens. In addition to specificity, the development of immunologic memory is a key characteristic of adaptive immunity.

4.2.2.1 CELLULAR IMMUNITY AND T-CELLS

4.2.2.1.1 T-Cell Development

BM precursors give rise to T lymphocytes, which are transported to the thymus for maturation, selection, and export to the periphery. The peripheral

T cell subsets include regulatory T (Treg) cells, which regulate immune responses, memory T cells, which develop from previously activated antigens and provide long-term immunity, and naïve T cells, which can respond to new antigens [33]. When naïve T cells encounter a pathogen and receive costimulatory signals from DCs, interleukin 2 (IL-2) is produced. Effector cells proliferate, differentiate, and migrate to various locations to assist in pathogen clearance by generating effector cytokines and cytotoxic mediators. Activated effector cells have a limited lifespan, but some differentiate into memory T cells that can migrate, localize to different tissues, and undergo self-renewal [34]. Although their lineage and ancestry are still unclear, memory subsets contribute to long-term immunization and protective responses. Common lymphoid progenitors from the fetal liver or BM give rise to T cells in the thymus. Platelet selectin glycoprotein 1 in progenitors and the integrin P-selectin on thymic epithelium interact to promote thymus seeding. Various cytokine receptors, including IL-2, IL-4, IL-9, IL-15, and IL-21, share the common γ chain, which is encoded on the X chromosome. Under the influence of IL-7, newly arriving cells rapidly expand [35]. Mutations in this polypeptide cause X-linked SCID disorder, characterized by a lack of T lymphocytes. This early thymocyte proliferation is accompanied by the stimulation of Notch-1, as well as other transcriptional factors that bind cofactors to all T-cell lineages and stimulate the expression of genes crucial in T-cell receptor (TCR) development. A coordinated set of genomic rearrangements results in the production of functioning genes that encode the a and b or g and d domains of the TCR during the subsequent maturation of the increased reservoir of T-cell precursors or pro-T lymphocytes in the thymus. This process is antigen-independent [36]. If gene-segment rearrangements result in a gene generating a full-length TCR polypeptide without introducing stop codons, they are referred to as productive sequentially effective rearrangements resulting in the expression levels of ab or gd from two TCR genes. Pre-T cells change into double-positive T cells, which exhibit both CD4 and CD8, at the TCR. At the cell surface, the TCR chain is put together dynamically with the proteins that comprise CD3, including g, d, e, and z chains. Both negative and positive selection incidents surrounding antigens and MHC molecules control the further maturation of these double-positive lymphocytes, which are situated in the thymic cortex, into single-positive T lymphocytes, which are located in the medulla. When self-MHC (complexed to self-peptides) on the thymic epithelium is bound by the TCR of the double T cells with low avidity, positive selection takes place. The TCR-positive double-positive cells that do not bind to self-MHC are destroyed [37]. The

T-cell receptor (TCR) of double-positive T cells, on the other hand, binds to the self-MHC peptide with a very high affinity, preventing the maturation of autoreactive T-cell progenitors (central tolerance). The activity of the autoimmune regulator (AIRE) gene, which promotes the expression of genes with broad tissue specificity in the thymic epithelium, facilitates the elimination of T-cell clones interacting with peptides ordinarily produced in distant organs [38]. Dysfunction of this gene is directly responsible for certain self-reactive T cells escaping and can result in autoimmune polyendocrine disorders. By further interacting with lymphoid cells' epithelial MHC class I proteins, double-positive thymocytes that successfully navigate both positive and negative selection grow into CD81 single-positive T cells, while those selected on MHC class II develop a CD41 single-positive morphology [39].

4.2.2.1.2 T-CELL ACTIVATION

The concurrent interaction of the T-cell binding site and a co-stimulatory protein, such as CD28 or ICOS, mostly on T cells by the complex of major histocompatibility (MHCII) polypeptide and co-stimulatory proteins on the APC, results in the activation of CD4+ T cells. In the absence of co-stimulation, the T cell receptor activity alone causes anergy; both are necessary for the generation of an efficient immune response [40]. The PI3K pathway, which generates PIP3 at the plasma membrane and recruits PH domain-containing signaling molecules like PDK1 that are necessary for the activation of PKC and eventual synthesis of IL-2, is a typical signaling pathway engaged downstream from co-stimulatory molecules. CD4+ signaling is necessary for the optimal CD8+ T cell response. CD4+ cells help maintain the memory of CD8+ T lymphocytes after an acute infection and in the first antigenic stimulation of naïve CD8 T cells [41]. As a result, CD4+ T cell activation may enhance CD8+ T cell activity. The T cell receptor's interaction with its relevant peptide displayed on MHCII on an APC generates the initial signal. Nerve cells, B lymphocytes, and macrophages, to name a few, are examples of professional APCs that express MHCII. The peptides presented to CD4+ cells by MHC class II molecules are typically longer, ranging from 12 to 25 polypeptides in length. This is because the binding sites of MHC class II molecules are open [42]. On the other hand, the peptides presented to CD8+ T-cells by MHC class I molecules are shorter, around 8 to 13 peptides in length. The second signal for T-cell activation is provided through co-stimulation, where a small number of

stimuli, mostly pathogen products but sometimes cell breakdown products like necrotic aggregates or heat shock proteins (HREs), activate surface proteins on the antigen-presenting cells (APCs). Naive T-cells constitutively express the co-stimulatory receptor CD28, and co-stimulation is provided by the APC's CD80 and CD86 proteins, collectively known as the B7 protein (B7.1 and B7.2, respectively). OX40 and ICOS are additional receptors that are expressed upon T-cell activation, but their expression heavily relies on CD28. The second signal, provided by co-stimulation, authorizes the T-cell to respond to an antigen. Without this signal, the T-cell becomes anergic and finds it more difficult to activate in the future. Self-peptides are typically not accompanied by sufficient co-stimulation, which helps prevent inappropriate responses to self-antigens [43]. Upon proper activation (i.e., after receiving signals one and two), a T-cell undergoes changes in the expression of many proteins on its cell surface. HLA-DR, CD69, CD71, CD25 (also a marker for Treg cells), and CD25 are all markers of T-cell activation. Additionally, activated T-cells produce more CTLA-4, which competes with CD28 for binding to the B7 molecules. This serves as a checkpoint mechanism to prevent excessive T-cell activation. Multiple proteins constitute the complex that forms the T cell receptor [44]. The T cell receptor consists of distinct alpha and beta genes, which create two distinct peptide chains. The complex also includes CD3 proteins, such as CD3 homodimer, CD3 homodimer with six ITAM motifs, and CD3 heterodimer. Lck phosphorylates the ITAM patterns on CD3, attracting ZAP-70. Other molecules like CD28, LAT, and SLP-76 can also have their tyrosine residues phosphorylated by Lck and/or ZAP-70. This enables the formation of signaling complexes involving these proteins. When LAT is phosphorylated, SLP-76 is recruited to the membrane, where it can subsequently recruit PLC-, VAV1, Itk, and perhaps PI3K. On the innermost layer of the membrane, PLC-breaks down PI [4, 5] P2 to produce the active mediator's diacylglycerol (DAG) and inositol-1,4,5-trisphosphate (IP3). Additionally, PI3K phosphorylates PIP2 to generate phosphatidylinositol-3,4,5-triphosphate (PIP3). Some PKCs are bound and activated by DAG. PKC-, which is crucial for triggering the transcription factors NF-B and AP-1, is particularly significant in T cells. When PLC-releases IP3 from the membrane, it diffuses quickly and activates calcium channel sensors on the ER, causing calcium to be released into the cytosol. Low calcium levels in the rough ER enable STIM1 to cluster on the ER membrane and activate CRAC channels on the cellular membrane, allowing more calcium from the external environment to enter the cytosol. When calmodulin binds to the aggregated cytosolic calcium, calcineurin can

be activated. NFAT is then activated by calcineurin and binds to the nucleus. IL-2, a cytokine that promotes the long-term proliferation of activated T cells, is one of several genes whose transcription is induced by the transcription factor NFAT. Additionally, PLC-B can initiate the NF-B pathway [45]. PKC is triggered by DAG, which phosphorylates CARMA1, causing it to unfold and serve as a scaffold. Through the CARD domains, the cytosolic domain contacts the adaptor BCL10, which subsequently binds TRAF6, leading to ubiquitination at K63. Target proteins are not degraded by this type of ubiquitination. Instead, it recruits NEMO, IKK, and TAB1-2, and TAK1 to enable K48 ubiquitination, resulting in proteasomal degradation. TAK1 phosphorylates IKK-, IKK-, and IB. Rel A and p50 can then bind to the NF-B response element inside the nucleus. This fully activates the IL-2 gene when combined with NFAT signaling. While TCR recognition of the antigen is typically required for activation, other activation pathways have been reported. For instance, it has been demonstrated that Cd8 can stimulate cytotoxic T cells, leading to their tolerance. Reactive oxygen species regulate the activation of T cells [46].

4.2.2.1.3 Subsets of T-Cell Effectors

Despite the fundamental ideas guiding thymic evolution, most T cells follow the same activation pathways. However, there is a surprising variety of effector actions induced in response to activated T cells. These actions can directly contribute to the eradication of infected target cells and pathogens. T cells can also serve as helper cells, providing cognate or cytokine signals to enhance both T-cell and B-cell responses by triggering mononuclear phagocyte activation. T cells play a crucial role in controlling immunological responses, preventing tissue damage caused by highly inflammatory or autoreactive immune reactions. The CD41 ab TCR population constitutes the majority of activating T cells in the body [38]. These cells are classified as T-H cells, as the majority of them have a helper function. During activation, TH cells produce a variety of cytokines. Immunologists Robert Coffman and Tim Mossman discovered about 20 years ago that not every CD41 TH cell has the potential to produce the entire spectrum of cytokines known to be part of the T-cell repertoire. Instead, they showed that there are two primary types of TH cells, TH1 and TH2 cells, each of which mainly produces distinct panels of cytokines. This was demonstrated through the analysis of T-cell clones [35]. The CD41 ab TCR fraction of lymphocytes

also plays a crucial role in controlling T-cell responses, and it is likely that numerous regulatory cell types are involved in this process. It has been demonstrated that IL-10-producing regulatory T (TR1) lymphocytes, along with naturally occurring and induced CD251 CD41 T cells expressing the transcriptional forkhead box protein 3, suppress T-cell reactions. A severe multisystem proinflammatory illness results from the lack of forkhead box protein 3, which is encoded on the X chromosome. Recent literature has conducted a comprehensive evaluation of the intricacy of the T-Lymphocyte system [34]. NKT cells, a distinct subtype of T cells, recognize nonpeptide pathogens presented by non-classical MHC class of the CD1 family, similar to gd T cells. NKT cells are characterized by the simultaneous expression of NK cell antigens and T-cell (CD3, TCRab) antigens (CD56). The majority of NKT cells are classified as "invariant NKT cells" because they exhibit a single distinct TCRa arrangement, Va24-Ja18 with Vb11. Activated NKT cells have been associated with the etiology of allergies and are capable of rapidly producing cytokines, such as IL-4, in large quantities. The exploration of natural and pathogen-derived molecules that may promote NKT growth and activation is currently a highly researched topic [47].

4.2.2.2 HUMORAL IMMUNITY AND B-CELLS

4.2.2.2.1 B-Cell Growth and Development

Humoral immunity, also known as antibody-mediated immunity, is mediated by B lymphocytes. When a B cell encounters an antigen, it undergoes transformation into a specific cell that secretes antibodies. These antibodies, produced by plasma cells, closely resemble the receptors on progenitor B cells. Once released into the lymphatic system and blood, the antibodies attach to the target epitope and initiate neutralization or destruction. Antibody production can last for days or even months until the antigen is neutralized [48].

Memory B cells, distinct from other B cells, do not develop into plasma cells but play a crucial role in providing long-lasting immune memory. B-cell receptors (BCRs) are expressed on the cell membrane of B cells, distinguishing them from T lymphocytes and NK cells, the other two groups of lymphocytes. The BCRs enable B cells to recognize unfamiliar antigens and mount an antibody response. B cells originate from the bone marrow at the immature stage and undergo development to reach the mature or naïve stage [49]. The presence of IgD, in addition to IgM, on the surface of the cell indicates this. There is no interaction with any exogenous antigen during the

entire development process. Therefore, it is referred to as B-cell development independent of antigen. Any genetic changes that disrupt the structural aspects of the pre-B cell receptor or the signaling pathways connected to it result in immunodeficiency with agammaglobulinemia, as well as a lack of B cells [50]. Immunoglobulin genes are assembled in a similar manner to TCR genes, with light chains being assembled from three segments and heavy chains being assembled from four segments (VH, D, JH, and CH). The k and l genes are located on chromosomes 2 and 22, respectively, while the heavy chain sequences are located on chromosome 14. The chapter "Structure and Function of Immunoglobulins" provides a detailed examination of immunoglobulin structure [51].

4.2.2.2.2 Activation of B-Cells

After activation and exposure to an antigen, the second stage of B-cell development, known as the antigen-dependent phase, occurs. The activated cell will either develop into a memory cell, which can be triggered again in the future, or it will develop into a plasma cell that generates enormous amounts of antibodies, depending on the numerous interactions and cytokine stimuli it receives.

1. **T-Cell Dependent Activation:** Foreign proteins are examples of T cell-dependent (TD) antigens, to which B cells respond and become activated. They are called TD antigens because they cannot elicit a host response in organisms lacking T cells. B cell reactions to these antigens take several days, but the antibodies produced have a higher affinity and greater functional versatility compared to those produced through T cell-independent (TI) activation. Once a B cell receptor (BCR) binds to a TD antigen, the antigen is internalized through receptor-mediated endocytosis, destroyed, and presented to T cells as peptide fragments bound to MHC class II molecules on the cell membrane. T helper (TH) cells, particularly follicle T helper (TFH) cells, recognize and bind to these MHC-II-peptide complexes through their T cell receptor (TCR). T cells also express the membrane protein CD40L, as well as cytokines such as IL-4 and IL-21, following TCR-MHC-II-peptide interaction. CD40L, by binding to the B cell surface receptor CD40, acts as a crucial co-stimulatory component for B cell activation. It promotes B cell proliferation, class switching to IgG, biological hypermutation, and also plays a role in T cell development and differentiation [53]. T

cell-derived cytokines not only promote B cell differentiation, but also induce immunoglobulin class switching, stimulate somatic hypermutation, and enhance B cell proliferation. These signals are believed to activate B cells. After activation, B cells undergo a two-step process that results in the production of short-lived plasmablasts for immediate protection, as well as the generation of long-lived cells and memory B cells for sustained protection. The extrafollicular response, which is the initial stage, takes place outside of lymphoid follicles but within the secondary lymphoid organs (SLO). Activated B cells undergo proliferation, potential class switching of immunoglobulins, and differentiate into plasmablasts. These plasmablasts primarily produce early, low-affinity antibodies of the IgM class. In the second stage, activated B cells enter a lymphoid follicle and form the germinal center (GC), a specialized microenvironment where B cells undergo extensive proliferation, class switching of antibodies, and affinity maturation through somatic hypermutation. TFH cells within the GC provide crucial support for these processes. This results in the generation of long-lived plasma cells and high-affinity memory B cells. Plasma cells secrete significant quantities of antibodies, which can either remain in the SLO or selectively migrate to the bone marrow [40].

2. **T-Cell Independent Activation:** Foreign polysaccharides and unmethylated CpG DNA are examples of TI antigens, which activate B cells without the assistance of T cells. These antigens can induce an antibody response in individuals lacking T cells, hence the given name. Although the B cell response to these pathogens is rapid, the antibodies produced tend to have lower affinity and less functional diversity compared to those generated by TD activation. Similar to TD antigens, TI antigens also require additional signals from B cells for full activation. However, these signals are not delivered by T cells, but rather through the binding and recognition of prevalent microbial components to toll-like receptor sites (TLRs), or by strong binding of BCRs to specific epitopes on bacterial cells [55]. Following activation by TI antigens, B cells may undergo antibody class switching, proliferate in external lymphatic tissue follicles while remaining within SLOs, and differentiate into short-lived plasmablasts that produce early, weak antibodies, primarily of the IgM class, but also a small population of long-lived plasma cells [56].

3. **Memory B-Cell Activation:** The target antigens, which are also recognized and bound by the parent B cell, trigger the activation of memory B cells. While certain memory B cells, such as those that are virus-specific, may be triggered without the aid of T cells, others require it. Upon primary antibody, the memory B cell internalizes the antigen via receptor-mediated endocytosis, breaks it down, and then presents the fragments to T cells on the cell membrane in association with MHC-II molecules. These MHC-II-peptide complexes are recognized and bound by memory T helper (TH) cells, typically memory follicle T helper (TFH) cells, which have been generated from T cells stimulated with the same antigen [57]. The memory B cell is activated following TCR-MHC-II-peptide binding as well as the relay of additional signals from the memory TFH cell, and then undergoes differentiation into either plasmablasts or plasma cells through an extrafollicular reaction, or into plasma cells and more memory B cells via a GC reaction. It is unknown if memory B cells continue to develop their affinities within certain additional GCs. Memory B cells can be stimulated *in vitro* using a variety of activators, such as monoclonal antibodies (mAbs); however, researchers have discovered that the most effective activator is a combination of R-848 with recombinant human IL-2 [58].

4.3 CANCER IMMUNOTHERAPY AND CANCER VACCINES (CVS)

The remarkable success of immuno-oncology therapies in stimulating the innate autoimmune process towards tumor recurrence has rendered them effective therapeutic approaches. In addition to novel immunotherapies currently undergoing clinical and experimental investigations, the Food and Drug Administration (FDA) of the USA has approved a significant number of successful immunotherapies (Table 4.1), evaluated through various parameters of vaccine development and drug toxicokinetics, which have been utilized in clinical settings over the past decade (Figure 4.1). Promising cancer immunotherapies that have the potential to elicit potent antitumor immunity with minimal off-target effects in clinical scenarios include immune checkpoint blockades (ICB), chimeric antigen receptor T cells (CAR-T), cancer vaccines (CVs), and cytokine treatments. CVs, for instance, exemplify immunotherapies that can initiate tumor-specific immune responses by introducing antigens to lymph nodes (LN) containing antigen-presenting cells (APCs). All of these vaccines have demonstrated highly effective anti-tumor immunity, as

well as long-term immunological memory that inhibits tumor development, recurrence, or metastasis to other organs. Furthermore, CVs often incorporate immunologic adjuvants that enhance the activity of APCs and augment the body's natural anti-tumor immune responses. However, due to the inefficiency of the anti-CV cascade, the clinical translation of these vaccines remains challenging. The specific recognition of antigens, co-encapsulation of the antigen and adjuvant, LN-targeted antigen delivery, internalization, and transfer of the antigen, and cross-presentation of the antigen to T cells constitute the core five components of the cascade [59].

TABLE 4.1 List of Approved Cancer Vaccines and its Target

Vaccine Market Name	Vaccine Constitution	Country of Manufacture	Target Type of Cancer	Type of Vaccine	References
HybriCell	Dendritic cell	Brazil	Renal cell carcinoma; melanoma.	Therapeutic vaccine	[98]
CIMAVax EGF®	Protein	Cuba	Lung cancer	Therapeutic vaccine	[99]
M-Vax™	Tumor cell	USA	Melanoma	Therapeutic vaccine	[100]
DCVax®-Brain	Dendritic cell		Glioblastoma	Preventive vaccine	[54]
Oncophage®	Peptide		Renal cell carcinoma	Preventive vaccine	[94]

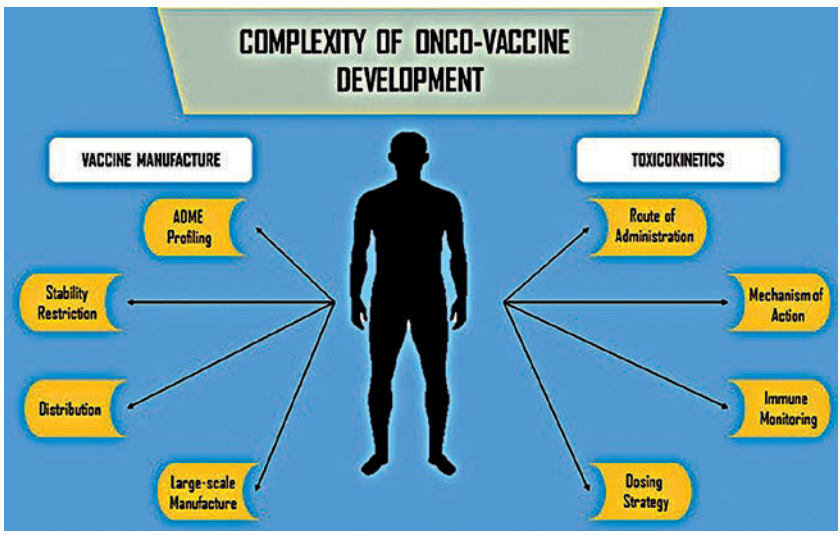

FIGURE 4.1 Complexity of onco-vaccine development.

Due to remarkable progress in cancer detection and knowledge, conventional treatments have largely remained unchanged. Unfortunately, there are instances where conventional medicines, such as surgery, chemotherapy, and radiotherapy, prove to be ineffective, resulting in the spread or recurrence of cancer cells. Recent studies indicate the need for a strategy like immunotherapy. Cancer immunotherapy has emerged as a key element in cancer treatment. One type of therapy, immuno-oncology, enhances the body's natural immune response to cancer by generating highly aggressive T lymphocytes that target and eliminate tumors. The most commonly used medications in cancer immunotherapy, immune checkpoint inhibitors, often have response rates of less than 30% in patients. Therefore, it is crucial to improve existing cancer immunotherapies [60].

4.3.1 CANCER VACCINES (CVS) THAT INFLUENCE THE PRODUCTION OF COMMON ANTIBODIES

The selection of the target antigen is arguably the most crucial factor in the development of a vaccine. In contrast to non-specific vaccinations, which use tumor lysate, the majority of studied vaccinations are specifically designed to stimulate T cell reactions, rather than targeting common TAs or antigens produced by cancerous cells and non-vital surrounding tissues. Typically, malignant cells activate these shared antigens, while healthy tissue or early embryogenesis express them in lower quantities. Examples include antigens commonly expressed during embryogenesis, antigens associated with the testes, and antigens that differentiate melanoma. As these proteins produce unaltered self-antigens, high avidity T cells that recognize them are likely eliminated during development due to the thymus's detection of the antigen. The limited pool consists of naive T cells that can respond to the vaccination. Targeting such antigens puts the specific vaccination system at an immediate disadvantage [61].

Therapeutic research, however, has demonstrated the feasibility of activating T cells in response to related antigens. Previous studies on patients with melanoma have shown that immunization with gp100, a common practice, only slightly increased the strength of gp100-reactive T-cell responses. On the other hand, modifying the peptide's immunogenicity through anchor residues significantly enhanced its binding affinity to MHC-I. This approach was employed in clinical research, where a modified gp100 peptide vaccination was administered with IL-2. As a result, gp100-reactive T lymphocytes were detected in the peripheral blood of melanoma patients [62].

Furthermore, in a phase III study investigating the same strategy, individuals who received the vaccination in combination with IL-2 showed significantly greater therapeutic responses compared to those who received IL-2 alone. These individuals also experienced a somewhat longer progression-free survival, with a median of 2.2 months compared to 1.6 months for the IL-2 alone group. It is worth noting that the use of vaccinations in cancer treatment extends beyond peptide vaccines [63]. DCs have been utilized as a vaccine to activate T cell responses against tumors. Early research conducted by Banchereau et al. demonstrated that DCs generated from CD34+ progenitors can elicit measurable T-cell responses to various antigens when loaded with peptides from Melanie/MART-1, MAGE-3, gp100, and tyrosinase [64]. Building upon this research, Palucka et al. showed that allogeneic DCs stimulated by monocyte-derived tumor lysis of cells can induce T cell reactions against common malignant cells, leading to partial or full recovery in some patients among a population of 20 immunized individuals [65]. Larger-scale clinical studies are currently underway to assess the overall therapeutic efficacy of this vaccination approach in treating various malignancies [66].

4.3.2 NEOANTIGEN-TARGETING ANTIBODIES

Neoantigens are a distinct category of tumor-associated antigens that have been the focus of recent research. The "non-self" peptides in this type of antigen are generated through alterations in the tumor genome that are not synonymous. Typically, these alterations are specific to the patient's tumor, making them a key aspect of personalized medicine. However, understanding "shared" neoantigens, which are multiple mutant epitopes observed in a patient, is crucial for the practicality of neoantigen vaccines. Several studies have linked the clinical effectiveness of checkpoint blockade and various types of adoptive cell treatment to neoantigens. Tumor cells are the sole producers of neoantigens, which exhibit a strong resistance to T lymphocytes that recognize the thymus as their most likely origin, evading deletion. Consequently, vaccines targeting these antigens overcome a significant barrier that hinders vaccines against shared antigens. Vaccines targeting neoantigens not only provide protection against them but also reduce off-tumor, on-target autoimmunity due to the tumor-specific expression of these antigens [67].

Targeting immunotherapies to neoantigens is effective in significant mouse model research. Castle et al. demonstrated the efficacy of a specific

peptide immunization against mutant regions such as Actn4 and Kif18b, which inhibited the growth of B16 tumors in a highly immunosuppressive mouse melanoma model. However, the mice could not be cured by immunization alone. Yadav et al. also showed that immunization with a peptide targeting mutants like Reps1, Adpgk, and Dpagt1 inhibited tumor development in a mouse colon cancer MC38 model [68]. This study found that inhibitory receptors PD-1 and Tim3 were highly expressed by neoantigen-specific T lymphocyte cells within the tumor, indicating T cell dysfunction. The expression of tumor receptors was decreased, although this may have been caused by the study's adjuvant. Duan et al. demonstrated that neoepitope vaccination targeting Tnpo3 resulted in tumor shrinkage in a preventive scenario using a novel method to identify immunogenic neoantigens and combined treatment [69]. The response to neoantigen immunization was enhanced by anti-CD25 and anti-CTLA4 antibodies. Preclinical studies are increasingly focused on precisely determining whether certain tumors include immunogenic neoantigens [70].

Neoantigen-specific vaccinations have been previously tested on several human subjects. Carreno et al. immunized individuals with three malignant tumors using neoantigen peptide-containing DCs. They demonstrated that this approach increased the quantity and range of neoantigen-specific T lymphocytes [71]. Two groups have more recently attempted this strategy with RNA and peptide vaccinations. Ott et al. administered 13–20 lengthy polypeptides with tumor-specific mutations that were predicted to bind human leukocyte antigen (HLA-A or HLA-B) to six high-risk melanoma patients. Contrary to expectations, CD4+ T cells predominated among the vaccine-stimulated T cells instead of the predicted CD8-positive T cells [72]. However, none of the six patients showed any signs of tumor recurrence 25 months later. Pembrolizumab was administered to two patients who still had the illness, resulting in full recoveries. Sahin et al. administered a cocktail of synthetic RNAs with anticipated neoantigens to patients with stage III and stage IV melanoma [73]. Unlike the study by Ott et al. [72], this investigation focused on selecting mutated sequences that could interact with both HLA class II molecules and HLA class I molecules. Both studies demonstrated that CD4+ T cells were preferentially activated in response to vaccination. Additionally, two patients showed clinically significant responses to immunization. One patient demonstrated disease progression after receiving the immunization. However, following pembrolizumab therapy, they experienced complete clinical recovery. This study provides compelling evidence that tumor-specific vaccinations do indeed elicit patient

immunity. The integration of immunization with the potential for immunotherapy significantly enhances the efficacy of the medication. Furthermore, several clinical trials are currently assessing the safety and feasibility of administering neoantigen vaccines, such as NCT02287428, NCT02510950, NCT02427581, and NCT02348320, to patients with glioblastoma or breast cancer who are concurrently undergoing chemotherapy or radiation therapy. These immunization efforts target homologous antigens for the respective antigen category treatment, potentially altering the benefits of immunogenicity prediction algorithms as they become more precise [74].

The perfect vaccine formulation is still unknown, even though finding the best therapeutic approaches is a top priority for researchers. Several organizations are currently investigating different techniques such as microbial vectors, recombinant peptides, or nucleotide vaccinations, each with varying degrees of success. However, due to the unique nature of antigens in each specific tumor type, it is challenging to directly compare different vaccine platforms. The multinational Human Immunome Project aims to bridge this knowledge gap by evaluating various delivery strategies targeting the same antigen, in order to determine the most effective immunogenic vaccination systems for cancer patients. The results of such trials have the potential to significantly enhance the therapeutic potential of cancer vaccines. Additionally, the Human Immunome Project also aims to define the innate immune capabilities in cancer patients. Early research has shown that, compared to cells from healthy individuals, developing cancer patients have fewer T cell stimulators in their DCs. A more recent study revealed that melanoma patients have higher concentrations of MDSCs and an immune-suppressive DC fraction that is BDCA1+CD14+, which inhibits T cell activation through an antigen-based system. These investigations shed light on the widespread immunological dysfunction experienced by cancer patients. Further characterization of this immunological dysfunction may lead to improvements in vaccination responses within the patient population [75].

4.3.3 DCS AS A COMPONENT OF A CANCER VACCINATION

DCs are indeed the main cell types that the aforementioned vaccinations target, as they play a crucial role in initiating the immune response against tumors. Therefore, the “immunization” of DCs is much safer than administering vaccinations, even if only a small number of vaccinations come into contact with DCs. The DC vaccines undergo this treatment and the distinct

DCs from a patient are isolated to create them. TAAs are added after the cells have been co-cultured with antigens such as proteins, peptides, mRNA, and DNA, which have reached maturity [75]. These adjuvants include TLR agonists or cytokines. After treatment, the patient receives the DCs again, which are then transferred to the LN, where the CD8 T cells are activated to initiate the anti-tumor immune response. The fundamental advantage of DC vaccinations is that, unlike traditional vaccines that require immediate delivery of vaccine components inside patients, off-target effects are much less of a concern because DCs are treated *in vitro*. The difficulty and high cost of producing cell procedures, as well as the batch-to-batch variation across vaccinations for different patients, pose significant obstacles to the standardized production of DC vaccines [76]. Although it has been approved by the FDA, its commercialization has only been achieved since Sipulencel T, the first DC-based CV, was created in 2010 to treat metastatic prostate cancer in a limited number of developed nations. This is mainly due to the expensive and stringent production requirements of vaccine factories. Comparing clinical studies and extrapolating their findings is challenging due to the wide variations in manufacturing processes in DC vaccines and the resulting composition. Silence T has been successful, and phase I and II clinical studies are producing encouraging preliminary results, although there have also been some significant failures. The dismal brief patients' condition is assessed, whereas encouraging findings from past studies have forced Argos Therapeutics to halt a DC vaccine for renal cell carcinoma that is now in the third phase of clinical trials. Similar findings from a phase III, DC melanoma vaccination clinical study revealed the drug's absence of an appreciable effect on patient mortality or recovery-related indicators. However, it is possible that the challenging procedure for acquiring, growing, and treating DCs [77, 78].

4.3.3.1 DCS' ROLE IN PROMOTING ANTI-TUMOR IMMUNITY

Multiple diverse subsets, including four main populations, make up DCs. These subgroups are most frequently categorized by ontogeny. These include MoDCs, plasmacytoid DCs, and conventional or traditional DCs, which were divided into types 1 and 2 (MoDC). The propensity for antigen presentation, migration, and cytokine release varies across each group. Therefore, choosing the appropriate DC vaccine development depends on the cell type [79].

4.3.3.2 DCS ARE PRODUCED FROM MONOCYTES

APCs, also known as MoDCs, are a diverse population that primarily develops in response to inflammation. Monocyte progenitors are generated from CD14+ monocytes or CD34+ HSPCs, allowing for the production of large numbers of mature MoDCs with antigens derived from peripheral blood through treatment with GM-CSF and IL-4. Most clinical trials utilize *ex vivo*-produced MoDCs (IL-4). Extensive testing of *ex vivo*-produced MoDCs in immunization trials has demonstrated their capacity for cross-priming T cells and the release of anti-tumor cytokines, including IL-12 [80].

A subset of patients who received this therapy exhibited anti-tumor activity, highlighting the potential of MoDCs as an effective component of vaccines. However, the clinical data for the majority of individuals' responses have been mild, possibly due to the fact that *ex vivo* differentiation techniques only partially replicate the physiological growth of MoDCs. It has been observed that GM-CSF and IL-4 treatment of both murine and human precursors leads to MoDCs that differ from their naturally occurring (primary) counterparts in terms of transcription and phenotype. This disparity may be attributed to limitations in *ex vivo*-produced MoDCs, such as reduced binding ability to CD11c+ DCs isolated from circulating blood. Additionally, MoDCs generated *in vivo* may have a diminished capacity to migrate into lymph nodes, which could explain the suboptimal efficacy of vaccines formulated with such MoDCs [81].

4.3.3.3 TRADITIONAL DCS OF TYPE 1

A growing body of research indicates that cDC1s are a crucial component of tumor immunity and could serve as a potential alternative cell type for vaccination. Although there are inconsistent definitions regarding cDC1-specific gene signatures, which may introduce some variability in our study, bulk whole cancer gene expression analyses reveal that cDC1 gene signatures are associated with improved outcomes in various types of cancer. Recently, strong evidence was published in a single-cell RNA sequencing (scRNA-seq) study of non-small-cell lung cancer (NSCLC), demonstrating that CDC clusters correlate with improved survival. In this analysis, immune cells were categorized using unmonitored grouping, despite the bias of reference genes. Numerous pre-clinical studies using cDC1 depletion models based on mice deficient in Batf3, a transcription factor essential for cDC1 development,

have shown the necessity of cDC1s for immunogenic tumor cell rejection and responses to immunotherapies like ICB and adoptive T cell transfer [82].

It appears that cDC1s mediate cancer immunity through various specialized tasks involving different processes. One of the most crucial processes is the ability of CD8+ T lymphocytes, a cell type closely associated with prognosis in multiple types of cancer, to cross-present exogenous antigens on major histocompatibility class I (MHCI) molecules to cDC1s. It has been established that tumor rejection requires cross-presentation, particularly via cDC1s. By utilizing a clustered regularly interspaced short palindromic repeats (CRISPR) screen, it was discovered that the BEACH domain-containing protein WDFY4 is essential for the cross-presentation of cDC1s in both primary splenic cDC1s and *ex vivo*-differentiated cells. Wdfy4-/- mice also exhibited an inability to mediate the rejection of a specific antibody fibrosarcoma tumor. Theoretically, WDFY4 modifies vesicular trafficking pathways that play a crucial role in antigen processing, although the exact mechanisms remain unclear. It is worth noting that the loss-of-function characterization of cell type specificity did not affect the presentation of TAAs by cDC2s or MoDCs, and only allowed for cross-presentation, as it had no adverse effects on cDC1 formation, chemokine production, or MHCII antigen presentation. This suggests that cross-presentation, particularly via cDC1s, is required for tumor rejection in the fibrosarcoma model. These research findings align with previous studies that describe various interactions involving DCs and the cDC1 subunit in cross-presentation processes [83].

According to multiple studies, the impact of cDC1s on the immune response against tumors goes beyond cross-presentation alone. Interferon regulatory factor 8 (Irf8VENUS), a crucial transcription factor necessary for cDC1 lineage commitment, which is typically regulated by BATF3-mediated autoactivation, can be expressed as a transgene to restore cDC1 development in Batf3-/- mice. This results in functional cDC1s that can be utilized in both cases, but fibrosarcomas are not effectively eliminated. Although some BATF3-dependent activity that is necessary for cross-presentation does not appear to be related to tumor rejection in cDC1s, the precise processes are not well understood. One mechanism involves the influence that cDC1s have on immune cell migration, which is facilitated by interactions between chemokines and receptors that bring different immune cells together within tumors and towards lymph nodes. For example, the infiltration of CXCR3+ T cells into the tumor is promoted by chemokine (C-X-C motif) ligands 9 and 10 produced by cDC1s (CXCL9), while XCR1+ cDC1s are attracted to XCL1 produced by tumors. This highlights the importance of tumor-produced

chemokines and their effects on the secretion and behavior of cDC1s. These chemokines, found within the tumor environment, activate cDC1-dependent cellular signaling and anti-cancer responses against various tumor types. DCs need to express C-C chemokine receptor 7 (CCR7) in order to be transported to lymph nodes that drain tumors. In melanoma models, where effector T cell priming occurs and leads to anti-tumor activities, cDC1s must express CCR7 for tumor-associated antigens (TAAs) to be transported to lymph nodes. Overall, these findings demonstrate the critical role of cDC1s in migrating towards lymph nodes, where they can present TAAs to effector T cells and infiltrate the tumor, thereby attracting more DCs and lymphocytes through the release of chemokines [84].

According to two recent investigations, the effectiveness of immunity checkpoint inhibition in colon cancer MC38 models adenocarcinoma is determined by cytokines secreted by cDC1s. One study utilized scRNA-seq in combination with intravital live cell imaging to demonstrate that cDC1-like cells secreted IL-12 after the release of interferon (IFN) from a CD8+ T cell-coated surface with an anti-programmed cell death protein 1 (PD-1) antibody. This reactivation of exhausted T lymphocytes, followed by anti-tumor responses, required interaction. The requirement for cDC1-derived CXCL9 for tumor regression on PD-1 blocking was also discovered. In contrast to its traditional role as a T cell chemoattractant, CXCL9, in this study, only served to promote CD8+ T cell proliferation and reactivation. Previous findings using anti-TIM-3, a different checkpoint blockade treatment, are consistent with this (also known as mucin-domain containing-3 and T-cell immunoglobulin). cDC2s did not affect CXCL10, a distinct chemoattractant that interacts with CXCR3 and predominantly increases the tumor's susceptibility to checkpoint inhibitors. These diverse findings demonstrate the crucial role of cDC1s in triggering the immunological generation of cytokines to activate [85].

4.3.3.4 TRADITIONAL DCS OF TYPE 2

Additionally, although the data's breadth is less substantial compared to MoDCs and cDC1, the immunotherapeutic potential of DC subsets is also diminished. cDC2s are experts at priming CD4+ T cells via antigen presentation on MHCII (Box 1), effectively polarizing TILs toward anti-tumor T helper 1 (Th1) or Th17 phenotypes. Furthermore, humans, as evidenced by cDC2s, can release IL-12, and under specific circumstances, CD8+ T lymphocytes can cross-present antigens. scRNA-seq analysis has shown that

intra-tumoral levels of cDC2s are favorably associated with NSCLC patient survival rates [86].

In a B16-F10 melanoma model, the presence of Tregs or regulatory T cells, which inhibit the migration of cDC2s, has been shown to stimulate CD4+ anti-tumor immunity and activity upon their removal. The anti-tumor effects of Treg depletion were not reliant on cDC1s, suggesting that cDC1s are not actively suppressed by Tregs in this context. Conversely, in the presence of PD-1 inhibition in the same tumor model, IL-12 produced by cDC1 was found to be necessary for an anti-tumor response, indicating the involvement of distinct and potentially synergistic cDC subsets in therapeutic processes. Another example is the potential of tumor-derived cDC2s to polarize CD4+ T cells towards a Th17 phenotype, which subsequently leads to a phenotypic shift of tumor-associated TAMs from pro-tumor M2 to anti-tumor M1. This elicited a potent anti-tumor response in a lung cancer model with significant TAM infiltration. However, the impact on cDC1s was less pronounced in a B16 model with low TAM invasion. If present, cDC1s exhibited a stronger anti-tumor response in the absence of TAMs, likely due to enhanced CD8+ T cell priming. Primary cDC2s derived from primary cDC2s were clinically evaluated in studies investigating vaccination against human blood melanoma, demonstrating their anti-tumoral effects. These findings have been observed in certain malignant tumors, leading to improvements in immunogenicity and patient survival in numerous cases [87].

4.3.3.5 THE PLASMACYTOID DCS

In response to viral infections, plasmacytoid dendritic cells (pDCs) are well known for producing significant quantities of type-I IFNs. On one hand, pDCs have been associated with immunosuppression in cancer, primarily through promoting pro-tumor Treg production of inducible costimulatory ligand (ICOS-L). This relationship between intra-tumoral tumors and pDC-level development in various malignancies is likely explained by this function. However, pDCs also exhibit a valuable early response to cancer vaccinations. It has been demonstrated that following the administration of an RNA vaccine in nanoparticle (NP) form, pDC-derived type-I IFNs are necessary for the maturation of activated effectors through cDC1s anti-tumor responses. This finding once again suggests that utilizing different DC subsets may be advantageous. Indeed, pDC-cDC1 crosstalk is beneficial in an anti-viral setting. Primary pDCs have shown the ability to produce type-I IFN, as well as release IL-12 and cross-prime T-cells. Therefore, in melanoma

patients, IFN-driven T-cell responses were induced by pDCs isolated from human blood. According to preclinical and clinical research, pDCs may serve as beneficial components for anti-cancer vaccination, despite potential concerns regarding PDC-mediated immunosuppression [88].

4.3.3.6 COMBINATION OF DC VACCINATIONS

The relative importance of each DC subset in anti-tumor responses will inevitably vary depending on the type of cancer (as demonstrated by cDC2-based model-dependent vaccinations) and the therapeutic approach. To fully leverage their complementary activities and intercellular communication, it is reasonable to hypothesize that an ideal vaccine would incorporate multiple DC subsets. Further research is needed to elucidate the specific functions through which each DC subgroup effectively prevents tumor growth; this knowledge could guide the development of multiplexed DC vaccinations. Additionally, improvements in *ex vivo* differentiation techniques are necessary to enable the generation of DC subsets with intact immunogenic capability [85].

4.4 ELEMENTALS OF CANCER VACCINES (CVS) AND IMMUNOTHERAPY

Through the reactivation of innate defense mechanisms, which include immune cells, tumor immunotherapy aims to treat cancers. Innate immunity cells and macrophages have the ability to directly eliminate pathogens in innate immunity. T lymphocytes and B lymphocytes require activation by antigens in adaptive immunity to generate effector cells or antibodies that initiate an immune response. Antigen-presenting cells (APCs) process tumor cell-derived antigens before presenting them. Tumor cell death and antigen release serve as the starting point, leading to an enhanced immune response and further tumor cell death. The "cancer immunity cycle" refers to the progressive processes resulting from this closed-loop system, which theoretically creates positive antitumor feedback regulation. Based on this cycle, several tumor immunotherapies, including immune checkpoint inhibitors (ICBs) and CAR-T cell immunotherapy, have entered clinical studies. First-generation ICBs, such as anti-PD or PD-L1, rely on the presence of cytotoxic T cells (CTLs) that infiltrate tumors. However, the presence of myeloid-derived suppressor cells (MDSCs) with immunosuppressive properties, regulatory T lymphocytes, and tumor-associated macrophages near the tumor site

significantly impede the therapeutic benefits. The aforementioned approaches demonstrate effectiveness at the later stages of the cancer immunity cycle. By delivering and ingesting antigens prior to the cancer-immunity cycle, tumor vaccines, which are considered to be one of the most effective methods of enhancing immunity, induce CTLs and humoral immunological responses. The development of a tumor vaccine primarily involves four essential steps: selecting the vaccine's active ingredients, precise vaccine administration, antigen release or presentation, and T-cell immunological infiltration. The initial phase focuses on choosing the crucial vaccine ingredients, including the appropriate adjuvants and antigens. The immunogenicity of the adjuvants and antigens, particularly TA, plays a vital role in the anti-tumor immunological activity. In the second phase, effective distribution of the tumor vaccine relies on the ability of antigen-presenting cells (APCs) to promote endocytosis in lymphoid organs and minimize systemic side effects. The third phase involves intracellular delivery of the vaccinations, reducing the amount of antigen destroyed in lysosomes and endosomes. To maximize CD8+ T cell activation against solid tumors, the immunological response triggered by the vaccine must cross-present more foreign antigens through MHC class I molecules. The cancer vaccines precisely target the antigen-presenting cells (APCs), which are quickly phagocytosed and processed to enhance cross-presentation. Tumor vaccines can effectively improve the immunosuppressive environment, promote lymphocyte infiltration, and enhance the efficacy of tumor vaccines when combined with other therapies to eradicate the tumor microenvironment. The tumor's dependence on a gradual cascade response triggers an immune response against the tumor. Several guiding principles are laid out to guide the development, design, and ongoing improvement of successful tumor vaccines, including the conditions required to prime the immune response. To maximize the CD8+ immune response and eliminate tumor tissues, adjuvants, and antigens must be delivered simultaneously via dendritic cells (DCs) and used as intelligent reactive elements. This approach enables cross-presentation and facilitates T lymphocyte infiltration [89].

The loop between cancer and immunity during tumor immunotherapy. Step 1: In step 2, APCs endocytose the relevant antigens after the tumor cells have died and released their antigens. DCs are among the APCs that are most proficient at consuming and handling cancer antigens at this stage. Step 3: Mature DCs are formed when the lymph nodes are the destination of immature DCs that have absorbed the antigen. DCs then activate antigen-specific cytotoxic T cell responses by presenting antigen fragments to CD8+ or CD4+ T lymphocytes through the MHC I or MHC II pathway of the MHC.

Step 4: Effector T cells that recognize an antigen migrate towards tumor tissues. Step 6 follows step 5 to recognize, or rather, bind to tumor cells. Step 7 involves the release of granzyme and the destruction of tumor cells [89].

4.5 TYPES OF CANCER VACCINES (CVS) AND THEIR TARGET

For cancer patients, immunotherapy has always been a desirable and potentially effective treatment option. Vaccines are medications that support the body's immune system. They can educate the immune system to recognize and eliminate harmful microorganisms and cells. Throughout your life, you receive various vaccinations to protect against common illnesses. There are also additional vaccinations available for cancer. Both cancer prevention and cancer treatment vaccines are available [90].

4.5.1 THERAPEUTIC CANCER VACCINES (CVS)

Therapeutic cancer vaccinations are designed to enhance the patient's adaptive immunological response to a specific tumor, aiming to regain control over tumor progression, induce shrinkage of existing tumors, and eliminate minimal residual disease. The key principles for effective therapeutic vaccination against a tumor include delivering substantial amounts of high-quality antigens to dendritic cells (DCs), optimal stimulation of DCs, induction of robust and persistent CD4+ T helper cell and CTL (cytotoxic T lymphocyte) responses, infiltration of the tumor microenvironment (TME), and ensuring durability and maintenance of the immune response. These goals can be achieved through various approaches, such as utilizing immune checkpoint inhibitors (ICIs) to reverse tumor-induced immune exhaustion, administering cancer antigens with adjuvants to activate DCs and effector T cells, or employing autologous DCs loaded with specific tumor antigens for vaccination [91].

4.5.2 PREVENTIVE CANCER VACCINES (CVS)

Other potential antigens for preventative vaccinations must be examined as objectives for malignancies, as the infectious underlying etiology is either unclear or non-existent for the majority of cases. Since most tumor-associated antigens (TAs) are composed of self-molecules, inducing a strong immune response against them has been considered to violate self-tolerance and increase the risk of developing autoimmunity. As a result, most efforts to generate cancer vaccines have been focused on treating advanced cancer,

where the risks associated with the choice of target molecules have been found to be more manageable [92].

4.6 MECHANISM OF ACTION AND IMMUNOLOGICAL EFFECTS OF CANCER VACCINES (CVS)

The immunological mechanism of CVs and their approach to tumor cell death has not been clearly understood, yet the process of immunogenic cell death (ICD) has been deduced in various cancer research. Adequate communication between immunological and non-immune components of the cancer microenvironment (CME) is necessary for effective antitumor immunity (Figure 4.2). Antigen-presenting cells (APCs), such as dendritic cells (DCs), collect and cross-present the peptides produced by cancerous cells, activating T lymphocytes. In the CME, natural killer (NK) cells, neutrophils, and macrophages of the innate defense mechanism play a critical role in the early identification and destruction of tumor cells. When cancer cells die spontaneously or as a result of therapies like certain forms of chemotherapy, they produce tumor-associated antigens (TAs) and molecular patterns that are generally associated with various risk factors [93].

Predominantly, CVs involve the exogenous infusion of specific TAs along with functionally active DCs or DCs activating proteins. The objective of immunotherapeutic vaccines is to stimulate the patient's adaptive immune response against specific TAs, thereby halting tumor development, causing existing tumors to regress, and eliminating any minimal residual disease. Key elements required for effective therapeutic vaccination against cancers include significant doses of high-quality cancer peptide delivery to DCs, optimal activation of DCs, induction of potent and long-lasting CD4+ T helper and CTLs responses, infiltration of the CME, robustness, and restoration of the immune response [75]. This can be achieved through various approaches, such as using immune checkpoint antagonists to reverse tumor-induced immune depletion, introducing cancer proteins with molecules to activate DCs and effector T lymphocytes, or utilizing autologous DCs loaded with specialized tumor antigens for vaccination. Alternatively, the tumor's endogenous immune system can be extensively stimulated to induce malignant cell destruction and enhance the accessibility of cancer antigens through *in situ* vaccines (ISVs). The *in-situ* approach generates the vaccine within the CME by harnessing antigens from dead or injured cancer cells. This differs from traditional immunization, where antigens are carefully selected, purified, or prepared before administration to patients [95].

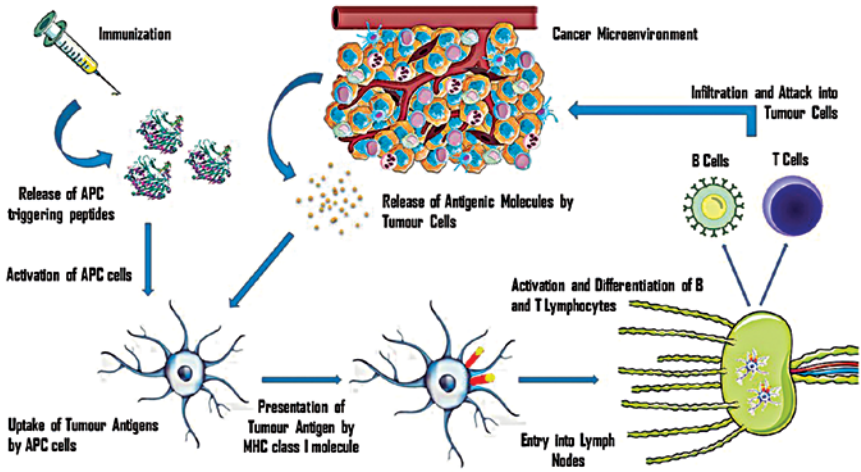

FIGURE 4.2 General mechanism of action of cancer vaccines (CVs).

4.7 FUTURE PERSPECTIVE OF CANCER VACCINES (CVS) IN CANCER IMMUNOLOGY

Although a vast number of cancer-related antigens have been discovered, questions have been raised about their limited immune activation and suitability for use in the creation of cancer vaccines. The security of these vaccinations will continue to be an issue. While the current technical trend of using pure recombinant proteins instead of whole-cell or subunit pathogens has increased vaccination safety, it has also resulted in decreased immunogenicity due to the absence of recognizable entire pathogens [96]. However, the utilization of recombinant vectors could potentially offer a platform. It is necessary to investigate new targets and routes for producing efficient immune responses. Overall, there is enormous promise for developing innovative paths for more effective immune adjuvants, particularly those that are effective against the devastating illness of cancer [97].

4.8 CONCLUSION

One of the biggest successes of immunotherapy so far has been its acceleration of developments in cancer research, significantly influencing anticancer therapy. Numerous new insights into how the immune response affects cancer have been revealed through research, justifying the FDA's authorization of

immunotherapeutic drugs. Oncologists now utilize novel immunotherapies that modify immunological checkpoints, utilize infections to trigger an efficient immune reaction, employ CVs to stimulate the immune system, and develop T-cell treatments for specific antigens. Importantly, individuals who previously had limited treatment options have shown dramatic improvements in their prognoses due to these immunotherapy techniques. Despite the fundamental change in anticancer therapy and its emergence as the fifth element of anticancer drugs, immunotherapy still faces challenges. These obstacles, including immunosuppressive CME, T-cell stimulation, and loss of responsiveness, reduce the effectiveness of immunotherapy. However, they also provide opportunities for unique medication interactions and novel drug designs. Ongoing research may lead to the development of novel immunotherapy delivery systems, the identification of fresh immunotherapy potentiation pathways, a deeper understanding of the immune-cancer interaction, and most importantly, continued improvement in clinical achievements.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

KEYWORDS

- **antigenic stimulation**
- **cancer vaccine**
- **immunological checkpoints**
- **multifactorial antigens**
- **mutations**
- **pattern recognition molecules**
- **tumor-infiltrating-lymphocytes**
- **vascular endothelial growth factor**

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CHAPTER 5

Cancer Vaccine for Lung Cancer

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ABSTRACT

Cancer is the leading cause of death worldwide, claiming the lives of millions of people each year. The most prevalent types of cancer include lung, breast, colorectal, prostate, and pancreatic cancer. Treatment options for cancer encompass chemotherapy, radiotherapy, surgery, and the use of inhibitors that can impede cancer signaling pathways. However, cancer remains a significant health concern. In the past decade, scientists have made advancements in the development of cancer vaccines (CVs) that stimulate the body's immune system. These CVs fall under the category of immunotherapy, which triggers an immune response against tumor-associated antigens (TAAs) and tumor-specific antigens. They are classified as either preventative or therapeutic vaccines based on the timing of administration.

Preventative vaccines, for instance, have shown a 70% reduction in the risk of cervical and hepatic cancer when used against HPV and HBV. On the other hand, therapeutic vaccines enhance the recognition of tumor antigens (TAs) while reducing immune tolerance. Although CVs hold promise, the development of lung CVs has been challenging due to the constant exposure

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of the lungs to antigens, dust, and microbes, as well as the difficulty in identifying the target antigen, among other factors. However, when combined with other conventional therapies, vaccines such as MAGE-A3 and L-BLP25 have shown benefits for patients. Combinations of vaccines and targeted drugs, such as immune checkpoint inhibitors (ICIs) and antiangiogenic therapy, have proven to be more effective. It is crucial to understand the tumor microenvironment (TME), pathways, and utilize personalized approaches in the development of vaccines to ensure the success of CVs.

Therapeutic vaccines have shown more promising results. To enhance the immune response of patients to viral vectors, multiple antigens, and boost strategies, new clinical trials are being standardized.

5.1 INTRODUCTION

Cancer is the leading cause of death worldwide, accounting for over 10 million deaths in 2020 alone. According to the World Health Organization (WHO), one out of every six deaths in the world is due to cancer. Among all cancers, lung cancer is the second most common, affecting people globally. In females, it is the second most common after breast cancer, while in males, it is the most common. The American Cancer Society states that the causes of cancer can be genetic, dietary, related to exposure to radiation or chemicals, or due to infection.

The lungs are vital organs in the human body, especially in the respiratory system. However, smoking is the major cause of lung cancer, accounting for more than 80% of cancer diagnoses. Non-smokers are also at risk of developing lung cancer due to factors such as secondhand smoke exposure and other factors [1, 2]. Accurate identification of primary bronchial carcinoma is crucial for treatment purposes. Despite significant progress in the development of treatment options over the last century, widespread effectiveness has not been achieved. Understanding and classifying cancer is essential for the development of effective treatments.

There are two main types of lung cancer: small cell lung carcinoma (SCLC) and non-small cell lung cancer (NSCLC). Furthermore, NSCLC and SCLC are further classified into various subgroups, including squamous cell carcinoma, adenocarcinoma, large cell carcinoma, and more [3]. When examined under a microscope, specific types of tumors such as Pancoast tumors (upper sulcus tumors) and carcinoids, which are composed of specialized cells called neuroendocrine cells, can be identified. We will discuss these in more detail in the upcoming paragraph. Approximately 10–15% of tumors

are SCLC, while 85–90% are NSCLC, identified through histopathology of the tumor [4].

The chances of detecting cancer at an early stage are about 15%, and more than 75% of lung cancer cases are detected at the metastasis stage. The treatment for the primary stage includes surgery in addition to chemotherapy, but for the advanced stage, combined therapy is required, which includes targeted therapy, pathway inhibition, radiotherapy, and immunotherapy [5]. Advanced strategies, along with conventional approaches, are being used, such as mutational activation targeting the epidermal growth factor receptor (EGFR) and the ROS proto-oncogene treatment. However, the outcome is disappointing mainly due to delays in diagnosis and low response to current approaches [6, 7]. Screening methods for lung carcinoma include lung biopsy, chest X-ray, low dose computed tomography, and sputum cytology. Low-dose CT is highly sensitive but may expose patients to radiation. Sputum exfoliative cytology is a rapid, specific, practical, and economical diagnosis method, although its sensitivity is reduced [8, 9]. The need of the hour is to find the right diagnostic tool with high specificity and sensitivity. In the year 2000, Hanahan & Weinberg discovered the six hallmarks of cancer, which include inducing angiogenesis, activating invasion and metastasis, resisting cell death, unlimited replicative potential, evading apoptosis, and sustaining proliferative signaling. In 2011, two more hallmarks were added due to our understanding of epigenetics and crosstalk between cancer cells, which are reprogramming cellular metabolism and avoiding immune destruction.

In recent years, the most debated topic in the field of cancer research has been cancer immunotherapy, which has led to the discovery of new approaches for its treatment. With the increased use of immunotherapy in cancer, such as Monoclonal antibodies (mAbs) like trastuzumab and alemtuzumab, there is enormous potential in using the cellular arm as a cancer vaccine (CV) [10]. Initially, there were mainly three types of cancer treatment, but Professor Lloyd J. Old noticed that cancer cells are different from normal cells in our body. He predicted in the early 1900s that cancer immunotherapy, along with surgery, chemotherapy, and radiotherapy, would treat cancer more effectively [11]. After decades of extensive research and intense clinical trials, cancer immunotherapy was recognized and became the fourth pillar of cancer treatment. Immunotherapy utilizes the body's own defense mechanism to fight against the disease, revolutionizing the way cancer is treated. There are four main types of immunotherapies based on the type of cell used for treatment: checkpoint inhibitors, cytokines, CAR T-cell therapy, and CV. The use of nanotechnology to develop nano-vaccines has also shown great potential,

although there is still a long way to go. We will discuss their mechanisms of action on our body and current research in later paragraphs. With the invention and development of vaccines, global pandemics have been significantly reduced, saving millions of lives. Vaccines teach our immune system to fight against all types of foreign microorganisms. Although vaccines have prevented certain types of cancer, such as the Human Papilloma vaccine (HPV) and Hepatitis B vaccine (HBV), which have the potential to cause hepatocellular carcinoma (HCC), intensive research is ongoing to develop vaccines targeting existing cancers, known as CV. Cancer cells have the property of escaping from immune cells and preventing our immune system from attacking the tumor by producing immunosuppressive checkpoints [12]. Vaccines given after the onset of infection are called therapeutic vaccines. In the case of cancer, they are mainly divided into autologous and analogous CV. The first type is taken from the patient, processed, multiplied, and injected, whereas the second one is grown from non-self-cells [13]. Autologous CV provides numerous benefits, such as being a source of tumor antigen (TA), activating CTLs and Th cells, and even offering personalized treatment options for the development of personalized vaccines.

In this chapter, we will discuss the role of CV therapy in detail for lung cancer. As lung carcinoma progresses, the survival rate decreases, highlighting the importance of early diagnosis. In stage III and IV, systematic cytotoxic chemotherapy is the primary treatment option. Although targeted therapies based on mutations have been utilized, their success is limited. Therefore, cancer immunotherapy remains the most powerful approach [14]. CV therapy, which aims to induce T cell infiltration into the tumor, is considered the most effective strategy. Various types of vaccines, including peptide vaccines, dendritic cell (DC) vaccines, vector-based antigen-specific vaccines, whole cell vaccines, and vaccines targeting tumor-specific T cells, have been developed [15]. Tumor eradication can be achieved by stimulating the immune system to produce CD4+, CD8+, and cytotoxic T lymphocytes (CTLs) that target tumor-associated antigens (TAAs). An example of a therapeutic vaccine is CIMAvax-EGF, developed in Cuba for NSCLC, as EGFR is present in more than 50% of patients. This vaccine, licensed in 2008, induces antibodies against autologous EGF [15]. Recent approaches involve the use of adjuvants that enhance the immunogenic response to vaccines. These adjuvants further activate antigen-presenting cells (APCs), which in turn activate tumor-specific T cells [16]. Finally, we will explore the causes of lung tumors and how mutations contribute to the development of cancer.

5.2 LUNG CANCER

In the case of lung cancer, the cells of the lung behave abnormally and divide uncontrollably, forming a tumor that can metastasize to other organs of the body. The accumulation of molecular abnormalities over a prolonged period of time is involved in the pathogenesis of cancer. External factors, such as tobacco smoking, account for approximately 80% of all cases [17]. Other factors include air pollution and exposure to harmful gases. Understanding the molecular characterization of lung cancer will aid in the development of better clinical approaches.

There are two different types of small cell lung cancer (SCLC): combined SCLC and small cell carcinoma. These types are classified based on their appearance under a microscope. SCLC is commonly associated with smoking and is typically treated with chemotherapy. On the other hand, non-small cell lung cancer (NSCLC) accounts for a higher percentage of cases, grows slowly, and can metastasize to different parts of the body. NSCLC is classified into adenocarcinoma, which is usually found in the outer lining of the lung; squamous cell carcinoma, which is found near the bronchus in the center of the lung; and large cell carcinoma, which can develop in any part of the lung but grows faster. Under a microscope, specific types of lung cancer, such as Pancoast tumors, can be identified. These are rare and can develop due to certain disease conditions, such as tuberculosis or lymphoma. Carcinoid tumors are also uncommon and grow slowly. They are composed of specialized cells called neuroendocrine cells. Treatment for lung cancer often involves surgery. Let's understand the types of lung cancer and the molecular genetic behind cancer development (Figure 5.1).

5.2.1 TYPES OF LUNG CANCER

Mainly lung cancer is classified into two types of SCLC and NSCLC. About 15% of lung cancers are attributed to SCLC, which exhibits a high proliferation rate and poor prognosis. SCLC can be further classified into two subtypes: small cell carcinoma and combined mixed cell carcinoma. These subtypes are distinguished based on their microscopic appearance. The primary cause of SCLC is strongly associated with smoking [18]. By examining the genetic profile, it is primarily caused by the inactivation of two tumor suppressor genes, namely RB1 and TP53 (p53), which is different from NSCLS where oncogenic mutations are essential for tumor formation.

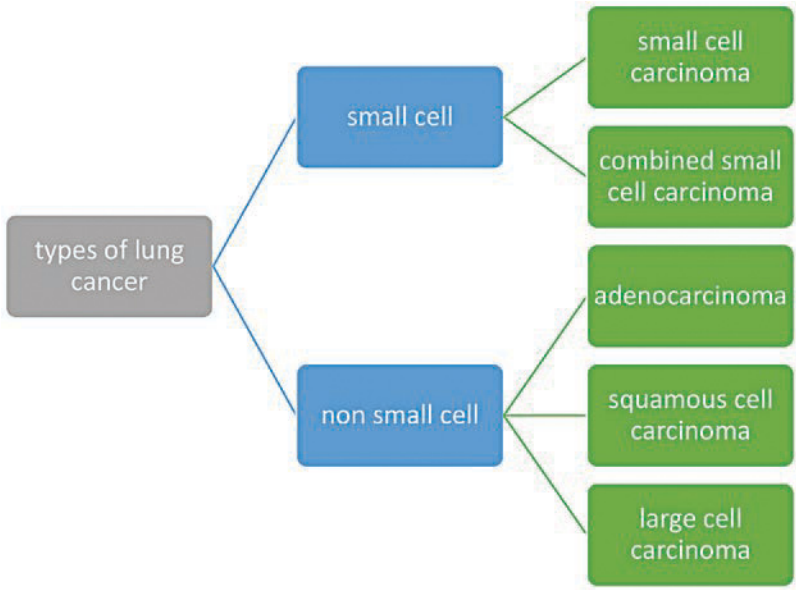

FIGURE 5.1 Two main types of lung cancer (NSCLC and SCLC) and its further classification.

Changes in the tumor microenvironment also contribute to the development of SCLC [19]. Overall, the mechanisms by which extrinsic and intrinsic factors contribute to the formation and proliferation of SCLC are not yet fully understood. SCLC is characterized by early metastasis and rapid growth.

Let's look at the gene which contribute to the formation of tumor along with their occurrence in SCLC (Table 5.1).

Another gene that is observed in SCLC patients, although very rare, is FAT1, NF1, and APC, occurring in 4% [20]. The prognosis for a patient with SCLC is generally poor. However, 30% of patients have LS-SCLC, which refers to limited stage disease where the tumor is confined to one side of the chest and can be treated with radiation. The remaining 70% of patients have ED-SCLC, which indicates an extensive stage diagnosis.

Now, let us delve into the causes of NSCLC, a complex and the most common type of cancer. It is classified into three major types: adenocarcinoma, which originates from secretory cells and is predominantly found in the outer lining of the lungs, especially in smokers; squamous cell carcinoma, which arises from squamous cells located in the central part of the lung; and large cell carcinoma, which is challenging to treat due to its rapid spread throughout the body, large size, and being referred to as large cell undifferentiated carcinoma [21].

TABLE 5.1 Types of Gene Mutation Which is Responsible for Causing SCLC Cancer and Their Function in Normal Condition

Gene Name	Occurrence (%)	Type of Mutation	Function	References
TP53	89%	Deletion	Stress response, transcription regulation, tumor suppressor.	[20]
RB1	64%	Deletion	Transcription repression, tumor suppression, cell cycle regulation.	[20]
KMT2D	13%	Deletion	Tumor suppression, chromatin modeling, histone modification.	[20]
PIK3A	7%	Activate mutation	Oncogene, PTEN-mTOR.	[20]
PTEN	7%	Deletion	Tumor suppressor, m-TOR.	[20]
NOTCH1	6%	Inactivate mutation	Tumor suppression.	[20]
CREBBP	5%	Deletion	Tumor suppressor, chromatin remodeling.	[20]

The most common mutation in NSCLC is TP53, which occurs in approximately 50% of patients. Additionally, mutations in EGFR account for about 10%–35% of cases, leading to irregularities in the AKT and MAPK pathways, further promoting cell proliferation. The increasing molecular heterogeneity in NSCLC tumors necessitates complex treatment procedures. Let's examine the different genes responsible for NSCLC mutations in patients (Figure 5.2) (Table 5.2).

TABLE 5.2 Genes Causing Mutations in NSCLC Patients and Their Occurrence Percentages

Gene	Causes	Occurrence	References
TP53	Mutation	50%	[22]
EGFR	Mutation	10–35%	[22]
KRAS	Mutation	15–27%	[22]
ALK	Fusion	5–10%	[22]
HER2	Overexpression	20%/2%	[22]
PIK3CA	Mutation	2%–8%	[22]
AKT1	Mutation	1%	[22]
BARF	Mutation	5%	[22]
MET	Mutation	5%	[22]

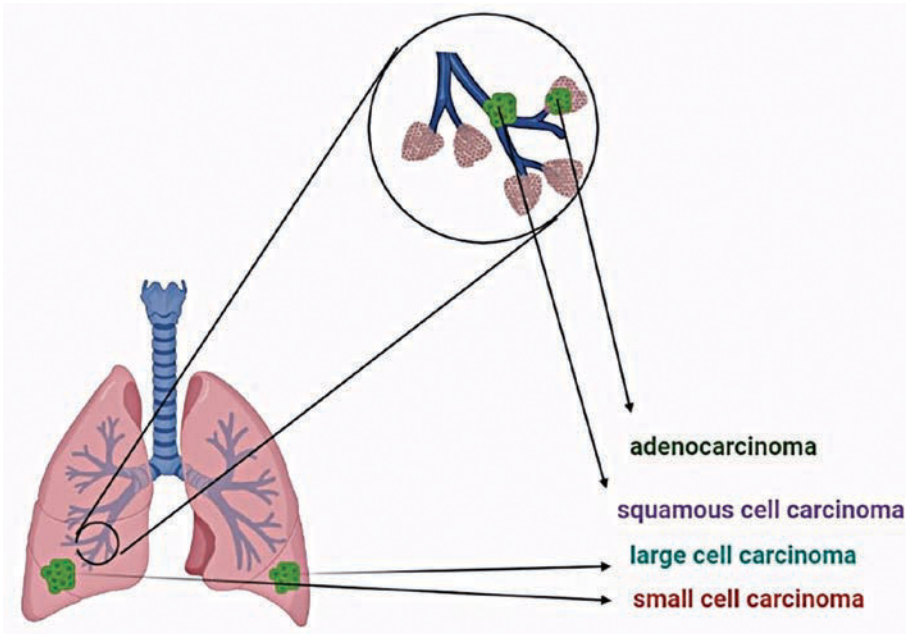

FIGURE 5.2 The most common type of lung cancer is adenocarcinoma, which typically grows slowly. Large cell carcinoma, on the other hand, is fast-growing and mainly found in the outer part of the lung. Squamous cell carcinoma begins in the bronchi, usually in the center of the lung. Small cell carcinoma has a lower occurrence compared to other non-small cell lung cancers.

Let's look at the differences between different types of cancer along with their histology of all types of cancer (Table 5.3).

TABLE 5.3 Lung Cancer is Characterized by Uncontrolled Cell Division, and There are Various Types of Lung Cancer with Different Histology and Additional Symptoms Beyond the Common Ones

Type	Location	Causes	Histology	Presentation	References
Adenocarcinoma	Periphery	KRAS, ALK, EGFR mutation	Pneumocytes type II and Clara cell.	Clubbing	[23]
Squamous cell carcinoma	Periphery	Mainly smoking	Pneumocytes (giant and undifferentiated)	Gynecomastia	[23]
Large cell carcinoma	Central part	Mainly smoking	Intercellular bridge and keratin pearls	Hypercalcemia	[23]
Small cell carcinoma	Central part	Smoking	Kulchitsky cell (undifferentiated)	Crushing syndrome, A	[23]

5.3 CANCER IMMUNOTHERAPY

The tumor microenvironment (TME) in the case of cancer contains different cells such as immune cells, endothelial cells, stromal cells, etc. However, through a series of different mechanisms, cancer cells evade immune surveillance. Based on this, several approaches have been developed to modify immune cells that can recognize and attack cancer cells [24]. Cancer immunotherapy is a type of treatment that involves using our body's immune system to fight against cancer. In the last decade or so, it has shown remarkable results in cancer patients. Successful clinical trials have been conducted against both NSCLC and SCLC. However, the percentage of responders is low. To overcome this issue, non-genetic and genetic determinants of patients should be evaluated. The genetic profile can be accessed using next generation sequencing along with some advanced technology [24].

Moreover, cancer immunotherapy can be classified into four main different categories:

- Checkpoint inhibitors;
- Cancer vaccine;
- Cytokines; and
- CAR T-cell therapy.

Let's discuss about them in detail and their effect on cancer cells (Figure 5.3).

5.3.1 CHECKPOINT INHIBITORS

Cancer cells, noncancerous cells such as stromal cells, immune cells, and various other cells, along with the extracellular matrix (ECM), constitute the tumor microenvironment (TME). The growth of cancer is dependent on the interaction between tumor cells, such as tumor-associated macrophages (TAM), T cells, natural killer cells (NK cells), tumor-associated neutrophils (TANs), innate lymphoid cells (ILCs), and mast cells. These cells influence the process of angiogenesis by producing enzymes such as cytokines and chemokines and establish immunosuppression [25].

The communication between immune cells occurs due to molecules such as cytokines, which play a crucial role in modulating cancer progression. Immune checkpoint inhibitors (ICIs) release T cells, which act as brakes and further activate the immune system by generating antitumor immune responses. Additionally, they activate the adaptive and innate immune systems, leading to an effective response against tumors and peripheral

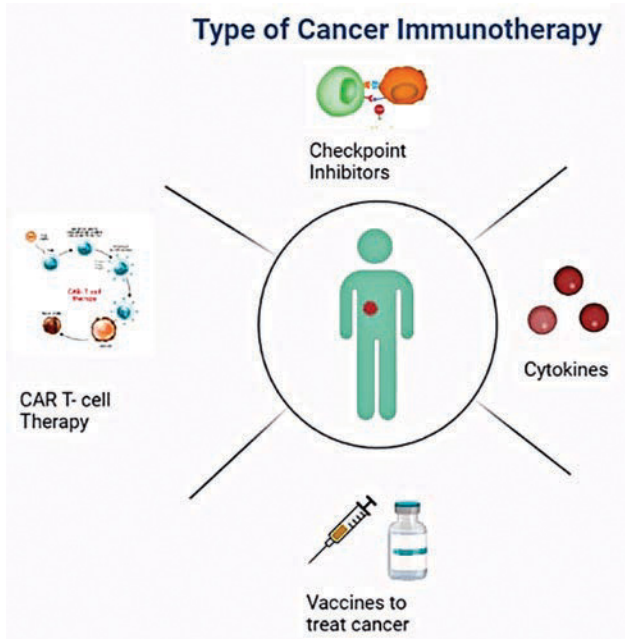

FIGURE 5.3 There are different types of cancer immunotherapy used to treat tumors, such as checkpoint inhibitors, CART T-cell therapy, vaccines, and cytokines. Additionally, monoclonal antibodies and oncolytic viruses are also used as immunotherapies to target specific types of cancer.

organs like blood and LN [25]. To illustrate their role, let's consider an example: in the case of myeloma bone disease, mesenchymal stem cells, stromal cells of the bone, and other bone cells contribute to the development of the disease [26]. The interaction between cytokines and cell adhesion leads to the formation of new blood vessels, promoting further proliferation [27]. *In vivo* models have demonstrated that defective immunosurveillance results in cancer proliferation [28]. Cancer-associated fibroblasts (CAFs) also facilitate tumor formation by secreting cytokines and chemokines that interact with the tumor microenvironment (TME) and contribute to immune evasion by increasing checkpoint activity and immunosuppressive cytokines, thereby promoting cancer proliferation [28]. Recent progress in both clinical and preclinical settings has shown that checkpoint inhibitors are an effective method of cancer therapy.

The Food and Drug Administration (FDA) has approved three molecules for use in humans. Ipilimumab is the first drug approved for metastatic melanoma, acting as an antibody against CTLA-4 (CTLA associated protein 4).

The second drug blocks the inhibitory receptor for programmed cell death-1 (PD-1), which interacts with PD-L1 and PD-L2. Two drugs, pembrolizumab and nivolumab, have been approved as anti-PD-1 antibodies. The third category of drugs consists of anti-PD-L1 antibodies, including avelumab, atezolizumab, and durvalumab. These PD-L1 blocking agents are used for different types of cancer such as non-small cell lung cancer (NSCLC), Merkel cell carcinoma, and urothelial carcinoma. These drugs are often combined to achieve higher efficacy and better results [29, 30]. Despite these efforts, many patients do not respond to these therapies and develop resistance, although the mechanism behind this is not fully understood.

5.3.2 CAR-T CELL THERAPY

CAR-T, or Chimeric Antigen Receptor-T cell therapy, has demonstrated effective clinical responses. These engineered receptors have the ability to target and eliminate cancerous cells that express tumor-specific antigens. The therapy involves two crucial components: autologous T cells, which are isolated from the patients' peripheral blood, and CARs created through genetic engineering. The patients' T cells are isolated and genetically modified to express CARs that specifically target the tumor-specific antigens. Subsequently, the modified CAR-T cells are reintroduced into the cancer patients. These cells can identify and bind to tumor cells expressing the particular antigen, leading to their elimination [31]. CAR-T cells are primarily composed of four parts: an intracellular signaling domain, a spacer/hinge domain, an extracellular antigen binding domain, and a transmembrane domain. Each component is designed to optimize CAR activity [32, 33]. The intracellular domain facilitates signal transduction and T cell proliferation, while the extracellular signaling domain targets the cancer cell-specific antigen. Additionally, CAR-T cells are engineered to overcome the immunosuppressive tumor microenvironment (TME) by secreting various cytokines [34].

There are several surface antigens currently undergoing preclinical trials. Let's discuss them one by one.

1. **EGFR:** These are receptor tyrosine kinases that belong to the HER/ErbB family. They function by transducing extracellular signals. Approximately 60% of NSCLC patients exhibit EGFR mutations, making it possible to target these EGFRs for CART cell therapy. *In vitro* experiments have shown a significant decrease in tumor size and a promising potential [35].

2. **Mesothelin (MSLN):** It is a tumor-associated antigen (TA) that is expressed at very low levels in normal cells compared to its overexpression in solid cancers. Results have shown that it decreases cancer cell invasion and proliferation. In a mouse model, it demonstrated slow tumor growth when injected into the tail vein [36].
3. **ROR1:** Or receptor tyrosine kinase-like orphan receptor 1, sustains pro-apoptotic and survival signals in cases of lung carcinoma. Overexpression of ROR1 is observed in lung carcinoma. *In vitro* testing has shown its anti-cancer activity [37].

   There are various other targeted antigens. Let's examine them in Table 5.4.

TABLE 5.4 Targeting Antigens for SCLC and NSCLC Lung Cancer for CAR-T Cell Therapy in Their Clinical Phase

Type of Cancer	Target Antigen	Phase	Status	References
NSCLC	MUC 1	Phase 1/2	Unknown	[36]
NSCLC	TnMUC1	Phase 1	Recruiting	[36]
NSCLC	PD-L1	Phase 1	Unknown	[36]
NSCLC	Mesothelin	Phase 1	Recruiting	[36]
NSCLC	PD-L1	Early phase 1	Terminated	[36]
NSCLC	Alpha-PD1, MSLN	Phase 1	Recruiting	[36]
NSCLC	ROR 1	Phase 1	Recruiting	[36]
NSCLC	MUC1, PD-L1	Phase 1/2	Recruiting	[36]
SCLC	DLL3	Phase 1	Suspended	[36]
SCLC	GPC3	Phase 1	Recruiting	[36]
Lung cancer	CEA	Phase 1/2	Recruiting	[36]
Lung cancer	CD276	Phase 1	Not yet recruiting	[36]
Lung cancer	HER2	Phase 1	Recruiting	[36]

CAR-T cell therapy has wide potential. However, patients face some issues including toxicities on targets, evasion of antitumor response, and off-tumor toxicity. The treatment of solid tumors is even more challenging due to factors such as immunosuppressive TME and difficulty in infiltration of CAR-T cells into tumor sites. Successful implantation has enhanced the effectiveness of treating solid tumors through continuous modification and evolution in CAR structures, leading to reduced toxicities [37]. However, more clinical trials are required to enhance the efficacy and efficiency of CAR-T therapy in lung cancer. Now, let's turn our attention to the third type of cancer immunotherapy, which is cytokines.

5.3.3 CYTOKINES

Cytokines are produced by immunocytes and non-immunocytes as messenger molecules to communicate with other cells. These proteins have a molecular weight below 30 kDa and play various roles in cancer immunotherapy, including T cell activation, infiltration, and presentation. They also assist in the differentiation of CD4+ cells into regulatory T cells and helper T cells, which upregulate the activity of tumor progression [38]. This type of immunotherapy is based on extracting cytokines that enhance the activity of our immune system to suppress cancer. IL2 was the very first cytokine used for cancer treatment. IL-2, IL-12, IFN type I colony-stimulating factor (CSF), ligand (CCL)21, and chemokine (C-C motif) are all types of cytokines with properties that suppress cancer. However, some cytokines, such as IFN-gamma, IL-1, and TNF-alpha, have dual roles [39].

Recent research shows the use of various other types of cytokines, but Th1 or helper T cells have the most potent antitumor activity when tested in animal models. Combination immunotherapy can be used, such as combining it with CV to achieve effective results [40].

Let's look at different cytokines that are being used (Table 5.5).

TABLE 5.5 Different Types of Cytokines and Their Different Types Which are Being Produced by Immune Cells Mostly Having Anti-Cancerous Activity

Type	Receptor	Produced by	Activity	References
IL-18	TLR	Macrophages, DC, epithelial cell.	Activate T-cell and increase activity of Th1.	[40]
IL-2	CD-25	T cell, DC, and NK cell.	Promote T-cell growth.	[40]
IL-12	IL-12R beta 1	Macrophages and DC.	Differentiation of Th 1.	[40]

The efficacy of cytokines as drugs on clinical outcomes is limited. One possible reason is the short lifespan of cytokines in the blood, requiring high doses to achieve the desired effect. For example, the effective dose of IL-2 is 6,00,000 IU/kg, which needs to be administered every 8 hours for a period of 5 days. This high dosage often leads to adverse conditions in patients, including musculoskeletal pain, fatigue, fever, and chest pain. More serious conditions can include cardiac arrest, immune system disorders, and gastrointestinal disorders. To address these drawbacks, researchers are exploring the use of nanomaterials as carriers to target oncogenic tissues. Nanomaterials in chemotherapy offer significant benefits, such as improved circulation time, water solubility, and targeted deposition at the site of cancer [41]. For

example, PjME/GM-CSF coated with chitosan showed greater effectiveness in DC recruitment compared to naked Pjme/GM-CSF. Encapsulation of IFN-alpha with poloxamer blend microspheres allowed for sustained release in the blood over a month [42]. The growth of tumors was delayed when IL-2 and TGF-beta inhibitors were loaded with liposomal polymeric gels. Encapsulation of PEG liposomes with TNF-alpha and BN175 has also shown potential in reducing toxicity [43]. Additionally, studies have shown that adjuvants can enhance the activity of cytokines, although clinical results have not been consistently encouraging. Careful design of cytokine-based anti-tumor immunotherapy is critical, as cytokines can also have suppressive and anti-inflammatory effects in certain cases. Lastly, we will discuss CV, which is the main focus of this chapter. Before delving into CV, it is important to note that there are other types of immunotherapies, including the use of monoclonal antibodies, oncolytic viruses, metabolic inhibitors, molecular agonists, and antibody-drug conjugates.

5.4 CANCER VACCINE (CV)

The CV on which this chapter is based involves the exogenous administration of specific tumor antigens (TAs) combined with certain proteins to activate our body's immune system. This stimulation of the biological system activates the adaptive immune system, which is specific to a particular TA. Successful vaccination against cancer requires large and high-quality dendritic cells (DCs), their activation, long durability of their response, strong and sustained activation of CD4+ CTL T helper cells, infiltration of the tumor microenvironment (TME), and a maintained response.

Other methods can be used to achieve these mechanisms, such as using immune checkpoint inhibitors (ICIs) or utilizing the TME to induce cancer cell death. Another approach called *in situ* vaccine (ISVs) can be used to activate tumor cell death. In the recent approach of vaccination, specific antigens are selected, purified, and administered into the host [44, 45]. To increase effectiveness, TAs are combined with adjuvants, which further activate DCs to stimulate the patient's adaptive immune system. This helps gain control over the growing tumor by inducing regression, establishing long-lasting anti-tumor memory, eradicating the disease, and avoiding adverse or non-specific reactions. However, these approaches pose challenges such as monoresistance and immunosuppression, which will be discussed in the upcoming paragraph.

The CV demonstrates significant potential in inducing long-lasting and specific antigens against a specific cancer known as TA. The primary objective of CV is to educate the patient's immune system to eliminate tumor cells and specifically identify them. The targets for immune response can be TAAs, which are antigens present in both normal and oncogenic cells, or tumor-specific antigens (TSAs), which are exclusively found on tumor cells. TSAs exhibit high specificity in their action and consist of mutant proteins resulting from somatic mutations in the normal protein sequence. These antigens provide a major advantage as they are crucial for oncogenesis and tumor progression [46], but personalized treatment is required for each cancer type due to the varying nature of these mutations. On the other hand, TAAs are expressed in different types of tumors that share a common origin and some histology. However, TAAs are immunologically weak due to tolerance developed by our immune system during the developmental phase [47] (Table 5.6).

TABLE 5.6 TAs Types and Examples [49]*

TA Type	Subtype	Example	References
TAAs (tumor associated antigens).	Product of silent genes	MAGE-1, NY-ESO1	[48]
	Universal tumor ag	Her2/neu, telomerase, survivin	
	Oncoviral TAAs	HPV E6, E7, EBV-latent	
	Differentiation antigens	Gp100, tyrosinase, Melan-A	
Mutational antigen	Neoantigen	Case-specific mutation	[48]
	(TSA) Product of mutated oncogene	P53, Ras, Bcr-Abl	

*TAs are classified into two categories: TAA and mutational antigens. To better understand, let's compare these two types of TAs.

5.4.1 TYPES OF CVS

There are different types of CVs that have been developed to elicit an immune response in order to prevent its occurrence and progression. CVs can be either composed of cell-based or non-cell-based products. Cell-based vaccines include DC, whole cell vaccines with adjuvants, peptides, vaccines pulsed with nucleic acid, DC pulsed with immune stimulators, whole cell genetically modified tumor vaccines (GMTVs), and tumor cells fused with B cells or DCs or TAAs. Non-cell-based CVs include particle-based vaccines, viral vector-based vaccines, DNA vaccines, or anti-idiotypic antibody vaccines [50].

5.4.1.1 BACTERIAL/VIRAL-BASED CVS

To combat bacterial infections, bacteria were the first to be utilized as vaccines to stimulate cellular or humoral immune responses. Killed or weakened bacteria have long been employed as prophylactic vaccines. With recent advancements in the field of molecular biology, the lifestyle of pathogenic bacteria, as well as genetically engineered bacteria, has been modified to enable them to serve as antigen delivery vectors. Attenuated bacteria have significant potential to induce a broad range of immune responses and elicit strong danger signals through their pathogen-associated molecular patterns (PAMPs) such as flagellin, CpG, lipopolysaccharides (LPS), and lipoproteins. PAMPs further bind to pathogen recognition receptors (PRRs) including C-type lectin-like receptors (CLRs), RIG-like receptors (RLRs), NOD-like receptors (NLRs), and Toll-like receptors (TLRs). These interactions directly or indirectly activate antigen-presenting cells (APCs), which in turn direct adaptive immune responses. Additionally, toxins produced by bacteria reinforce effector or memory responses [51] (Figure 5.4).

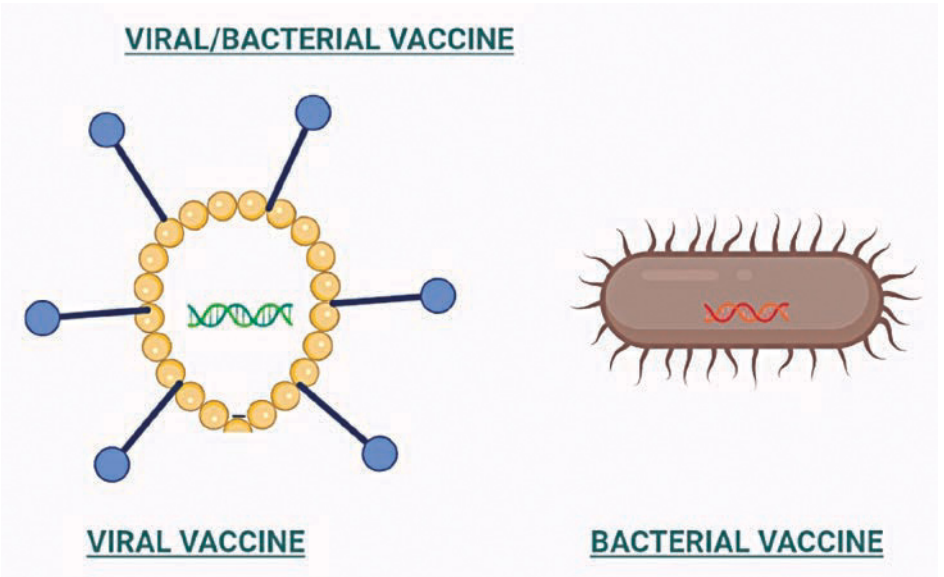

FIGURE 5.4 For the treatment of lung cancer, viral or bacterial vaccines with biological or non-biological gene delivery vectors are used to protect foreign DNA. These vaccines have very high immunogenicity and are easier to produce, but they also possess potential risks and the possibility of unwanted infections.

Several viruses have been used in the cancer vaccine (CV) process, such as poxvirus, alphavirus, and adenovirus. The benefit of using a virus-based vaccine is that it activates both the adaptive and innate immune systems, resulting in a strong and long-lasting effect. However, the anti-viral immune response can neutralize the vector, requiring repeated doses of the cancer vaccine. To address this issue, a heterologous prime-boost strategy is employed. In this strategy, the patient is first injected with one virus, followed by a boost using a different viral vector, such as a DNA plasmid. An example of this approach is PROSTVAC-VF/Tricom, where the priming is done with a vaccinia virus encoding PSA, followed by six booster doses of PSA with fowlpox virus [52]. During phase II clinical trials, 125 men with metastatic colorectal cancer (mCRC) were given PROSTVAC in combination with GM-CSF. Unfortunately, the results could not be replicated in phase III, and the trial was stopped [53]. The reason for this could be that the vaccine alone was not able to suppress the immunosuppressive tumor microenvironment (TME) and was not potent enough. Currently, trials are ongoing to combine PROSTVAC with various checkpoint inhibitors (CPI) to enhance its efficacy. To further increase the potency of the cancer vaccine, various vaccine-based immunotherapy regimens (VBIR) are being developed. These regimens consist of heterologous prime-boost vaccines, where the priming is achieved using non-human specific viruses and replication-defective chimpanzee adenovirus. Boosting is done through intramuscular electroporation [54].

5.4.1.2 CELL-BASED CV

The concept of cancer cell vaccines previously assumed that tumor cells would provide TAAs, which can elicit a specific type of immune response. However, immune cells such as B cells, which secrete antibodies, T cells, and DCs, play a crucial role in the development of cell-based cancer vaccines. B cells not only produce antibodies but also serve as a source of chemokines and cytokines, thus regulating the immune response. Only when B cells are appropriately activated, they become professional antigen-presenting cells. The first time CD40 and its ligand CD40L were explained, they were identified as the most important stimuli for B cell activation. Activated CD4+ cells express CD40L and further activate B cells, enhancing the humoral and cell-mediated immune response [55]. Preclinical trials conducted on CD40 B cell-based cancer vaccines have shown strong rationale. For example, LCMV antigen-pulsed CD40B cells have demonstrated a delay in tumor growth in C57BL/6 mice and many more [56].

Now, let's discuss DCs. They induce a strong immune response against both self and non-self-antigens. DCs have various roles, such as micropinocytosis, phagocytosis, detection of tumor cells or pathogenic cells through environmental sensors, co-stimulation of T cells, and providing crosstalk between macrophages, B cells, and NK cells. Based on the potential of DCs, the first clinical trials were conducted between 1995 and 2004 using GM-CSF + IL-4, which are derived from DCs. They were tested in various ways to promote antitumor activities, such as in the case of melanoma patients or the characterization of shared TAs like gp100 and MART-1 [57]. These were also tested on leukemia, myeloma, or hepatocellular cancer. Provenge, an FDA-approved cancer vaccine targeting prostate cancer, is based on GM-CSF [58] (Figure 5.5).

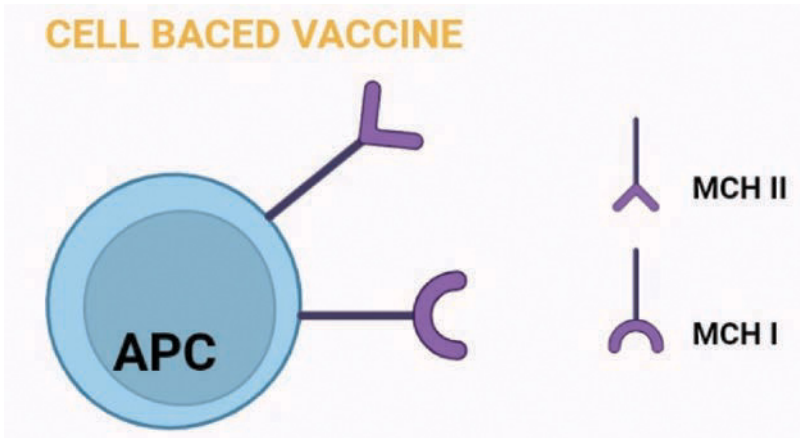

FIGURE 5.5 Cell-based CVs, such as DCs, play a role in activating the innate and adaptive immune systems. They can control antigen presentation and are highly immunogenic, but they are expensive to produce.

5.4.1.3 GENE-BASED CV

Type vaccines are a great tool for eliciting an immune response, even in the case of TAs. Gene-based vaccines are classified into two main types: viral vaccines and DNA plasmid vaccines. With advancements in the field of immune-oncology, DNA CVs are considered the best CVs for activating our immune system [59]. They activate a wide and broad range of immune responses, but in clinical trials, their effect was modest, possibly due to the immunosuppressive nature of developing tumors. To increase response efficacy, optimizing the antigens is required, or DNA therapy can be combined

with other therapies to attenuate the immunosuppressive nature of the TME. Various clinical trials focus on enhancing these two strategies to overcome limitations in CV (Figure 5.6).

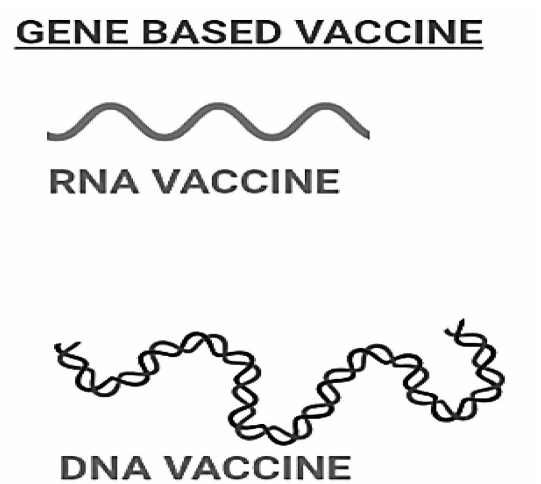

FIGURE 5.6 Gene-based vaccines, such as RNA and DNA, have the ability to deliver multiple antigens and induce both cellular and humoral immunity. However, RNA-based vaccines require specific transportation and storage conditions.

5.4.1.4 PROTEIN/PEPTIDE-BASED CVS

Most of the peptide vaccines are based on epitope peptides that stimulate CD4+ or CD8+ T helper cells, targeting tumor-specific antigens or TAAs. There are numerous nanomaterials and adjuvants that can enhance the efficacy of the peptides, offering several benefits such as ease of production, lower cost, high stability, reduced carcinogenicity, and resistance to harmful pathogens. These peptides typically consist of 8–12 amino acids derived from TAAs, which are overexpressed during tumorigenesis. They can be internalized into DCs and further assembled into peptides that bind to HLA. Antigens used in peptide-based therapeutic vaccines for lung cancer include CDCA1, KIF20A, Lengsin, and URLC10, which are all tumor-specific antigens. TAAs such as STEAP, TERT, SOX2, and VGFR are also included. Peptide-based vaccines for different types of cancer are being tested in clinical trials. The first peptide-based cancer vaccine approved by the FDA is sipuleucel-T, used for prostate cancer. These vaccines often combine multiple epitopes with multiple targets [60] (Figure 5.7).

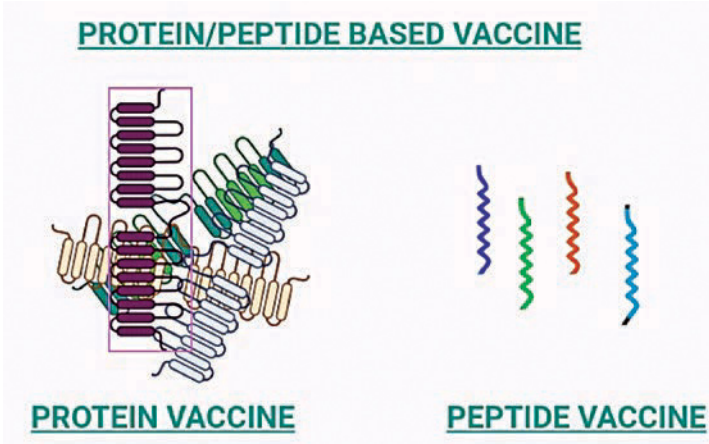

FIGURE 5.7 Protein or peptide-based cancer vaccine have low toxicity, but they have low to moderate immunogenicity.

5.5 BIOLOGICAL RATIONALE FOR USING LUNG CANCER VACCINE

The spread of tumor growth is not only dependent on the characteristics of cancer cells, but also on their interaction with our immune system [61]. Dr. William B. Coley was the first to explain the use of immunotherapy in the treatment of cancer. He used a streptococcal vaccine, later named 'Coley's toxin,' which was used to treat malignant diseases. A disease-free condition was observed in more than 50% of patients suffering from soft tissue sarcoma [62]. Despite these successes, immunotherapies in lung cancer have not been explored until recently, which has shown survival benefits [63]. With the positive response, there has been an interest in lung cancer immunotherapy, which works by helping cancer cells release the immune response or modulating the interaction of antigen-presenting cells and T cells. Additionally, cellular immune response can be induced by CTLs, but the success rate is very low. The limitations can be attributed to a variety of reasons, such as the lack of function or physical disabling of primed tumor-specific T cells, high infiltration of lymphocytes that are immunosuppressing the regulatory T cells such as CD25+ and CD4+, as they further secrete CTLA-4 (cytotoxic T-lymphocyte antigen-4) and TGF-beta, and priming of tumor-specific T cells, etc. [64].

Recent approaches in CV development mainly focus on immunogenic adjuvants that can be coupled with TAs or tumor cells and then administered into patients. These adjuvants induce an immune response by enhancing the APC response in the body. Furthermore, these APCs present antigens to T cells, resulting in the activation of tumor-specific T cell response.

Other methods of strengthening the immune system include genetic modification of allogenic cell lines or autologous tumor cells to secrete co-stimulatory molecules. Additionally, naturally secreted immune modulators can be added to vaccines. Another approach is to express TA as a viral vector to elicit the expression of cytokines and co-stimulatory molecules.

Priming of the patient's immune system can also be achieved by using CVs that contain autologous DCs loaded with TAs to elicit a specific CTL response.

In the case of NSCLC and SCLC, various therapies can be used in vaccine development. These include target protein-specific vaccines against MUC-1 (mucin 1), melanoma-associated antigen A3 (MAGE-A), epidermal growth factor (EGF), and whole cell vaccines such as belagenpumatucel-L [65].

5.5.1 RATIONALE FOR IMMUNOTHERAPY IN SMALL CELLS LUNG CANCER (SCLC)

SCLC, or small cell lung cancer, is an immunogenic tumor with a very poor prognosis despite extensive research. It primarily affects individuals who directly or indirectly consume tobacco, leading to somatic mutations and a suppressed immune response. This is attributed to a high level of non-synonymous somatic mutations known as TMB, or tumor mutational burden [60], which impacts the adaptive immune system. The presence of a specific mutation accounts for the highest rate of somatic mutation, even affecting DNA repair mechanisms and increasing the likelihood of developing tumor-specific neoantigens that can trigger the adaptive immune system. Defects in tumor mismatch repair genes can result in MSI, or microsatellite instability, which benefits PD-1 ICI and immunotherapy in various cancers [66].

Despite the high somatic mutation rate in SCLC, it exhibits an immunosuppressive phenotype characterized by low expression of major histocompatibility antigens, beta-2 microglobulin, and HLA. The loss of expression of different types of HLA-A, B, and C allows cancer cells to evade host immunosurveillance [67]. Additionally, SCLC shows low levels of TILs (tumor-infiltrating lymphocytes), myeloid cells in an immunosuppressive state, and a very low CD8/CD3 ratio.

With advanced research in the field of immunotherapy, the suppressed immune response can be elevated with the help of checkpoint inhibitors such as nivolumab, durvalumab, pilimumab, atezolizumab, etc. These inhibitors have shown anti-cancerous activity and improved survival rates in patients suffering from SCLC. Atezolizumab, when combined with chemotherapy,

has set a milestone in the field of cancer immunotherapy as it became the first drug to increase chances of survival in patients suffering from extensive stages of SCLC. Due to the success of checkpoint inhibitors, clinical trials were conducted for CVs, TLR9 inhibition, and the use of cytokines, among others. For CV, a different approach was taken, such as stimulating anti-tumor immunity to generate an adaptive immune response. This is achieved by exposing the tumor antigens to the immune cells. Various approaches are being tested in clinical trials for CV, which will be discussed in the upcoming paragraph. Different types of CVs have been tested in SCLC lung cancer patients.

A common cell membrane ganglioside called GD3 is mostly expressed in SCLC cancer patients and in a limited number of normal tissues. Around 15 patients who were given a dose of BEC2 (an anti-idiotypic monoclonal antibody that has shown to induce anti-GD3 antibodies) with bacillus Calmette Guerin (BCG) vaccine showed increased immunogenicity [60] when administered intradermally on weeks 0, 2, 4, and 6. All of them developed anti-BEC2 antibodies, while five of them developed anti-GD3 antibodies. In a randomized phase III clinical trial, a total of 515 patients with LS-SCLC were administered a combination of BEC2 and GD3 after chemotherapy. Unfortunately, there was no observed improvement in quality of life (QoL), overall survival (OS), and progression-free survival (PFS). Among the patients, 33% developed a humoral immune response, while others experienced prolonged survival [69]. Another anti-idiotypic monoclonal antibody, CV 1E1O, was developed to elicit an immune response against GM3 in SCLC, breast, and melanoma cancer [70]. Phase I clinical trials were conducted on nine patients after their initial chemotherapy. The treatment was administered every two weeks, followed by six doses every 28 days, resulting in significantly extended survival. The modified version of 1E1O, now known as racotumomab, is primarily used in NSCLC and has been discontinued for SCLC patients.

In the case of SCLC patients, N-polysialic acid (polySA), a side chain attached to the neural cell adhesion molecule, is commonly found [71]. During phase I clinical trials, polySA was conjugated with the keyhole limpet hemocyanin (KLH) vaccine to induce an increased antibody response in SCLC patients. Additionally, MHC class I expression is induced by mutated p53 protein in SCLC patients, which can serve as an efficient target for immune recognition [69]. A tumor vaccine consisting of the wild-type p53 gene and DCs, carried by adenovirus, was tested in phase I and II trials involving 29 patients. After the initial round of chemotherapy in ES-SCLC

patients, the gene specific to p53 was found to be detached in approximately 57% of cases [73]. This vaccine was combined with all-trans retinoic acid (ATRA), which suppresses the growth of myeloid-derived cells, and tested in clinical trials. Positive responses were observed in 20% of the patients and 43.3% of those who received both treatments [74] (Table 5.7).

TABLE 5.7 In Case of SCLC Various Agents Such as Bec2 and Others as Illustrated Above Studied on Clinical Trials

Agents	Phase	Study	No. of Patients	Endpoint	Result	References
Adjuvant Bec2/ BCG	III	Randomized	515	OS	14.3 and 16.4 month in vaccination and observation arm.	[75]
KLH-conjugated N-propionylated polysialic acid.	II	Single group	20	Antibody response	8/9 with IgG and 9/9 with IgM.	[75]
Autologous DC adenovirus p53 vaccine.	II	Palatinum-based chemotherapy then vaccine.	29	P53-specific response.	57.1% with p53 specific response.	[75]

5.5.2 RATIONALE FOR IMMUNOTHERAPY IN NSCLC

Other approaches to immunotherapy, such as checkpoint inhibitors, have revolutionized the field of cancer immunotherapy, especially in the case of NSCLC. Unfortunately, not all patients exhibit the same durability, and the prognosis is very poor. To further enhance effectiveness, strategies need to be improved. One way is to use therapeutic oncogenic vaccines to achieve a long-lasting effect against specific tumor antigens with minimal side effects. The main benefit of using these vaccines is that they can boost anti-cancer tumor activity and work synergistically with immune checkpoint inhibitors (ICIs). Therapeutic vaccines can be either preventive or therapeutic, used for early treatment of cancer or in combination with various other types of cancer drugs during treatment. They are designed to act against tumor antigens, increase immunosurveillance of cancer cells, and primarily trigger T cell responses. Recently, it has been found that neoantigens play a crucial role in developing therapeutic vaccines, and targeting these antigens is of utmost importance. Many clinical trials are being conducted based on this concept. Now, let's discuss the different types of therapeutic vaccines in the case of NSCLC and their mechanisms of action. As we have already

discussed, there are different types of therapeutic vaccines, such as cellular, viral vector, protein, and peptide-based vaccines. To enhance the delivery of these vaccines, more immunogenic antigens, such as neoantigens, are being selected. These antigens can broaden tumor-specific T cell responses and enhance anti-cancer activity [76].

Now let's discuss the interaction between immune cells and lung cancer TME. It primarily involves the suppression of immune cells that aid in the growth, expansion, and metastasis of tumor cells. Various interactions take place in the TME to stimulate the heterogeneity of tumor cells, including mechanical pressure, induction of hypoxia, hypoglycemia, and various biochemical signaling such as auto, para, and endo to interact with cancer cells [77]. The activation of the patient's body to fight against cancer has been the most appealing method, but the restrictions imposed on the TME can affect immunotherapy. In the pre-malignant stage of cancer, preventive measures are given, such as in the case of diseases like HBV and HPV, which can decrease the risk of cancer, such as HCC or cervical cancer caused by these viruses. Despite the development of many therapeutic vaccines, the outcomes are unsatisfactory. The reasons for the low efficacy can be attributed to the immunosuppressive nature of the TME, difficulty in identifying TA, overexpression of self-antigens, low affinity with T cells, and many more [78, 79].

Let's explore various CVs for NSCLC along with their latest updates. These CVs can serve as adjuvants in both unresectable and resectable NSCLC by providing a response to various immunotherapies.

One such NSCLC therapeutic vaccine is CIMAvax-EGF, which was developed in Cuba. The research for this vaccine began in 1995 and it was licensed by the Cuban Regulatory Agency in 2008. CIMAvax-EGF consists of a recombinant protein carrier from Neisseria Meningitidis B, human EGF conjugated with P64K, and Montanide ISA51 as an adjuvant. Upon induction, it produces antibodies against EGF, preventing it from binding to its receptor and inhibiting tumor growth. So far, it is considered safe for NSCLC patients. To achieve better control over cancer growth, a combination of CIMAvax and ICIs is being administered to patients in clinical trials.

Another therapeutic vaccine is UV1, which is composed of three long polypeptides covering an epitope-rich sequence containing the active catalytic site of human telomerase reverse transcriptase (hTERT). hTERT is a TAA that is overexpressed in approximately 85% of solid tumors [80]. It is crucial for the immortality of tumor cells, making it an important target for anticancer therapy. UV1 peptides have been extensively analyzed in the

bloodstream over a long period of time. When the first-generation hTERT vaccine called GV1001 was clinically tested, it demonstrated a high amount of CD4+ responses against hTERT peptides [81]. Upon induction of UV1 peptides, it elicits a wide spectrum of immune responses, such as Th1, interleukin-2 (IL-2), interferon-gamma (IFN-γ), and tumor necrosis factor alpha (TNF-α), which collectively stimulate and activate other cells like CD8+ T cells and effector cells [82]. Phase I clinical trials were also conducted using UV1 and GM-CSF (granulocyte-macrophage colony stimulating factor) for melanoma and prostate cancer [83].

As we discussed earlier, there are four types of cancer vaccines based on their composition: peptide/protein, viral, genomic, and cell-based vaccines. Now, let's categorize these vaccines based on their types specifically for NSCLC (Table 5.8).

TABLE 5.8 In the Case of NSCLC, Clinical Trials Have Been Conducted for Vaccine Development Using Different Compositions Such as Virus, Cell-based, and Peptides

Name	Composition	TAA	Phase	Patients No.	Drug Stage	Endpoint	References
TG4010	Virus-based	MUC1	II	65	IV	Tumor response	[84]
LV305	Virus-based	NY-ESO-1	I	47	IV	Safety	[84]
GVAX	Cell-based	Autologous tumor cells.	II	86	IV	Safety	–
1650-G	Cell-based	Allogenic cell	II	12	I-IIB	Immunogenic response	[84]
Belagenpumatucel-L	Cell-based	Allogenic cell	III	532	IIIA-B/IV	OS	[84]
PRAME	Peptide based	PRAME	I	60	IB, II, IIIA	OS	[84]
Tecemotide (L-BLP25)	Peptide-based	MUC 1	III	1513	III	OS	[84]
Racotumomab alum	Peptide-based	NeuGcGM3	III	1082	IIIA-IV	OS	[84]
CIMAvax-EGF	Peptide-based	EGF	III	579	IIIB-IV	OS	[84]
MAGE-A3	Peptide-based	MAGE-A3	III	2312	IB-IIA	PFS	[84]

5.6 MECHANISM OF ACTION OF CANCER VACCINE ON LUNG CANCER

CV induces a cell-mediated and humoral immune response against specific tumor antigens (TA). Upon injecting CV, it triggers cell-mediated immune

responses that begin with antigen-presenting cells such as dendritic cells (DC) and uptake of TAAs. First, the TAAs are processed and displayed on the surface of cells associated with major histocompatibility complex (MHC) class I and class II. The interaction of processed TA on MHC complexes with T cell receptors (TCR) further activates T helper cells such as CD4+ and CD8+, which secrete interleukins like IL-2, IL-12, and INF-γ. Collectively, they secrete cytotoxic T lymphocytes (CTLs), which recognize antigens on cancer cells and initiate cellular immune attack leading to apoptosis of tumor cells. Additionally, CD4+ T cells enhance the activity of natural killer (NK) cells, which engage in phagocytic activity and stimulate B cell response. These B cells produce antigen-specific antibodies, resulting in a humoral immune attack (Figure 5.8).

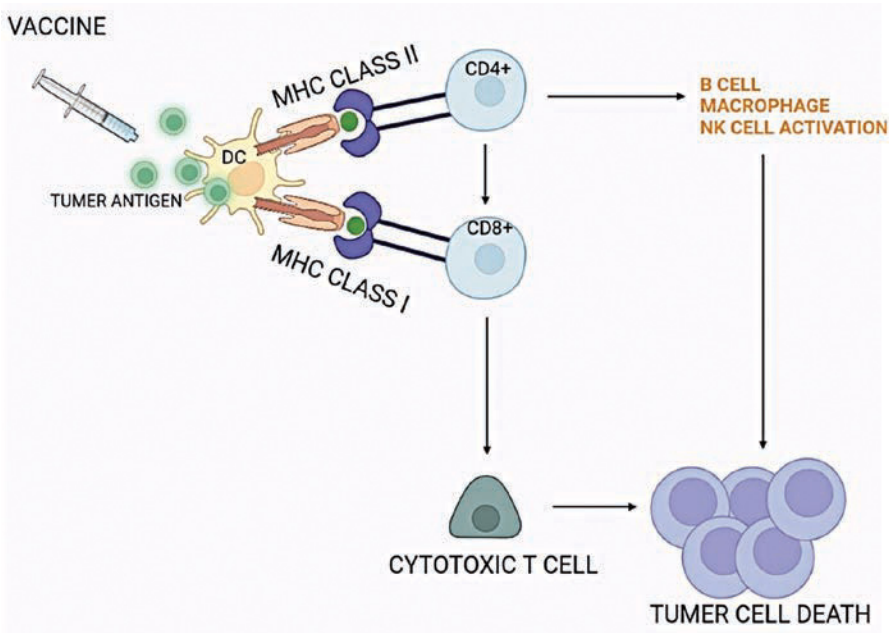

FIGURE 5.8 An example of how a therapeutic CV works in the case of lung cancer: Tumor antigens (TAs) from cancer cells are taken up by antigen-presenting cells (APCs), which process these antigens and present them on the surface of MHC Classes I and II molecules.

In the case of lung cancer, the interaction between TCR (T cell receptor) and MHC I complex in the tumor lymph node activates CD8+ T helper cells, CTLs, and antigens that recognize CTLs. Furthermore, CTLs secrete pro-inflammatory mediators (IFN-gamma and TNF-alpha) via different pathways such as perforin and granzyme. The interaction between MHC

class II and TA further stimulates the release of different subsets of T cells and, in addition, activates CD4+ cells which produce antibodies against TA by B cells and enhance tumor clearance.

5.7 OPTIMIZING VACCINE STRATEGIES FOR LUNG CANCER

The main goal of a cancer vaccine is to stimulate the immune response against a specific set of antigens. This can be achieved by enhancing the function of antigen-presenting cells, which in turn triggers a T cell response leading to antibody production and activation of T cytotoxic cells. This approach, known as immunotherapy, has already shown promise in CAR-T cell therapy and immune checkpoint inhibitors (ICIs). It is now time for cancer vaccines to play a role in eradicating cancer, particularly focusing on lung cancer.

There are two keyways to leverage the cellular immune response and unlock the potential of vaccines in cancer treatment. The first is to induce a T cell response with higher specificity and efficacy, ensuring that the vaccine effectively reaches the site of the cancer and performs its intended function. Various approaches can be used to optimize tumor antigen (TA) targeting, including the use of whole tumor-derived mRNA, allogeneic tumor cell lines, autologous tumor cells, and irradiated autologous tumor cells [85]. However, these approaches present challenges in terms of standardization and require adherence to good manufacturing practices (GMP).

Adjuvants offer numerous benefits in vaccine development. These substances are used in combination with target antigens to elicit a strong immune response. However, finding the right adjuvant for CV poses several challenges. They are poorly immunogenic and must pass the polarized cell-mediated immunity. Adjuvants are further classified into two major types:

- Particulate adjuvants, which act on antigens as delivery systems or depots; and
- Immunomodulatory adjuvants, which can trigger the innate immune response.

Immunomodulatory molecules possess the capability to mimic pathogen-associated patterns and interact with Toll-like receptors (TLRs) on antigen-presenting cells (APCs) such as B cells, dendritic cells (DCs), and macrophages. Several noteworthy adjuvants for optimizing vaccine candidates (CVs) are discussed below. TLR4 is an immunostimulatory adjuvant that aids in APC activation [86]. It is used in combination with ASo4 adjuvant, primarily in vaccines against HPV and HBV. Stimulation

of TLR also activates homeostatic counterregulatory mechanisms, including IL-10, PD-L1, and others. Another adjuvant is Saponin, derived from the soapbark tree (*Quillaja Saponaria*), which contains a pro-inflammatory molecule called QS21. It is widely used in vaccine development to elicit a robust Th1 and humoral response. Clinical trials have shown no convincing evidence of cell-mediated immunity, but humoral immunity was observed [82]. Another example is the stimulator of interferon genes (STING), located in the endoplasmic reticulum, which triggers IFN responses. Activation of STING by bacterial cyclic dinucleotides in preclinical trials resulted in apoptosis of tumor cells at high doses, although other studies have unfortunately shown that its activation also leads to apoptosis of T cells [87]. Therefore, its implementation in CVs should be approached cautiously or used in combination with other therapies. Other examples include granulocyte-macrophage colony-stimulating factor (GM-CSF), the most commonly used ingredient in CV development. It is beneficial for DC recruitment, maturation, and antigen presentation [84]. It is also utilized in monocyte-derived immunotherapy. Heat shock proteins (HSPs) are endogenous proteins with immunogenic activity that are released during stress and can be delivered to DCs, which can cross-present and induce T cell-based immune responses [89]. There are several ongoing trials combining HSPs with other therapies to combat cancer. An example of a particulate adjuvant is alum, which has been used for decades and is known to induce innate immune responses. In the case of cancer vaccines (CVs) aiming for sustained long-term immune responses, they are being used in combination with type 1 polarizing agents such as recombinant IL-12, IISA 51, and MPL (GSK's ASo4) [90]. Other particulate adjuvants include Freund's adjuvant, which becomes less toxic when combined with squalene or oleate. One therapeutic CV that combines all these components is Montanide ISA-51, also known as incomplete Freund's adjuvant. Another effective approach to achieve this goal is through virosomes, microspheres, liposomes, and ISCOMs [91]. Virus-like particles (VLPs) can fuse with the membrane of target cells. One successful VLP-based vaccine is Gardsil®, which is composed of capsid proteins of HPV serotypes and induces a long-term humoral immune response. Other strategies for optimizing targeted CVs include improving effector T cell access into the tumor, combating immunosuppressive cells in the tumor microenvironment (TME) that elicit T cell immune responses, enhancing tumor visibility to the immune system for rapid induction of immune responses, releasing T cells from negative checkpoint signals, and addressing the immunosuppressive metabolic tumor environment [92].

5.7.1 INFUSING NANOTECHNOLOGY IN LUNG CANCER VACCINE

Nanotechnology has various effects on lung cancer treatment. Due to its good biocompatibility, it can carry cancer drugs without causing any toxicity. Nanoparticles (NPs) with high surface area to volume ratio enhance the presentation of antigen-presenting cells (APCs) to tumor antigens (TA). For example, CE6-doped mesoporous silica NPs, modified with PEG and glycol chitosan (GC) via a linker, were loaded with CpG oligonucleotide. When these NPs were applied to the tumor, the linker dissociated, releasing CpG/GC into the tumor environment. Subsequently, photodynamic therapy (PDT) generated reactive oxygen species (ROS), which released the TAAs. These TAAs were internalized by dendritic cells (DCs), enhancing antigen presentation [93].

Another method of developing cancer vaccines involves fabricating nano-vaccines based on fluoropolymer, which activate the toll-like receptor 4 (TLR-4) signaling pathway, inducing rapid maturation of DCs. Personalized cancer vaccines can be developed by grafting polyethyleneimine (PEI) with fluoroalkane, combined with cancer cell membrane isolated from autologous tumors. These cell membranes express tumor-specific neoantigens, which can also be used to produce nano-vaccines [67].

5.8 CURRENT PERSPECTIVE AND FUTURE DIRECTION

It is important to develop anti-cancer immunotherapy composed of various different types of treatment methods so that more effective anti-cancer therapies can be developed, which are effective on a large scale of patients. The cancer immune cycle can be corrected using a combination of multiple therapies that revolve around various immune cells, disorder development, and their causes [72]. To achieve success in anti-cancer immunotherapies, it is essential to build and assess the immune status of each cancer and the therapies that can be induced. In the case of SCLC cancer, adoptive cellular therapy (ACT), i.e., the infusion of T cells into cancer patients, has shown various potential in specific immune response. A variation of ACT known as chimeric-antigen receptor T cell or CAR-T therapy has been widely used. Various clinical trials are being conducted for SCLC patients to evade cancer [68]. Combining immunotherapy with CV is used to develop next-generation vaccines, and the coupling of checkpoint inhibitors with antitumor signals has shown interest in immunotherapy. Examples under clinical trials include the galinpepimut-S vaccine in combination with pembrolizumab and the Ad. p53 DC vaccine with ipilimumab and nivolumab. New predictive biomarkers

are under development as a low response in immunotherapy is a major challenge as it can guide and enhance immune response. One well-known biomarker is PD-L1, its expression and tumor mutational burden (TMB) are best characterized and significant in NSCLC compared to SCLC [69]. Its expression was measured using the tumor proportion score (TRS) and the percentage of positive staining by immunohistochemistry. Response rates to nivolumab monotherapy or with ipilimumab were compared between PD-L1 negative and PD-L1 positive expression.

As we are aware, the immunosuppressive ability of tumors weakens the body's immune control. However, recent vaccines primarily focus on antigen presentation rather than immunosuppression of the lung cancer microenvironment (CME) [67]. There are various pathways responsible for DC anergy in lung tumors, such as the expulsion of cDCs from the lung cancer lesion, molecular expression, and downregulation of functional markers. In the future, vectors could be utilized to deliver DC activation genes. For instance, a lung cancer patient was administered a DC-targeting lentiviral vector that can deliver a gene capable of effectively responding to T cells [72]. Despite the potential applications of DC vaccines, their adaptation for clinical use remains a challenge [68]. Clinical trials II were conducted after immunization with TP53-transfected DC vaccine in SCLC patients, but no survival difference was observed. As mentioned, antigen-loaded DC vaccines fail to migrate to the lymph nodes and induce a T cell immune response due to the immunosuppressive environment observed in patients. However, the common blockade of these immune checkpoints includes PD-1 or PD-L1, which have been extensively explored. In a study conducted by Chen et al. PD-1 blockade-activated DC-CIK showed great potential in solid tumors, even in the case of NSCLC patients. Understanding the physiology of DCs and how to generate an immune response through novel approaches is the future requirement, in combination with other immunotherapies. Non-immunotherapies and immunotherapies, such as checkpoint inhibitors, CAR-T therapy, and CV, have been utilized in numerous clinical trials to enhance the efficacy and treatment of lung cancer.

5.9 CONCLUSION

The complexity of the tumor-associated immunosuppressive microenvironment poses challenges for the delivery of CV. Data from clinical and preclinical settings suggest that vaccines have the potential to eliminate cancer cells. One of the challenges we face with CV is developing a roadmap for

our vaccine to overcome the immunosuppressive environment. Additionally, we need to address the challenge of developing technologies in immunomics that provide a wide range of biomarkers for designing therapeutic vaccines.

Both NSCLC and SCLC are highly challenging diseases, particularly SCLC, where immunotherapy can help patients by inducing antitumor responses. However, the immunosuppressive phenotypic environment of SCLC hinders vaccine development. The combination of nanotechnology and nano-vaccines offers unique advantages, such as eliminating half-life, achieving peak drug concentration, improving clearance rates, and ensuring biocompatibility. This makes nanotechnology a powerful tool for vaccine development.

Furthermore, we should explore the role of CD4+ T cells in anti-CV development and investigate whether gut microbes hinder the efficacy of immunotherapy. We also need to determine the optimal therapy to improve the survival and quality of life of patients. These challenges are difficult to address, but the rewards can revolutionize immune therapy by establishing long-term immunological memory and transforming the way we treat lung cancer.

KEYWORDS

- **cancer vaccine**
- **combination therapy**
- **immune response**
- **immunotherapy**
- **lung cancer**
- **non-cell small cell lung cancer**
- **reactive oxygen species**
- **tumor antigens**

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CHAPTER 6

Synergistic Effect of Vaccines and Chemotherapeutic Agents in Combating Different Carcinomas

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ABSTRACT

Cancer vaccines (CVs) are agents that assist the body in combating cancer. They stimulate the immune system to identify and eliminate harmful pathogens and cells. There are two types of vaccines: (i) Preventive vaccines (such as the HPV vaccine and Hepatitis B vaccine); and (ii) Therapeutic vaccines (such as the Sipuleucel-T vaccine and BCG live for bladder cancer). Chemotherapy is a treatment that involves the use of one or more anticancer drugs to halt the growth of tumor cells, either by killing them or preventing them from dividing. The potential of using vaccines in conjunction with traditional chemotherapeutic medicines to treat neoplasms is being investigated through various animal and human studies. T-cells face challenges in infiltrating large tumor masses due to the lower expression of Major Histocompatibility Complex (MHC) class 1 molecules in these tissues, and the tumor cells themselves outcompete the antigen-specific T-cells produced within the host cell system. The combination of chemotherapy and immunotherapy has opened new avenues in cancer management. Depending on the cytotoxic agent and the specific vaccination used, this synergy can be achieved through various mechanisms. Chemotherapeutic drugs can alter the phenotype of tumor cells

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by modifying the expression of TAAs, MHC-I, ICAM-1, and APM, making them more susceptible to immune-mediated attack. The combination of these two approaches may therefore be more effective than using each strategy alone. Vaccination against a specific target structure can specifically target cancer cells that are resistant to chemotherapy. In this chapter, we discuss the synergistic effect demonstrated by chemotherapy in combination with immunotherapy for the effective treatment of cancers.

6.1 INTRODUCTION

One of the most significant public health issues in the world is cancer. With the evolving nature of the disease and the aging population, the global burden of cancer has become increasingly prominent. Cancer is responsible for one-fourth of deaths in the United States, and many cases are not effectively treated by current conventional therapies. The primary causes of cancer are believed to be mutations in oncogenes, tumor suppressor genes, and genes related to genome stability. Cancer is commonly seen as a cell-autonomous genetic disease. There are two types of cancer: benign and malignant, with malignant cancer having the ability to metastasize. The process of metastasis, which involves the transformation of cells into a malignant form, is driven by various genetic and epigenomic changes due to increased genomic instability. These changes lead to abnormal cell cycles, which in turn affect the characteristics of other cells and tissues [1].

Cancer vaccines (CVs) have the potential to complement standard cancer treatments in a non-overlapping manner by inducing a therapeutic host anti-tumor immune response. Currently, primary neoplasms are managed with a variety of treatments, including surgery, local radiation, immunotherapy, and chemotherapy in the majority of cases. Recent preclinical and clinical research has validated the rationale for combining vaccinations with chemotherapy in patients with advanced tumors. Patients who receive anticancer vaccination monotherapy only demonstrate modest clinical effectiveness in terms of tumor burden. Chemotherapy, immunotherapy, and radiation are examples of palliative cancer treatments that have limited efficacy [2]. To improve survival and reduce mortality during cancer therapy, new strategies need to be developed [3]. Depending on the type of cancer, chemotherapy has been used to reduce tumor burden, accelerate tumor shrinkage, limit metastasis, and prevent cancer relapse. Alkylating drugs, antimetabolites, mitotic spindle inhibitors, and topoisomerase inhibitors are among the most commonly used

therapeutic agents [4]. Chemotherapy has adverse effects on both healthy and malignant cells, which means it can also impact normal cellular activities. Chemotherapeutic agents may have drawbacks such as rapid clearance and poor pharmacokinetics, in addition to various side effects, due to their nonspecific distribution and multidrug resistance (MDR) [5].

6.2 IMMUNOTHERAPY

Immune-oncology, also known as tumor biotherapy, aims to utilize the precise strength and precision of the immune system to treat tumors by stimulating the person's own immunity. Cancer is fought by the body's defense system with the assistance of immunotherapy. The body's immune system helps defend against infections and other disorders. It consists of organs in the lymphatic system and white blood cells. Based on the condition of the patient's immunity and the mechanisms of immunotherapeutic drugs, there are two types of immune cell therapy: active and passive [6].

6.2.1 HOW DOES IMMUNOTHERAPY WORK AGAINST CANCER?

The body's defense system recognizes and removes aberrant cells, stopping or slowing the progression of many malignancies. Immune cells, such as TILs, are occasionally observed in and around tumors, indicating that the body's defense system is responding to the presence of tumors. TIL-positive patients often have better outcomes in their fight against cancer compared to TIL-negative patients. There is great potential for CVs to complement conventional cancer treatments by inducing a therapeutic host anti-tumor immune response without interfering with them.

However, cancer cells have their own defenses against destruction by immune cells. For instance, tumor cells:

- They have genomic modifications that reduce their visibility to the body's defense system;
- They have polypeptides on their outside that prevent leucocytes;
- Change the normal cells in the tumor's vicinity so that they interfere with the immune system's response to the tumor cells.

Biotherapy improves the body's defense system's capability to fight tumor. Types of immunotherapies (Figure 6.1):

- Immune checkpoint inhibitors;
- T-cell transfer therapy;
- Monoclonal antibodies;
- Immune system modulators.

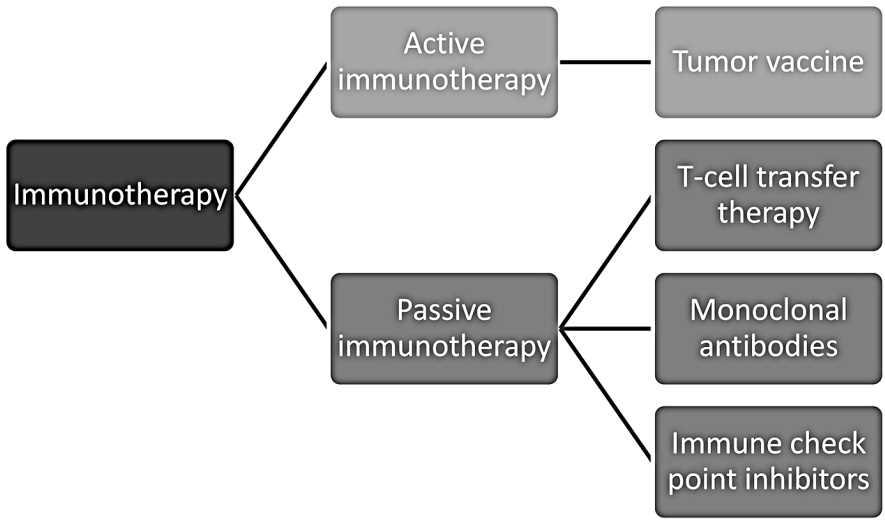

FIGURE 6.1 Different types of immunotherapies.

6.3 CHEMOTHERAPY

Paul Ehrlich, a German scientist who studied the use of pharmaceuticals to treat infectious disorders, coined the term "chemotherapy." The purpose of chemotherapy is to prevent tumor invasion and metastasis by inhibiting cell proliferation and tumor development. However, chemotherapy also has harmful effects on normal cells. Tumor development can be inhibited at various levels within the cell and its surroundings. Chemotherapy drugs generally disrupt the production or function of neoplastic cell proteins by interfering with DNA, RNA, or macromolecular synthesis, or by altering the proper functioning of preformed molecules [7].

6.3.1 IMMUNOSUPPRESSIVE ADVERSE EFFECTS OF CHEMOTHERAPEUTICS

Many of the current tumor chemotherapy drugs are also used as immunosuppressants to treat severe systemic autoimmune disorders [8]. Protein tyrosine

kinase inhibitors (TKIs) can also affect the T-cell component of acquired immunity. Imatinib mesylate, which specifically inhibits the interaction between the macromolecules KIT, c-ABL, and its tumorigenic combination derivative BCR-ABL, inhibits the protein tyrosine kinase LCK7. This is believed to be the mechanism by which imatinib mesylate inhibits T-cell proliferation and stimulation at high doses. Animal model studies have shown that imatinib mesylate preferentially inhibits the growth of memory CTLs but has no effect on initial T and B-cell responses [9].

Glucocorticoids are important components of chemotherapy regimens used to treat various lymphoproliferative diseases due to their ability to induce death in white blood cells (WBCs). Corticosteroids significantly disrupt dendritic cell (DC) development and antigen presentation, despite stimulating the expression of template receptors Toll-like receptor 2 (TLR2) and TLR4. Corticosteroids also suppress the transcription of genes encoding the TCR genes TCRA, TCRB, TCRD, and TCRG, as well as message transmitters and activators involved in the innate immune response. Glucocorticoids increase the expression of several TGF family members, which inhibits the effector functions of T-cells and natural killer (NK) cells. In addition to inhibiting the cytostatic property of NK cells promoted by the NK (p46), NK(G2D), or 2C4 receptors, glucocorticoids also decrease the growth of NK cells triggered by interleukin-2 (IL-2) and IL-15 and block the cell-surface expression of mainly two natural cytotoxicity receptors [10] (Table 6.1).

TABLE 6.1 Prominent Side-Effects of Cytotoxic Agents

Sl. No.	Cytotoxic Agent	Side-Effect
1.	Irradiation	Lymphocytopenia
2.	Alkylating agent's high dose	Lymphocytopenia
3.	Steroids	Suppression in production of cytokines and chemokines.
4.	Taxanes	Thymocytes and NK cell expansion and stimulation retardation.

6.4 CHEMOIMMUNOTHERAPY (CIT)

Chemoimmunotherapy (CIT) can integrate and apply both classic chemotherapy and modern immunotherapy methods to limit tumor development, metastasis, and relapse, even when a cure or symptom relief is not achievable in palliative care. Chemotherapy, in conjunction with surgery, is the primary treatment for tumor patients. To defeat cancer, one must not only devise

plans for effectively eliminating whole tumor cells by employing the ideal ratio and timing of anticancer medicines but also make an effort to elicit an immunological response so that the body's defense system can control any remaining cancer cells.

However, as the tumor grows, it develops treatment resistance, limiting the effectiveness of chemotherapy. The most common causes of resistance expansion are gene amplification by targeted drugs and point mutation in certain tumor cells, which allow the therapeutic agent's deadly impact to escape (e.g., T790M mutation in EGFR). Until just a few years ago, coupling chemotherapy with active immune treatment was unthinkable. The idea was that concurrent active immune treatment with chemotherapy would be useless because chemotherapy would impair any immunological responses, partly because dividing immune cells are susceptible to chemotherapeutic agents [11]. However, new therapeutic options for cancer have been made possible by combining immune cell therapy and drug therapy for cancer treatment, and early research shows that anti-cancer vaccinations and chemotherapy have a synergistic impact, as depicted in Figure 6.2.

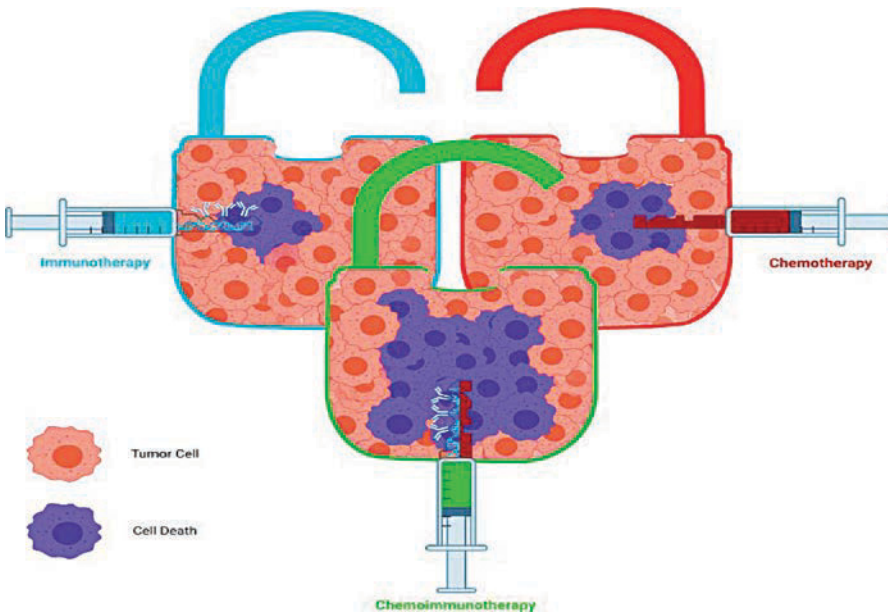

FIGURE 6.2 Mechanistic approach of CIT.

Chemotherapeutic drugs can induce various biological reactions that impact the survival and proliferation of tumor cells. Programmed cell death, a

crucial natural process for regulating healthy cell numbers during growth and disease, plays a significant role. It is evident that different medications can trigger typical apoptotic pathways, leading to the destruction of tumor cells. Currently, all cytotoxic anticancer drugs used in clinical settings, including nucleosides, DNA damaging agents, and microtubule-targeting drugs, promote apoptosis in cancer cells. However, the effectiveness of chemotherapy in treating cancer is limited by antineoplastic resistance. In addition to resistance to the specific treatment being used, tumors may develop cross-resistance to other drugs with different mechanisms of action [12]. This acquired resistance is a disappointing aspect of chemotherapy. Most chemotherapy agents eliminate target cells by inducing programmed cell death, also known as apoptosis, which was previously considered non-stimulatory or tolerable. Consequently, treatments that induce cell death may render cytotoxic T lymphocytes (CTLs) insensitive, even though CTLs are normally responsible for eliminating tumor cells. Vaccination against a specific target structure could selectively target chemotherapy-resistant tumor cells. Immunotherapy possesses the capability to address the shortcomings associated with significant drug resistance and limited specificity of chemotherapy drugs, while improving the responsiveness of tumor cells to chemotherapy medicines. Therefore, the synergistic effects of this combination system might boost treatment efficacy. Additionally, it has been shown that some chemotherapeutic medicines alone can have immunomodulating effects by causing immunogenic cell death (ICD), making tumor cells more susceptible to immune attack, and eliminating the cells that reduce immunity [13].

In an investigation of FDA-sanctioned goods for drug therapy, immune cell therapy, and CIT approaches, the importance of using and developing CIT for the prospect of increasing therapeutic application in cancer treatment was emphasized. Cancer immunotherapies work by immunomodulating the immune system, which involves boosting T cell activity, removing immune cells that suppress the immune system, and then boosting endogenous immunity to stop tumor growth. The use of immunomodulators is one of the potential cancer immunotherapy methods (Tables 6.2 and 6.3). The immunomodulation stops the spread of cancer by increasing cell activity by utilizing antibodies to inhibit or activate regulatory receptors. Currently, antibody-based immunotherapy focuses on leukocytes instead of tumor cells. The immunostimulatory effects of chemotherapy medicines, such as increasing alteration load and antigen expression burden, promoting thymocyte stimulation along with selection to a tumor, and increasing Major histocompatibility complex (MHC) expression to promote protein expression, may supplement

the immunomodulatory effects of Immune checkpoint inhibitors (ICIs). Clinical studies have demonstrated that adding ICIs to chemotherapy can increase anti-tumor efficacy in some cancer types [14].

TABLE 6.2 Combination of Chemotherapeutic Agent with Immune Adjuants

Chemotherapeutic Agent	Immune Adjuvant	Tumor Type	Synergistic Action
DOX	Arginine	Breast cancer	Increased effectiveness of treatments and suppression of carcinoma cells.
PTX	aGC+PD-L 1	Melanoma and lung metastasis.	Enhanced antimetastatic effect.
DOX	TLR 2 agonist	Breast cancer.	High therapeutic efficacy.
PTX	(TLR)7 agonist	Melanoma, lung cancer and cervical cancer.	Prevent cancer cell development.
DTX	TLR-9 agonist	Colon carcinoma	Highest effectiveness against tumors and minimal adverse effects that are not intended.

TABLE 6.3 Combination of Some Chemotherapeutic Agents and Monoclonal Antibodies

Chemotherapeutic Agent	mAbs	Synergistic Action
DOX	Anti-PD-1	Regression of colon carcinoma.
PTX	PD1\PD-L1 inhibitor	Significant anticancer activity.
DTX	Anti-PD-L1	Substantial cancer suppression.

6.5 CHEMOIMMUNOTHERAPY'S IMMUNE-BOOSTING TECHNIQUES

Biotherapy is divided into two categories: active and passive, focusing on the state of the individual's immune system and the mechanisms of immunotherapeutic medications. Passive immunotherapy, instead of inducing cancer cell death, enhances the body's defense system to fight tumor cells. This is achieved by using immunotherapeutic agents such as monocytes, specific monoclonal antibodies (mAbs) targeting malignant cells, autologous cell transplant therapy (ACT), and immune adjuvants. Passive immune cell therapy involves the frequent administration of molecules that bind to malignant cells, including certain immunotoxins, which can be used alone or in combination with drugs, toxins, or radionuclides, delivering them directly

to tumor cells. In contrast, active immunotherapy aims to generate long-term immunity. The most effective method of immunotherapy is ACT, also known as cell-based immunotherapy. It involves extracting tumor-specific lymphocytes from cancer patients, modifying, activating, and expanding them outside the patient's body. As a result, T lymphocytes can now target cancer cells. Cytokines, a diverse group of small soluble macromolecules, are released by various cells including mastocytes, thymocytes, B lymphocytes, and MPs. They play a crucial role in cell communication, stimulating the growth and differentiation of lymphocytes, and regulating the inflammatory or anti-inflammatory responses of different cell types. In the early stages of carcinogenesis, pro-inflammatory cytokines (PICs) exhibit anti-cancer effects by enhancing antigen activation, activating immune-enhancing cells, and increasing the quantity and antitumor effect of the immune system in the tumor microenvironment (TME) [15].

6.6 IMPACT OF ANTICANCER THERAPY ON HEALTHY CELLS

Until now, there has been significant focus on utilizing chemicals or cells that can enhance the body's immune response in immune cell therapy. However, there is now a growing emphasis on suppressor mechanisms due to the recognition that counterproductive cells play a role in defense mechanisms. MDSC and Tregs are two major contributors in cancer. MDSCs are innate immune suppressor cells that can hinder both the inherent and adaptive natural defenses of the body. Similarly, Tregs from the adaptive immune system can dampen immunological responses. Both types of cells can be found in significant numbers at the tumor site in cancer patients. Moreover, scientific evidence suggests that these two cell types are therapeutically meaningful and reliable indicators of patient survival rate [16].

6.7 CHEMOTHERAPY RESISTANCE AND IMMUNE TARGETING

Drugs used for cancer treatment can trigger a series of biological reactions that influence the growth and survival of tumor cells. Among these reactions, programmed cell death, a natural process that regulates cell counts during growth and illness, has been extensively studied. Many clinically available medications induce tumor cell death by activating similar apoptotic pathways [17]. Consequently, most cytotoxic anticancer treatments, such as drugs that bind to tubulin, compounds that impair DNA, and nucleoside

monophosphates, lead to the demise of carcinoma cells. However, the effectiveness of these treatments is often limited due to the development of drug resistance in cancer cells. One challenging aspect of adaptive resistance in cancer cells is that resistance to one type of anticancer therapy can lead to resistance to other drugs with different mechanisms of action [18]. Drug resistance significantly hampers the efficacy of chemotherapeutic treatments in managing metastatic tumors. Abnormalities in apoptotic pathways associated with carcinoma play a crucial role in resisting the drugs used for cancer treatment and radiation. Overexpression of RAP, a protein responsible for the survival of T-lymphocyte antigens and macromolecules from the Burkitt lymphoma-2 family, is a major contributor to compromised apoptosis. Another mechanism of drug resistance involves tumor antigen (TA) cytochrome P1B1, which can deactivate anticancer or cytostatic drugs, thereby affecting the effectiveness of treatment. ATP transporters, which remove drugs from the cell, also contribute to drug resistance [19, 20] (Figure 6.3).

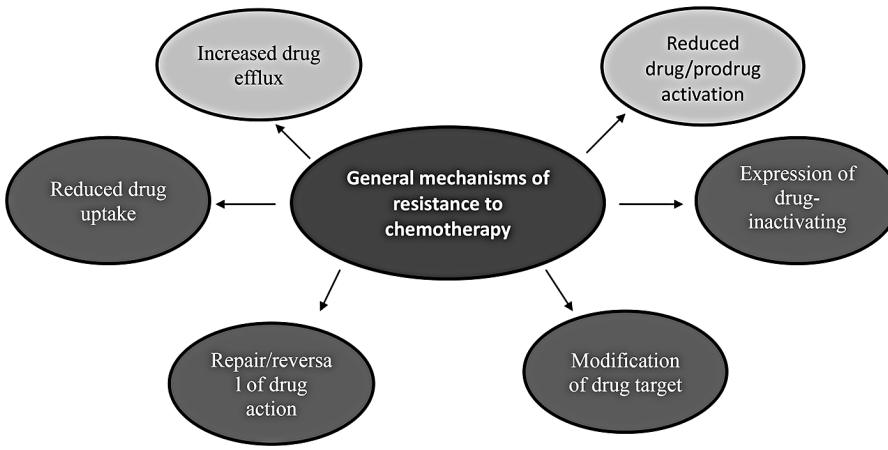

FIGURE 6.3 General mechanism of chemotherapy resistance.

6.8 VACCINE PLUS CHEMOTHERAPY

Recent research has demonstrated that, despite what might seem contradictory, vaccination treatment may not only be compatible but also beneficial when combined with certain chemotherapy regimens administered at the appropriate schedule. Medications such as interferon (INF) can upregulate several tumor-associated antigens (TAAs) and human leukocyte antigen (HLA) on the surface of cancer cells.

Furthermore, various medications commonly used in tumor treatment have shown the ability to overexpress tumor immunotoxins and/or histocompatibility immunogens. For example, Tegafur has been shown to upregulate Carcinoembryonic antigen (CEA) and HLA in tumor cells. In an experimental melanoma model, systemic cyclophosphamide (CTX) combined with locally and intratumorally injected DC completely regressed the tumor [21]. Preclinical mouse experiments have demonstrated that chemotherapeutic drugs such as chlorambucil, daunorubicin, paclitaxel, and vinorelbine enhance the anticancer immune response to total tumor-cell vaccination [22].

Moreover, cancer patients with a high tumor burden have been found to have higher levels of immunological regulatory T cells (CD4+/CD25 high). It is conceivable that systemic chemotherapy used to eliminate these Tregs may enhance the effectiveness of cancer vaccinations. Additionally, the death of cancer cells caused by certain chemotherapy drugs may promote DC uptake and subsequent activation of cytotoxic CD8+ T cells. It has been demonstrated that daunorubicin-induced CPP32-mediated cell death in colon carcinoma lines leads to a potent immune response. In contrast, cells destroyed with mitomycin C did not elicit an immune reaction [23].

Combining chemotherapy with vaccination presents several significant issues. Firstly, it is evident that patients with metastatic cancer who have undergone multiple administrations of various anticancer drugs have compromised immunity as a result. Therefore, combining vaccination with chemotherapy early in the course of the illness, when the body's defense system is still relatively functional, may be advantageous. Secondly, not all chemotherapy drugs may be used in conjunction with vaccines. Lastly, the timing of dosage may be crucial when combining vaccination with chemotherapy [24, 25]. To optimize the administration of anticancer medications and immunization concurrently, further research is undoubtedly necessary. A recent publication by Arlen et al. reported a phase II human trial involving individuals with cancerous AIPC who were randomly assigned to receive either the vaccination alone or the vaccine in combination with low-dose tesetaxel. The vaccination schedule included an initial priming shot with rV-B7-1 and rV-PSA combined, followed by periodic booster shots with rF-PSA. The vaccinations were administered locally using colony stimulating factor-2 (CSF-2). The main objective of the study was to determine whether concurrent tesetaxel therapy had any impact on the development of an immunological response to the vaccination, as measured by the relative shift of PSA-specific CD8 T-cell progenitors from day 0 to day 80. Clinical results and the safety of the combined therapy were considered secondary objectives. After 3 months of

treatment, both groups showed a 3.33-fold median increase in thymocytes precursor to gamma-seminoprotein or kallikrein-3 (KLK3). Additionally, an immunogenic reaction to other tumor-associated antigens (TAs) linked to prostate cancer was discovered. Eleven patients who progressed on vaccination alone were allowed to switch to receiving tesetaxel upon progression. This investigation demonstrated, for the first time in clinical studies, the safety of combining tesetaxel with vaccination without suppressing vaccine-specific thymocyte responses. Furthermore, the findings of this study provided initial evidence that prior immunization prolongs patients' response to tesetaxel compared to tesetaxel alone. As a result of these investigations, the NCI has recently initiated a randomized phase II trial to evaluate the therapeutic benefit of tesetaxel + biotherapy versus tesetaxel alone for individuals with metastatic breast carcinoma [26].

The most intriguing component of vaccination treatment is its potential to initiate a strong immunogenic response in the host, which can be utilized in subsequent treatments. Clinical experiments have demonstrated this tendency in multiple cases. Out of 10 patients who did not develop immunity from the initial vaccination, nine went on to receive additional treatments. In contrast, five individuals who did establish vaccination immunity surprisingly exhibited significant responses to salvage treatment administered after disease progression. Salvage treatment typically lasted for at least a year. Other researchers have also discovered that inducing or enhancing the immunogenic response to immunotherapy can improve the clinical response to chemotherapy.

6.9 VACCINE WITH HORMONE THERAPY

Mixing androgen-deprivation therapy (ADT) with vaccination to treat prostate carcinoma is gaining popularity. Research by Kwon et al. demonstrated that T-cell infiltration was easily detected in benign prostate hyperplasia and malignancies in the prostatic gland. Thymocyte in the prostate showed limited thymocyte receptor use, indicating a local oligoclonal reaction that became noticeable after 1 to 3 weeks of treatment. Other studies have shown that ADT causes the thymus to increase, expands the T-cell repertoire, and eliminates the body's defense system's tolerance to the prostate [27]. These findings have significant implications for administering vaccination in combination with hormone therapy for treating hormone-dependent cancers, including prostate carcinoma. Currently, there is no standard of care for patients with prostate cancer [26]. Patients who have not undergone castration surgery continue to receive ADT and are randomly assigned to

either vaccination or ARA treatment with flutamide. Patients with increasing PSA levels and no metastases after six months may receive both therapies concurrently. The main objective of the trial was to compare the duration of therapeutic failure between those who received the immunization and those who received ARA. Secondary outcomes included vaccination immunological response, immunotherapy efficacy, and the impact of combining the two modes in individuals with developing physiological insufficiency but lacking metastases. At the time of PSA development, flutamide was given to 12 individuals in the vaccination arm. The combination therapy had a mean duration to therapeutic failure of 426.116 days, for a total of 791.359 days from the beginning of treatment. In comparison, vaccination was administered to 8 participants in the flutamide arm at the time of gamma-seminoprotein or kallikrein-3 (KLK3) development. The average duration of the combined treatment was 158.272 days, and the overall trial period was 486.99 days. Although both the vaccination plus flutamide seemed to have therapeutic efficacy, individuals appeared to react more favorably to flutamide after receiving the immunization. To our knowledge, this was the first clinical research that focused on prostate cancer patients and provided detailed support for the idea that vaccination and hormone treatment may work better together than separately. According to a post-five-year review of this trial, patients who received the vaccination first and then the flutamide supplement had a 60-month survival rate of 74.9%, compared to patients who underwent the vaccination earlier and then the flutamide supplement and had a five-year survival rate of 43% [28].

As a result of the previous study, the NCI has recently initiated a clinical trial in prostate cancer patients that combines PSA-TRICOM vaccinations + GM-CSF with second-line androgen antagonist (flutamide). Patients will be randomly assigned to receive either the vaccination with androgen antagonist or the androgen antagonist alone. Flutamide will be discontinued if prostate specific antigen (PSA) levels increase, and patients will either resume the vaccine trial or be removed from the regimen due to excessive toxicity. The objective of the vaccine test is to determine if a combination of vaccine and androgen antagonist prolongs the duration until therapy failure compared to the androgen antagonist alone. The secondary goals of the trial include evaluating toxicity and the response of PSA-specific thymocytes in individuals with PSA precursor. The study will also investigate immunological patterns that may be altered by the treatment, such as the immunogenic effects of discontinuing flutamide. The findings of this investigation may provide the basis for a definitive phase III trial using this therapeutic approach.

6.10 BIOTHERAPY WITH ANTI-CTL 4 MAB

An insufficient impulse from the thymocyte macromolecule is not enough to achieve optimal activation of T-cells for most weak antigens, such as TAAs [7]. Activation of the T-cell specific to the target antigen requires a secondary immune-modulatory signal transmitted from B7 on the APC through CD28 on the T-cell. Two to three days after activation, CTLA-4 is also produced on the surface of the T-cell and binds to B7. This results in a negative signal due to the increased affinity of CTLA-4 binding to CD28, thereby suppressing the immune response. In addition to blocking CTLA-4's inhibitory effect, anti-CTLA-4 mAb also promotes T-cell clones with higher affinity [2, 38]. In murine tumors that were highly immunogenic or moderately immunogenic, anti-CTLA-4 mAb demonstrated anticancer effects. However, the development of weakly immunogenic tumors like MC38 is not significantly influenced by anti-CTLA-4 mAb alone [29]. A clinical investigation using anti-CTLA-4 as a treatment approach was published by Hodi et al. We evaluated the biological efficacy and toxicity of vaccination therapy in nine advanced cancer patients who had previously received treatment for either ovarian carcinoma (n=2) or melanoma (n=7). A dose of 43.3 mg/kg of MDX-CTLA-4 was administered. T-cell reactions against normal melanocytes were observed, but no significant harm occurred. Three patients with melanoma, who had previously undergone treatment with an autologous GM-CSF secreting tumor cell vaccination, exhibited significant tumor regression accompanied by immune cell infiltration. This study revealed that the use of an anti-CTLA-4 antibody may enhance past immunological memory responses. Recent preclinical research has explored the potential of anti-CTLA-4 mAb to modulate the number and/or avidity of antigen-specific T cells when combined with vaccination. In order to enhance T-cell responses, preliminary experiments were conducted to determine the optimal dosage regimen of anti-CTLA-4 mAb in combination with both rV-CEA-TRICOM and rF-CEA-TRICOM. Vaccination of mice with rV-CEA-TRICOM alone or in combination with anti-CTLA-4 mAb resulted in T cells with comparable frequencies of tetramer-positive precursors. Although mice vaccinated with rV-CEA-TRICOM exhibited a 2-fold increase in CEA-specific T cells compared to those receiving rV-CEA-TRICOM plus anti-CTLA-4 mAb, there was a significant difference in tetramer dissociation and a 10-fold increase in functional avidity in T cells receiving both rV-CEA-TRICOM and anti-CTLA-4 mAb. The combination of rV-CEA-TRICOM, anti-CTLA-4 mAb, and rF-GMCSF resulted in a synergistic reduction of CEA-expressing tumors in preclinical

mouse tumor studies. In clinical trials involving patients with melanoma, the combination of anti-CTLA-4 mAb (ipilimumab; Medarex, Princeton, NJ) with a peptide vaccination demonstrated anticancer efficacy, along with severe but transient immunological breakthrough events such as colitis and panhypophysitis [30]. Additionally, preliminary findings were revealed from a complete tumor-cell vaccination combined with ipilimumab. Patients with asymptomatic metastatic AIPC who had not undergone chemotherapy were recruited and treated with GVAX® (Cell Genesys, South San Francisco, CA) every 2 weeks, along with ipilimumab dosage escalation in groups of three patients every four weeks for up to 24 weeks. PSA levels decreased by more than 50% in five out of six individuals who received the top two dosage levels (3 mg/kg and 5 mg/kg). One patient, with a prestudy PSA level of 50 ng/ml, was among the responders who experienced an immunological breakthrough event. After two months of therapy, this patient's retroperitoneal adenopathy resolved, and their PSA level dropped to 0.5 ng/mL. Another patient showed considerable improvement in bone scan lesions when their PSA dropped by more than 50%. Compared to each treatment method alone, the combination therapy resulted in a higher percentage of individuals with PSA reductions. It is interesting to note that some of these individuals initially experienced a deterioration in their condition, as indicated by PSA, before mounting a significant response that coincided with the onset of an immunological breakthrough event.

Additionally, new research supports the idea that clinical responses are less frequent in patients with rapidly advancing diseases who have already undergone chemotherapy, as their immune systems are comparably less functional than those who have not. This is more likely to occur after several months of therapy. Patients who have not previously undergone anti-cancer therapy were much more likely to experience a clinical response and participate in the NCI's ongoing ipilimumab and vaccination trial (05-C-0167) for a longer period of time.

6.11 THERAPEUTIC CANCER VACCINES

Specified Antigens: The decision to target a specific neoplasm antigen has received a lot of attention in the context of improving anticancer vaccines. Targeted antigens continue to be the focus of discussions, despite the extensive investigation of several cancer vaccination techniques. They can be divided into two primary groups. Tumor-specific immunotoxins, such as CTA, upregulated immunotoxin (Her2/neu, survivin, MUC-1), and differentiation

antigens, serve as indicators for both healthy and tumor cells. Examples of tumor-specific immunotoxins include Mart1, PSA, and PAP. While normal cells do express certain TAAs, their immunogenicity leads to specific T-cell reactions. However, TAAs are subject to a certain level of self-tolerance. This central thymic tolerance is bypassed when it comes to tumor-associated antigens (TAAs), as the immune system interprets them as foreign antigens. This includes the carcinogenic viral genome in virally induced malignancies and specific antigens created by non-silent genetic variations of healthy genes.

6.12 SYNERGISTIC EFFECTS OF VACCINES AND CHEMOTHERAPEUTIC AGENTS

Vaccines used to treat cancer are known as treatment vaccinations or therapeutic vaccines. These vaccinations are part of an immunotherapy cancer treatment. To activate the host defenses, treatment vaccinations primarily include tumor-associated antigens (TAAs) and tumor-specific immunotoxins. In theory, the vaccine might induce both specific cellular immunity and a humoral immune response, preventing tumor development and eventually eradicating tumor cells.

6.13 COMBINATION OF DC WITH ANTI-CANCER THERAPY TO ACHIEVE A SUPRA-ADDITIVE EFFECT

Anti-cancer therapy, along with surgery, is the primary treatment for tumor patients. However, as the tumor grows, it develops treatment resistance, limiting its effectiveness. The most common causes of resistance expansion are gene amplification by targeted drugs and point mutation in certain tumor cells, which enable the therapeutic agent to evade its deadly impact. A combination of chemotherapy and immunotherapy is employed to combat tumor drug resistance and immune evasion [31].

6.13.1 WHAT CAUSES ANTICANCER THERAPY AND DC TO WORK TOGETHER SYNERGISTICALLY?

The anti-neoplastic efficacy of chemotherapeutic drugs can be enhanced through immunomodulation. Immunomodulation can be achieved through various means, including (Figure 6.4 and Table 6.4):

- Promotion of immunogenic tumor cell death to facilitate DC phagocytosis (cyclophosphamide, cisplatin, doxorubicin, irinotecan, 5-FU).

- Inhibition of tumor-induced immune suppression by Tregs and MDSCs (Capecitabine, busulfan, tesetaxel).
- Activation of cytotoxic T-cells such as NK LGL, macrophages, and tumor-specific cytotoxic thymocytes (busulfan, tesetaxel, oxaliplatin, capecitabine).

In one trial, low-dose tesetaxel was combined with DC vaccination administered within a tumor to treat mouse lung cancer. In terms of tumor development suppression, T cell tumor cell collection, and production of anti-cancer immunological reaction in local lymph glands, the therapeutic impact can be improved compared to the vaccination or paclitaxel alone treatment groups. Non-toxic levels of chemo agents influenced the endogenous molecules. Changes in DC activity was associated with changes in GTPase levels. Another study discovered that paclitaxel, doxorubicin, mitomycin C, and methotrexate increased DCs' capacity to deliver antigens to Ag-specific T lymphocytes. The enhanced secretion of IL-12p70 expression was related to activated DC activity [32, 33].

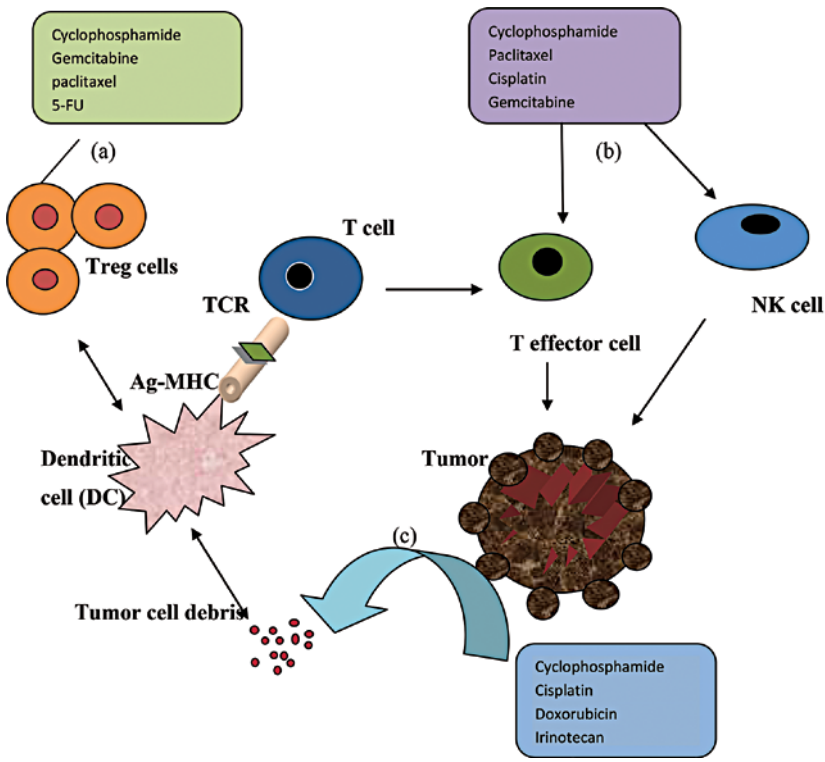

FIGURE 6.4 Probable immuno-mechanism of chemotherapeutic agents.

TABLE 6.4 Chemotherapeutic Agents and Their Impact on the Immune System

Agent	Class	Immunogenic Effect
Busulfan	Alkylating agent	By reducing the immunological inhibition brought on by Tregs, increase CD8 T cell activation.
Daunorubicin	Anthracyclines	Expanding tumor immunogenicity.
Tesetaxel	Taxanes	Increase DC activation and mannose-6-phosphate receptor expression on tumor cells, both of which are necessary for granzyme B-mediated death.
Capecitabine	Anti-metabolites	Lowers Tregs, improves CD8 activation, and reduces tumor-induced immunosuppression.
Cisplatin	Platinum compound	Enhance tumor immunogenicity.
5-FU	Antimetabolites	Enhance tumor immunogenicity.
Vincristine	Taxol	Overexpression of DC maturation factors like CD40, CD80, CD86, MHC-II, secretion of IL-6, IL-12.

6.14 CONCLUSION

A variety of recombinant vaccines have been created as a result of research in molecular biology and immunology. These vaccines consist of viral-based immune adjuvants, along with thymocytes co-activation molecules or lymphokines, for active biotherapy. There is increasing evidence that vaccinations can complement conventional cancer treatments such as radiation, chemotherapy, and surgery. Therefore, it is necessary to conduct relevant non-human and early human research to investigate these strategies. In order to better understand how they enhance cancer immunity and validate specific tests that correlate with human study responses, future human studies will also need to incorporate more comprehensive immune response monitoring. Additionally, individuals with advanced-stage diseases have been included in almost all clinical studies for cancer vaccinations. Further research is needed to determine if these vaccinations can improve survival rates in individuals with early-stage disease and minimal tumor burden.

KEYWORDS

- **cancer**
- **cancer vaccinations**
- **chemotherapy**
- **dendritic cells**
- **immunotherapy**
- **major histocompatibility complex**
- **T-cells**
- **tumor**
- **vaccines**

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CHAPTER 7

Cancer Immunotherapy Using mRNA

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ABSTRACT

Cancer immunotherapy has gained tremendous momentum in the past few years due to its wide range of benefits and its ability to overcome the limitations of conventional treatments. mRNA vaccines have emerged as a promising new option in the battle against cancer. This is because they activate the innate immune system and deliver antigens through co-stimulation. One advantage is that mRNA vaccines cannot enter the nucleus, thus leaving genetic expression unaltered. In the initial stage of mRNA therapy, genetic mutations in a patient's tumor cells can lead to the identification of neoantigens. *In-silico* approaches then determine which neoantigens are likely to elicit a response from the immune system by binding to T cell receptors (TCRs). Synthetic mRNA has proven effective in immunizing patients against cancer using this method. One of the main goals of personalized cancer immunotherapy with mRNA vaccines is to stimulate T cells that can recognize specific malignant cells based on the unique molecular characteristics of the disease. However, a significant obstacle to its widespread therapeutic application is the instability of mRNA and its inefficient transport to the target region. Recent advances

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suggest that protecting mRNA from degradation by RNases can be achieved through nanoparticulate formulations, which ultimately improve the uptake of antigen-presenting cells *in vivo*. This chapter provides an overview of the background and current advances in mRNA-based immunotherapy for cancer treatment, as well as insights into future efforts to enhance mRNA vaccines for cancer treatment.

7.1 BIOLOGY OF SYNTHETIC MRNA

Messenger ribonucleic acid (mRNA) is a subtype of RNA found in the nucleus. It is responsible for carrying genetic information from DNA to proteins through the processes of transcription and translation. Synthetic mRNA, made possible by technological advancements and research investments, exhibits remarkable stability when introduced into mammalian cells. It can be effectively translated into proteins and possesses exceptional properties such as fluorescence emission, ligand attachment via click chemistry, and photocross-linking. There have been reports indicating the effectiveness of synthetic mRNA against various biological conditions. These include restoring deficient proteins to mitigate severe diseases, immunizing cancer patients, and making specific alterations in DNA through genome engineering [1].

7.1.1 STRUCTURE

The three fundamental components that make up the core structure of mRNA are as follows: (i) the middle part is an open reading frame (ORF) that codes for protein. It is flanked on both ends by untranslated sections that are 5′ and 3′ in length (UTRs); (ii) a section called “a cap” that is located in the front, which is structurally a 7-methyl-guanosine residue attached to the 5′-end by a 5′-5′-triphosphate; and (iii) the 3′-end of the poly(A) tail, the ORF region improves protein expression and codon optimization with sequence changes. The UTRs are considered the regulatory elements that improve the efficacy of the translation process with their structure and length. The 5′ moiety increases the overall stability of the mRNA molecule. The length of the poly(A) tail influences translational efficacy significantly [2]. The mRNA structural elements have been presented in Figure 7.1.

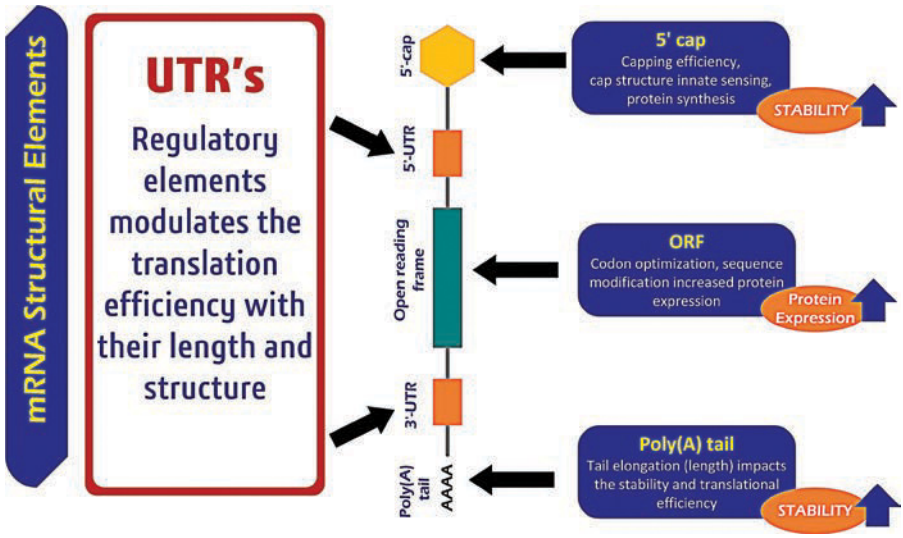

FIGURE 7.1 mRNA structural elements.

7.1.2 PROCESSING SYNTHETIC MRNA

By utilizing complementary DNA (cDNA) as a template, the SP6 *in vitro* transcription (IVT) of cDNA can generate synthetic mRNAs that are highly valuable. Plasmid DNA (pDNA) is specifically employed for this purpose, along with RNA polymerase as a bacteriophage [3]. The structure of mRNA consists of an open reading frame (ORF), multiple untranslated regions (UTRs), a 5′ cap motif, and a poly(A) tail, as previously discussed. The pDNA template contains an ORF, a bacteriophage promoter, and a poly(d(A/T)) sequence that is translated into the poly(A) tail to facilitate IVT. Additionally, a unique restriction site is present for the linearization of the plasmid, enabling precise specification of the transcription endpoint. In the initial step of the procedure, the linearized pDNA is transcribed into mRNA using recombinant RNA polymerase in a mixture containing nucleoside triphosphates. To obtain capped mRNA during transcription, a cap analog such as the dinucleotide m7G(5′)-ppp-(5′)G can be added to the reaction mixture. This is crucial for achieving the desired outcome. When the cap analog is abundant compared to the amount of GTP, transcription commences with the cap analog instead of GTP, resulting in the production of capped mRNA [4]. Following transcription, DNase digestion is performed on both the pDNA template and bacterial DNA. The sample, which contains numerous additional components along with mRNA, is purified through

precipitation and extraction processes [5]. The strategies for the capping reaction are illustrated in Figure 7.2.

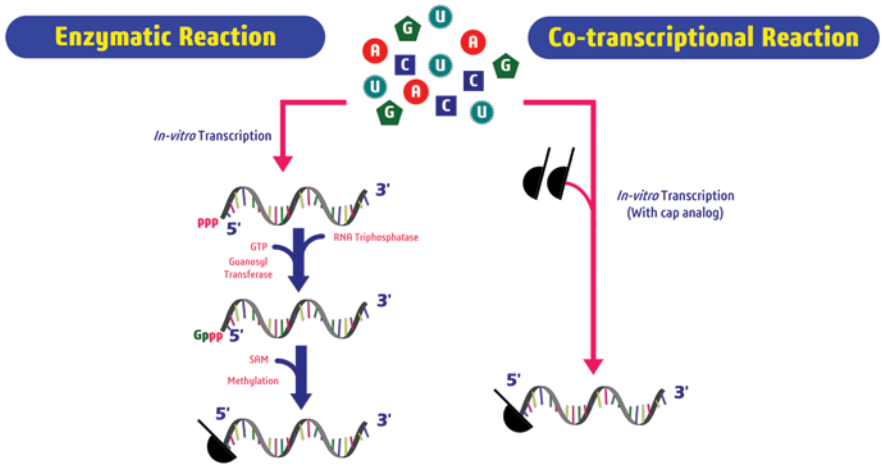

FIGURE 7.2 Strategies for capping reaction.

7.1.3 HOW CAN SYNTHETIC MRNA CONTRIBUTE TO THE CANCER TREATMENT?

Cancer immunotherapy studies have shown that mRNA is a promising vaccine candidate. The main aim behind this approach is to activate the host's immunity against tumor suppression, ultimately increasing the survival rate among cancer patients [6]. The mRNA vaccines that specifically target tumor-specific antigens have the ability to combat malignant cells that overexpress these antigens, leading to tumor regression through immunologic memory. Functional mRNA vaccines primarily enhance immunological reactions through adaptive immune responses. Upon exposure to synthesized mRNA, antigen presentation cells (APCs) present the translated encoded proteins to CD4+ T cells via the major histocompatibility complex-II (MHC-II). Subsequently, CD4+ T cells present them to CD8+ T cells via MHC-I, triggering a robust immunological response [7]. The major advantages of immunotherapy using synthetic mRNA are that these vaccines can activate both B cell-mediated immunity (humoral immunity) and CD4+/CD8+ T cell-mediated immunity. This aids in the active elimination of malignant cells from the tumor microenvironment (TME). Furthermore, mRNA poses no genetic risks as it cannot integrate into the genome sequence, making it highly tolerable [8].

7.1.4 POSSIBLE LIMITATIONS

The degradation of mRNA and the hindrance of protein synthesis are the primary obstacles to the treatment of cancer through mRNA-based immunotherapy. Additionally, the protein expression of mRNA may be diminished due to inadequate *in vivo* delivery of mRNA-based vaccines. These limitations have severely restricted the clinical use of mRNA vaccines for cancer immunotherapy. Other challenges that impede the successful implementation of this approach include excessive immunogenicity (which unnecessarily triggers the immune response) and a lower safety profile compared to inactivated vaccines. Furthermore, it is less effective than DNA vaccines [9].

7.2 MODIFICATION OF SYNTHETIC MRNA

In order to overcome the aforementioned limitations, scientists worldwide have consistently addressed these issues and have developed synthetically modified mRNA. Significant modifications have been made to the nucleobases, RNA terminals, and ribose structure. One approach involves creating cap mimics at the 5′ position to enhance stability, translational efficiency, and reduce immunogenicity. Another modification includes substituting oxygen with sulfur at the β position of the triphosphate bridge in m^7GpppG, resulting in reduced sensitivity to the decapping complex DCP1/DCP2 and increased affinity of the cap for eIF4E [10]. Furthermore, the tetraphosphate bridge has shown greater affinity for eIF4E compared to the triphosphate moiety. Chemical alterations of 0.2–0.4% in the pyrimidine bases have been found to confer resistance against RNase cleavage and activation of toll-like receptors (TLRs). For instance, replacing natural uracil/cytosine (U/C) with m5C improves resistance to RNase [11]. However, replacing some or all of the A with m6A reduces the binding affinity of TLR3/7/8 with synthetic mRNA, enabling it to evade recognition by the innate immune system. Additionally, modifying the ribose component of synthetic mRNA with 2′-OMe or 2′-O-fluoro may reduce inflammatory reactions. *In vitro* studies have demonstrated that 2′-O-fluoro-modified synthetic mRNA exhibits higher affinity for the RIG-I receptor compared to regular mRNA, as observed through a comparative analysis of the two RNA types [12].

7.2.1 FOR ANTIGENIC RECEPTORS

In the early 1980s, high dosages of IL-2 infused into melanoma patients alongside tumor infiltrating lymphocytes were shown to be curative for the

disease [13]. As conventional cancer treatments have proven ineffective, scientists have been working to improve outcomes by engineering T cells to express cancer-specific T cell receptors (TCRs) or chimeric antigen receptors (CARs). Several years of research have shown that electroporation of CAS9 mRNA into human T cells is a powerful alternative to direct integration of CD19-specific CAR to the T cell receptor constant (TRAC) locus [14]. This increased the potency of T cells and led to the uniform expression of CARs. In addition to this, when chemically modified synthetic mRNA, i.e., 1 mψ mRNA, was electroporated with murine T cells, it was found that upregulation of major checkpoints such as PD-1 and LAG-3 was drastically reduced. The primary advantage of mRNA encoded CARs or TCRs is that they can prevent the development of cytokine release syndrome [15].

7.2.2 IMMUNOMODULATORS

Synthetic mRNAs serve as a highly effective tool for delivering a wide range of immunomodulators, thereby enhancing the immune response of T cells in malignant conditions. For instance, the delivery of stimulatory cytokines such as IL-12, IL-15, and GM-CSF, in conjunction with mRNA encoding tumor antigens (TA), can effectively counteract immune suppression caused by cancerous cells. Moreover, the co-expression of TA mRNA with immunostimulatory molecules like constitutively active TLR4, CD40L, and CD70 in melanoma patients triggers T cell activation and facilitates dendritic cell (DC) maturation [16]. Furthermore, intra-tumoral administration of mRNA encoding two cytokines, namely IL-36γ (an alarm signal against foreign pathogens) and IL-23 (a coordinator of immune responses to danger signals), elicits a robust immune response against various tumor models. Combining mRNA-based vaccines with immune checkpoint inhibitors (ICIs) such as anti-CTLA4 and anti-PD-1 targeting antibodies has been demonstrated to suppress tumor development by reducing PD-1 expression on antigen-presenting cells (APCs), enhancing T cell activation and proliferation, and restricting tumor growth [17].

7.2.3 ANTIBODIES

Monoclonal antibodies (mAbs) are extensively utilized as potent therapeutic agents against cancer. This is due to their ability to primarily target stromal or vascular growth factors that deprive tumor cells of essential oxygen and nitrogen, ultimately leading to their destruction. However, the main challenges

associated with their usage are the high production cost and the site of action of these proteins. Research suggests that *in vitro* transcribed mRNA presents a viable solution to overcome these challenges. It is evident that mRNA is capable of encoding any given protein, including fragments of antibodies and full-size mAbs [18]. The subsequent distribution of this mRNA to cells and the synthesis of these proteins ensures post-transcriptional modification and optimal assembly. Over the years, significant effort has been dedicated to determining whether messenger RNA (mRNA) can effectively function as a vector for delivering mAbs. This strategy holds utmost importance as a suitable immune response against the tumor is crucial to trigger a cascade of immune reactions that contribute covalently to the fight against cancer, thereby improving the quality of life (QoL) for cancer patients [19].

7.3 DELIVERY STRATEGIES OF SYNTHETIC MRNA FOR THE CANCER IMMUNOTHERAPY

Significant technical advances over the past few decades have allowed for the development of medicines that utilize mRNA as a revolutionary step towards cancer immunotherapy treatment. The development of more efficient materials and delivery systems has assisted in removing significant barriers to eliciting the necessary immune response to combat a particular form of cancer [16]. Once the host cell has taken up the synthetic mRNA by endocytosis or the cell membrane, it will begin translating the mRNA into the mature form of the target peptide in the cytoplasm. Post-transcriptional changes cause these protein constructions, which are indistinguishable from the results of endogenous mRNA, to be degraded by intracellular compartments. Antigen-presenting cells (APCs) use major histocompatibility complex (MHC) presentation to transfer these peptides to effector cells present in the immune system of hosts, where they activate helper T lymphocytes and NK cells while also stimulating the production of cancer-specific killer T cells [20]. Exogenous mRNA not only generates a cancer-specific immune response but also aids in maintaining an immune-friendly tumor microenvironment (TME) by activating retinoic acid-inducible gene I (RIG-I) and TLR, which results in the secretion of type I interferon (IFN) and other inflammatory cytokines (RIG-I) [21]. The production of CD8+ cytotoxic T cells that are specific to antigens can be stimulated. The ratio between active CD8 cells and immune suppressive Tregs can be increased, and memory T cells can be induced for a long-standing immune response by engineering mRNA constructs to express proinflammatory cytokines such

as IL-2, IL-7, IL-12, and IL-15 [22–24]. Furthermore, mRNAs are used to encode monoclonal antibodies (mAbs), which have been demonstrated to play a crucial role as passively targeted immunotherapy methods for various cancers, including lymphomas and breast cancers. *In-vivo* research suggests that mRNA encoded monoclonal antibodies (mAbs) can indeed elicit a more prolonged antitumor effect than their recombinant equivalents in animal models. *In vivo* research suggests that mRNA-encoded mAbs can indeed elicit a more prolonged antitumor effect than their recombinant equivalents in animal models [25, 26].

7.3.1 DELIVERY STRATEGIES FOR MRNA

The utilization of mRNA technology in cancer immunotherapy has demonstrated potential, yet there remain numerous challenges to be addressed in order to generate an effective immune response. The large RNA molecule carries a negative charge and must first penetrate the cell membrane, which poses a significant obstacle to intracellular release due to its negative charge. Ribonucleases, which are abundant in the skin and bloodstream, degrade mRNA upon reaching the cell. Naked mRNA can be delivered intradermally, subcutaneously, or intramuscularly (I.M). However, these methods have limitations as they cannot efficiently enter intracellular compartments, resulting in a short half-life, rapid breakdown, and inadequate immune response. Therefore, the establishment of a robust delivery system is crucial for achieving an optimal therapeutic effect. In order to ensure adequate translational capacity, advancements in mRNA technology have been linked to the development of innovative nanotechnology-based delivery techniques [6, 27, 28].

7.3.2 SYNTHETIC SYSTEMS

7.3.2.1 DELIVERY SYSTEMS BASED ON LIPIDS

The most studied delivery strategies for RNA-based therapies are lipid-based materials. These structures, known as lipid nanoparticles (LNP), are composed of a cationic or, more recently, a pH-dependent lipid layer (ionizable); a polyethylene glycol (PEG) component; cholesterol; and phospholipids [29]. The ionizable amino lipid coating of the liposome is designed to acquire a positive charge as the pH decreases, facilitating easier endosomal absorption while maintaining the encapsulation of the negatively charged mRNA molecule. In addition to its role as a stabilizer with cholesterol, the PEG molecule also plays a crucial role in preventing breakdown by

macrophages [30–32]. It is well-known that the construction of the amino lipid component greatly influences delivery efficiency, tolerability, and tissue clearance [33]. Attempts to enhance mRNA vaccine delivery through LNP have achieved effective RNA release in cell lines and robust, long-term humoral immune responses for various viral infections in mouse models [34–36]. Although the follow-up period for clinical trials of two mRNA vaccines based on LNP for SARS-CoV-2 during the COVID pandemic was relatively short, positive outcomes were confirmed, indicating good effectiveness in diverse populations, and ultimately leading to regulatory approval [37, 38]. However, the design of LNPs needs to be improved to ensure that the mRNA cargo specifically targets antigen-presenting cells (APCs) in cancer vaccines (CVs). This will help prevent degradation and maintain optimal translation processes [39–42].

7.3.2.2 DELIVERY SYSTEMS BASED ON POLYMERS

mRNA vaccines against deadly viruses such as Ebola, HIV, Zika, and H1N1 influenza have gained popularity due to the use of polymeric polymers and dendrimers modified with nanotechnological fatty side chains. These modifications help reduce harmful effects and prevent enzymatic breakdown *in vivo* [43–47]. Studies using murine models have shown that an antiangiogenic RNA sequence delivered through polymeric structures, enclosed in a PEG outer shell, can inhibit pancreatic cancer growth [48]. Similar mRNA vaccines have demonstrated the ability to effectively produce tumor-related antigens *in vivo* [49]. In a castration-resistant prostate cancer model, administration of an RNA vaccine based on a polymer encoding PTEN resulted in decreased tumor growth, as reported by Islam et al. [50]. This was achieved by restoring PTEN's normal function.

7.3.2.3 DELIVERY SYSTEMS BASED ON PEPTIDES

Cell-penetrating peptides (CPPs) must be cationic in order to transport proteins, small chemical molecules, nanoparticles (NPs), and oligonucleotides through the cell membrane. CPPs are a potential family of non-viral delivery vectors due to their low toxicity and high transfection efficiency [51–53]. However, the limited selectivity of cells and tissues, as well as inefficient cargo absorption across multiple layers of cells, are limitations to the widespread implementation of this technique in a medical context

[54]. Recent research has focused on discovering the most effective CPPs to enhance immunological activation. Cationic peptides like protamine can protect RNA from degradation in lysosomes during transport. Activation of TLR 7 with a protamine-based delivery system has been shown to induce a robust immunological response [55, 56]. Biotech innovations have also made progress in mRNA release using peptides. For example, a powdered form of the cationic KL4 peptide complex, formed by PEGylation, is effectively utilized as an aerosol-based delivery system to the lungs [57]. Effective uptake and delivery of mRNA encoding the Ova peptide linked to the GALA peptide by antigen-presenting cells (APCs) resulted in increased antigen-specific T cell activation and DC maturation, compared to naked RNA or other peptide complexes [58].

7.4 SUCCESS STORIES OF MRNA IN TERMS OF CLINICAL TRIAL

Many successful milestones have been achieved in the translation of mRNA-based immunotherapeutics from bench to bedside. However, each milestone has presented new challenges in order to make it more suitable and convenient for patient care.

There has been significant interest in cancer immunotherapies following the approval of six immune checkpoint blockers and CAR-T cell immunotherapies. Notably, the USFDA approved an immune cell-based vaccine (PROVENGE) for the treatment of hormone-refractory prostate cancer in 2010 [59]. Personalized cancer vaccines are being investigated in combination with checkpoint blockers or cytokine therapies for various solid or metastatic cancers, showing promising clinical results [60].

The majority of mRNA-based immunotherapeutics based in Washington, D.C. were explored in clinical trials. Additionally, various biotech companies such as CureVac, BioNTech, and Moderna investigated promising anti-tumor responses from mRNA-based immunotherapies delivered by non-viral vectors in pre-clinical studies. To suppress the tumor microenvironment (TME), mRNA-based immunotherapeutics encoding immunostimulants such as IL-12, IL-32, OX40L, CD40L, and CD70 were injected at the target site [6].

Numerous studies were conducted to analyze the platform, antigens, delivery methods, clinical end goals, and preliminary clinical data. Significant corporations carried out clinical trials of mRNA treatments. Table 7.1 illustrates some of the clinical investigations that have been conducted [62].

TABLE 7.1 ClinicalTrials.gov Registered mRNA-based Cancer Immunotherapy Trials

NCT No.	Sponsors	Antigen Platform	Delivery System	Indications	Combination Therapy, If Any	Status
NCT03289962	BioNTech and Genentech	Neoantigen mRNA	RNA lipoplex, i.v.	Multiple solid tumors.	Atezolizumab	Phase I, recruiting, 2020 completion.
NCT03815058	BioNTech and Genentech	Neoantigen mRNA	RNA lipoplex, i.v.	Melanoma	Pembrolizumab	Phase 2 recruiting, 2022 completion).
NCT03897881	Moderna and Merck	Neoantigen mRNA	Lipid nanoparticulate system; i.m.	Multiple solid tumors.	Pembrolizumab	Phase 1, recruiting, 2022 completion.
NCT03948763	Moderna and Merck	TAAs mRNA	Lipid nanoparticulate system; i.m.	Colorectal, NSCLC, pancreatic.	Pembrolizumab	Phase 1 recruiting, 2024 completion.
NCT03380871	Neon and Merck	Neoantigen peptides	Polymeric, s.c.	NSCLC	Pembrolizumab, pemetrexed, carboplatin.	Phase 1 active, not recruiting, 2021 completion.
NCT02897765	Neon therapeutics and BMS.	Neoantigen peptides	Polymeric, s.c.	Solid tumors	Nivolumab	Phase 1 active, not recruiting, 2021 completion.
NCT03639714	Gritstone oncology and BMS.	Neoantigen and self-amplifying mRNA.	Viral vector, i.m.	NSCLC, colorectal cancer, endometrial cancer, breast cancer.	Nivolumab ipilimumab	Phase 1/2 recruiting, 2022 completion.
NCT03953235	Gritstone oncology and BMS.	Neoantigen	Viral vector, i.m.	NSCLC, colorectal cancer.	Nivolumab ipilimumab	Phase 1/2 recruiting, 2023 completion.
NCT03164772	CureVac, Boehringer Ingelheim, and MedImmune	Neoantigen mRNA.	Lipid nanoparticles, intradermal injection.	NSCLC, colorectal cancer.	Durvalumab, tremelimumab	Phase 1/2 recruiting, 2024 completion.

7.5 POSSIBILITIES OF PERSONALIZED ONCOTHERAPEUTICS WITH THE MRNA-BASED VACCINE

Cancer development is a unique process, and this characteristic could be utilized to personalize treatment based on individual needs. The development of tumor resistance can lead to therapeutic failure with immunotherapy, radiotherapy, and chemotherapy. The identification of specific neoantigens, tumor microenvironment (TME), and cancer stem cell (CSC) markers can aid in stratifying the population and targeting treatment accordingly [63]. Considering the flexibility in designing mRNA-based vaccines, tailoring them to the specific stage of cancer becomes feasible. To achieve effective antitumor effects, the mRNA encoding specific antigens must be delivered properly and translated within dendritic cells (DCs). Activation of TLR4 on DCs triggers the adaptive immune response, including the generation of targeted T cell subsets and subsequent production of tumor-specific antibodies [64]. mRNA-based oncotherapeutics have shown promising results in lung adenocarcinoma [65], bladder cancer [66], and other malignancies. The mRNA-based vaccine development process can deliver various types of neoantigens suitable for highly heterogeneous tumors. It can even compensate for the limitations of immunotherapy in treating solid tumors [67]. The synthesis of mRNA-based vaccines can be achieved through an *in vitro* transcription (IVT) process, which delivers relatively stable mRNA after specific chemical modifications [68]. IVT can optimize the immunogenicity of mRNA and prevent early degradation by the innate immune system [61]. Ongoing development of personalized mRNA vaccines by Moderna, Argos, and BioNTech holds promise for positive outcomes in the future.

7.6 CONCLUSION AND FUTURE PERSPECTIVES

As we have observed, mRNA serves as a highly effective and adaptable platform for the development of CVs. Prior to 2020, no mRNA vaccine had received global approval. However, in response to the rapid spread of the deadly COVID-19 disease, the USFDA authorized the use of multiple mRNA vaccines against it, highlighting the advantages of swift and efficient mRNA vaccine production in combating newly emerging infectious diseases. In a rapid response to the pandemic, therapeutic CVs were

created by leveraging the scientific, clinical, manufacturing, and regulatory knowledge accumulated over decades of mRNA research. Studies have demonstrated that mRNA-based vaccinations elicit a favorable immune response in cancer cells, leading to apoptosis and inhibition of tumor growth. Notably, mRNA-based CVs have shown superior efficacy and reduced toxicity compared to conventional cancer treatments such as chemotherapy and radiation. Unlike vaccines for infectious diseases, which target well-characterized antigens for preventive immunization, most tumor-targeting antigens exhibit significant variation among individuals, are rare, and remain poorly understood. Consequently, concerns have been raised regarding the safety and efficacy of mRNA CVs. More and more clinical trials, particularly those focusing on tailored vaccinations, are opening the door to the creation of mRNA vaccines against a variety of malignancies. The therapeutic potential of mRNA vaccines has been hindered by their instability and the need for *in vivo* delivery. Despite significant progress made in addressing these limitations over the past few decades, the design of mRNA vaccines still faces challenges. It is crucial to intensify efforts to enhance the stability of mRNA vaccines in order to facilitate their widespread use as cancer treatments. While there is still much work to be done, mRNA therapy shows great promise. If this research is successfully translated into clinical practice, it will significantly enhance our ability to combat cancer.

Investigating and harnessing the seemingly contradictory innate immunity of mRNA, developing novel and well-tolerated delivery systems to improve antigen expression and presentation efficiency, and engineering mRNA structures to achieve prolonged and controlled expression are all areas that require further investigation. Future studies should focus on technological research and clinical development. Cancer immunotherapy will benefit from the knowledge gained by advancing mRNA through all stages of drug development for the first time. This has sparked increased interest in mRNA-based vaccines as cutting-edge immunotherapies. mRNA vaccines have the potential to make a significant contribution to the fight against cancer. Moving forward, the scientific community will explore the potential of mRNA vaccines in conjunction with standard cancer treatments.

KEYWORDS

- **antigen presenting cells**
- **cancer immunotherapy**
- **chimeric antigen receptors**
- **immune mediated adverse events**
- **immune response**
- **messenger ribonucleic acid**
- **neoantigens**
- **T-cell**
- **tumor microenvironment**
- **vaccine**

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CHAPTER 8

Autologous Vaccines for Prostate and Pancreatic Cancer

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ABSTRACT

Vaccines are medicines that help the body fight against diseases and train the immune system to identify and eliminate harmful cells. Throughout their lives, people receive multiple vaccines. Cancer vaccines (CVs) provide a similar level of protection against these viruses. There are vaccines available for both treating and preventing cancer. CVs offer potent and clinically viable therapeutic approaches to reduce tumor burden, eradicate residual cancer cells, and prevent relapse. The development of new CVs has made remarkable progress in recent years, with techniques that induce strong, long-lasting, and cancer-specific immune responses, while also improving therapeutic efficacy and minimizing systemic side effects. For both solid tumors and blood cancers, autologous CVs are potential personalized immunotherapeutic treatments that rely on the patient's own cells to stimulate an immune response. Using autologous tumor cells as a source of tumor antigens (TAs), vaccination with these cells offers several advantages. This type of vaccine can be tailored to the patient and contains antigens that can activate both Th cells and Cytotoxic T lymphocytes (CTLs). As a result, they are considered personalized vaccines. However, the effectiveness of the

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vaccine will be limited if the tumor does not provide enough antigens. The current standard systemic treatment for prostate cancer consists of a prostate cancer vaccine combined with anti-hormonal and cytostatic medications. An autologous pancreatic cancer vaccine is created by genetically modifying pancreatic cancer cells unique to the patient to produce a cytokine called granulocyte-macrophage colony-stimulating factor (GM-CSF), which has potential immunostimulatory and anti-tumor effects.

8.1 INTRODUCTION OF AUTOLOGOUS CANCER VACCINE (CV)

The development of vaccinations has opened up new avenues for the prevention and treatment of infectious illnesses. The first vaccination was discovered in 1796 by Edward Jenner, who discovered that the cowpox vaccine may prevent smallpox infection [1]. Recent advancements in immune system-based tumor treatment methods have made immunotherapy a viable option for cancer treatment [2]. With the expansion of antibody-mediated immunotherapies like checkpoint blockade, cancer vaccines are once again emerging as potential therapeutics. Both in pre-clinical and clinical settings, T-cell responses to modified tumor epitopes are often associated with the success of immunotherapy [3]. Personalized cancer immunotherapy encompasses various techniques, but autologous tumor cell-based vaccines (ATVs) have shown significant promise in inducing potent antitumor immune responses [4]. Research into autogenous vaccines dates back to the early 1900s. The first autogenous vaccine was introduced by Sir Almroth Edward Wright in 1903, and various case reports about its manufacturing and applications were published in the years that followed [5]. An autologous vaccination is created by removing tumor cells from a person and using those cells to create a vaccine preparation at lab scale. Later on, it is given to the person whose tumor cells were removed. An autologous cell vaccination may induce a cytotoxic T-lymphocytic immune reaction to cell surface-expressed tumor-associated antigens (TAAs), leading to tumor cell death. This reaction is typically accompanied by an adjuvant immunostimulant [6]. An autogenous (autologous) vaccine, also known as an autovaccine, is a therapeutic vaccine created specifically for a patient with a history of recurring or chronic illnesses. In veterinary medicine, autogenous vaccines are frequently used to treat infectious illnesses that affect domestic and agricultural animals (stable or herd-specific vaccinations) [7]. In order to treat a variety of chronic illnesses, including skin infections, infections of

the respiratory system, infections of the colon, and infections of the urinary tract, autogenous vaccinations were administered to adults, children, and newborns. Additionally, autogenous vaccinations were applied in cases of bronchial asthma, sepsis, gonorrhea, candidiasis, and osteomyelitis, among others [7].

Vaccines used for cancer treatment are different from those used for virus prevention. These vaccines aim to stimulate a normal immune response to eliminate cancer cells in humans. Their purpose is to enhance the body's defenses against diseases rather than simply avoiding illness [8]. Some cancer therapy vaccines contain cancer cells, components of cancer cells, or purified antigens. In order to produce the vaccine, occasionally a patient's own immune cells are taken and exposed to these substances in a laboratory. Once the vaccine is prepared, it is administered to the body through an injection to enhance the immune system's ability to fight against cancer cells [9].

8.2 APPROVED CV AND ITS PROPOSED MECHANISM

CVs function by enhancing antigen presentation pathways to induce the T cell response. Antigen selection is a crucial stage in the development of CVs. The effectiveness of cancer vaccinations heavily relies on the T lymphocytes' ability to recognize tumor antigens (TAs) [10]. An ideal antigen for a CV should be highly immunogenic, specifically expressed in cancer cells (but not in normal cells), and essential for the survival of cancer cells [1]. There are two categories of TAs: tumor-specific antigens (TSAs) and tumor-associated antigens (TAAs). TSAs are a group of proteins exclusively found in tumor cells. They are also known as neoantigens, which are non-self-proteins resulting from specific mutations in tumor cells [11]. Neoantigens are solely produced by tumor cells, leading to a targeted T-cell response against the tumor with minimal "off-target" damage [12]. In recent years, the focus of tumor vaccination has shifted towards CVs targeting neoantigens. Promising results have been observed in recent clinical trials exploring neoantigen vaccinations, with improved patient survival [13]. The widespread use of next-generation sequencing (NGS) techniques enables the rapid and cost-effective identification of personalized neoantigens. Additionally, the development of epitope prediction algorithms for MHC I binding has greatly facilitated the discovery of potential novel immunogenic epitopes [14]. In recent years, researchers have expanded the pool of antigens for immunization by combining common antigens with neoantigens to enhance the

effectiveness of vaccines. For example, the APVAC1/2 vaccines have been successful in stimulating T-cell responses in the treatment of glioblastoma, as they contain both common tumor antigens (TAs) and patient-specific neoantigens [15]. Personalized neoantigen vaccines, when combined with PD-1 or PD-L1 inhibitors, have also shown promising anti-tumor effects in early clinical investigations. Tumor-associated antigens (TAAs) are versatile and can be used in a variety of patients. Initially, TAA-based cancer vaccines primarily targeted TAAs. However, T cells that recognize TAAs and potentially other autoantigens may be eliminated during development due to central immunological tolerance in the thymus, which can affect the efficacy of vaccination [16]. Therefore, TAA-based cancer vaccines need to be potent enough to "break the tolerance." Despite years of research on TAAs, clinical studies of TAA-based cancer vaccines have only achieved limited success, as shown in Figure 8.1. Additionally, the expression of TAAs in non-cancerous tissues poses the risk of vaccine-induced autoimmune damage [17].

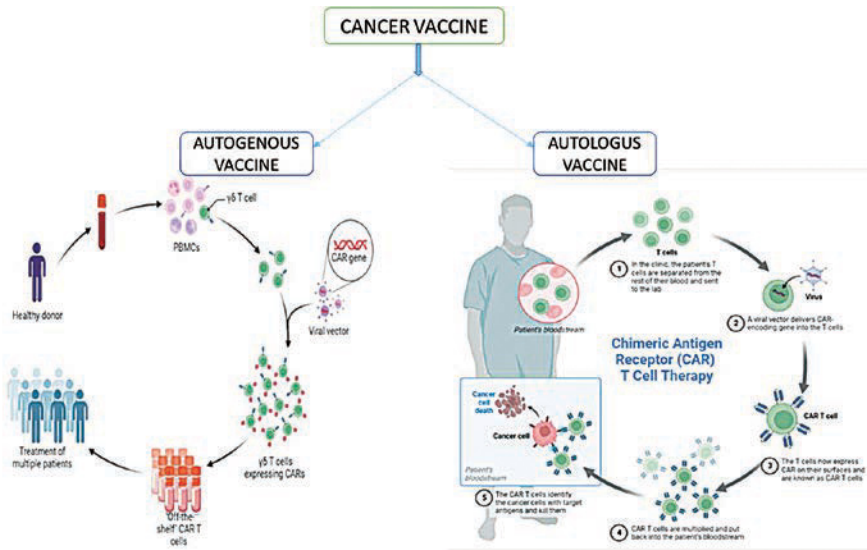

FIGURE 8.1 Types of CVs.

8.3 DEVELOPMENT OF AUTOLOGOUS CV

Over the past five decades, autogenous CVs have been extensively studied in clinical trials as exploratory immunotherapies with the goal of inducing or enhancing CD8+ cytotoxic T cell lymphocyte (CTL) TA-specific responses [18]. In the past, numerous pivotal studies and hundreds of autogenous CV

clinical trials were mainly unsuccessful in proving a distinct therapeutic advantage [19]. Several factors, including but not limited to: [1] insufficient antigens; [2] a lack of effective adjuvants; [3] insufficient immunogenic platforms; and [4] a lack of CTLs attempting to enter the tumor as a consequence of immunosuppression caused by a high burden of disease, insufficient immune wellness, or an immunosuppressive tumor cell microenvironment [20].

Neoantigen-based vaccines are customized treatments for specific tumors, often focusing on several tumor-associated antigens (TAs) that are unique to each patient [21]. A biopsy of the tumor is collected for whole-exome plus RNA sequencing to identify and confirm the presence of quasi-genetic abnormalities expressed in the tumor, which are then included in an autologous vaccine. Major histocompatibility class I (MHCI) motif prediction techniques are used to prioritize mutations after analysis. Ranked lists of candidate antigens are further refined based on the outcomes of an *in vitro* binding assay, where synthesized peptides are tested for their affinity to a relevant class I leukocyte antigen gene of interest [22]. Neoantigen-specific T lymphocytes are less likely to be eliminated during the acquisition of immune self-tolerance compared to T cells that are specific to tumor-associated antigens (TAAs), as some neoantigens are specific to the tumor. This enhances their ability to induce potent T cell responses, increases their immunogenicity, and expands and diversifies the immune responses [23]. Variant mutations can be targeted by neoantigen-based vaccines. These mutations can include frameshift mutations caused by the deletion or insertion of numerous nucleotides in the genome, as well as single nucleotide variants resulting from a shift of a single nucleotide from one base to another. The selected neoantigens can be clonal, present in all tumor cells, or subcloned, found only in a portion of tumor cells. Both types of neoantigens have an impact on immunoreactivity [24]. Furthermore, mutations can be categorized based on their effect on tumor development. Driver mutations, which provide growth advantages selected during tumor evolution, and passenger mutations, which lack inherent growth benefits, can be utilized to create customized cancer vaccines [25].

8.4 TREATMENT PROTOCOL BY CVS

8.4.1 RECENT TREATMENT PROTOCOL FOR PROSTATE CANCER

Due to tissue-specific antigens present only in the prostate gland, prostate cancer appears as an ideal target for this immunotherapy [26]. Additionally, the disease often progresses slowly because cells are constantly proliferating

[27]. This allows enough time for the immune system to respond to the vaccine, especially considering that repeated vaccinations are required for the best anti-tumor immunological response [28].

In terms of total cancer incidence, prostate cancer ranks fourth overall and second among males. In 2020, there was an increase in prostate cancer diagnoses to over 1.4 million [29]. Age and prognosis variables affect the initial treatment of patients with prostate cancer. Surgery or radiation treatment can be used to treat individuals with localized cancer when screening tests are not an option. However, disease recurrence can occur in up to 30% of patients, which is often indicated by a steadily rising blood level of prostate-specific antigen (PSA) [30]. Several novel hormone treatment strategies for metastatic or advanced prostate cancer have been authorized for clinical usage in recent years [31]. Despite hormone treatment's temporary ability to stabilize the disease, recurrent prostate cancer inevitably progresses to metastatic castrate-resistant prostate cancer (mCRPC). Patients with mCRPC have limited treatment options. Docetaxel was approved in 2004 due to its demonstrated survival advantage, despite its significant side effects. However, newer therapies such as cabazitaxel, abiraterone, and enzalutamide, which have been approved since 2004, are currently only indicated for patients who have already received docetaxel treatment. These therapies do not address the need for less toxic treatments in cancer patients without cancer-related pain who choose to avoid chemotherapy [32]. Furthermore, studies have shown that enzalutamide and the medication apalutamide can reduce the incidence of metastases in males with CRPC that has not yet spread to other parts of the body. Additionally, darolutamide has been shown to prolong the period of survival without cancer progression in men [33].

Two forms of immunotherapy are being explored for prostate cancer: vaccines and checkpoint inhibitors. Treatment vaccines are injections that stimulate the immune system to recognize and fight against tumors [34]. Only Sipuleucel-T, which received FDA clearance in 2010 for use in patients with asymptomatic or slightly symptomatic mCRPC, is a cancer immunotherapy vaccine that has been approved for use in clinical oncology [35]. Immune checkpoint inhibitors (ICIs) are a type of medication that disable proteins on immune cells, enhancing the immune system's ability to eliminate cancer cells [36].

8.4.1.1 CELL-BASED VACCINE

Autologous (patients' own tumor cells) or tumor cell lines are used to create cell-based vaccinations (allogeneic). In this case, a human GM-CSF gene

was transfected into two prostate cancer cells, LNCaP and PC-3, to produce the granulocyte macrophage colony-stimulating factor-transduced allograft prostate cancer cell vaccine (GVAX). However, in two clinical phase 3 studies, both when used alone and in combination with docetaxel, GVAX proved to be ineffective (VITAL-1; VITAL-2) [37].

8.4.1.2 PEPTIDE-BASED VACCINE

The creation of peptide vaccines requires the use of specific TAA epitope subunits. Similar to cell-based vaccines, techniques for peptide vaccinations can be based on either generic TAAs or specific peptide structures. Several peptide vaccines have undergone testing in early-stage 1/2 clinical studies, and the preliminary findings are highly positive. For example, a directed Ras homolog gene family member C (RhoC) elicited a strong and persistent T cell response in the majority of patients who had undergone radical prostatectomy (NCT03199872) [38]. In the majority of mHNPC patients who were not selected based on their HLA type, the human telomerase reverse transcriptase (hTERT) peptide vaccine UV1, in conjunction with GM-CSF, induced targeted immune responses with manageable side effects. Additional participants are needed for early-stage therapeutic trials using the B-cell lymphoma extra-large protein (Bcl-xl) Bcl-xl 42, a peptide fragment, and 42-CAF09b, an adjuvant that enhances immunostimulation [39].

8.4.1.3 VIRAL/BACTERIAL-BASED VACCINES

PROSTVAC (PSA-TRICOM), a human PSA vaccine, utilizes two distinct poxvirus vectors (PROSTVAC-V and -F). These vectors incorporate three T cell co-stimulatory molecules (TRICOM) to enhance the immunological response. However, the promising results observed in a phase 2 study (NCT01322490), which examined patients receiving PROSTVAC (n = 432) or PROSTVACplus macrophage colony-stimulating factor (n = 432) compared to placebo (n = 433), did not carry over to the phase 3 setting. There was no difference between the two treatment approaches in terms of overall survival (OS) or the percentage of patients who maintained their health [40].

8.4.2 RECENT TREATMENT PROTOCOL FOR PANCREATIC CANCER

One of the cancers with the highest mortality rates is pancreatic cancer. In the US, there were 45,750 fatalities and 56,770 diagnoses recorded in

2019 [6]. According to one research, by the year 2030, pancreatic cancer will surpass breast, prostate, and colon cancers as the leading cause of cancer-related death in the US [42]. These figures demonstrate the urgent need for new and innovative treatment options for individuals with this fatal condition. In fact, several initiatives are underway to alter the course of the disease and achieve better outcomes, similar to those seen in other cancers. Chemotherapy remains the primary treatment approach for pancreatic cancer at all stages of the disease. Based on recent evidence and approvals from the US Food and Drug Administration (FDA), the three main therapeutic groups are adjuvant, unresectable or metastatic, and neoadjuvant or induction treatments [42].Whole-cell vaccinations, peptide-based vaccines, DC vaccinations, new vaccines (plasmid immunizations, virus-based vaccinations, bacteria vectors, as well as fermentation recombinant vaccines), and mRNA immunization are all types of cancer vaccines that are now available on the market. However, these vaccines face significant challenges today due to the severity of pancreatic cancer (PC), the side effects of chemotherapy or radiation, and the weakened immune systems of patients. Some of the methods used in cancer vaccination to enhance anti-cancer immunity include the introduction of tumor antigens (TAs), often in combination with antigen-presenting cells (APCs) such as dendritic cells (DCs) and other immunological modulators that can alter the tumor. The main methods used to treat metastatic PC involve cytotoxic drugs or antibodies that target CD-8+ T cells, which can kill tumor cells or inhibit their reproduction. Nearly all cancer vaccines work by stimulating tumor-specific CD-8+ cytotoxic T cells through the injection of MHC class I-restricted peptide epitopes derived from antigens expressed exclusively on the tumor cells. In a recent multicenter Phase II study, patients with PC (n=30) who received the peptide cocktail vaccine OCV-C01 in combination with gemcitabine, the recommended first-line treatment, had a mean Disease-Free Survival (DFS) of 15.8 months, which was an improvement over gemcitabine monotherapy [43]. Therefore, therapeutic strategies that combine chemotherapy and vaccines may increase the number of melanoma-specific T cells in immunogenic tumors, leading to more favorable outcomes [44].

Clinical studies have demonstrated the safety and effectiveness of DC-based vaccinations for prostate immunotherapy in inducing tumor-specific immune responses. The clinical results of DC vaccination, a potential immunological treatment for prostate cancer with metastatic spread, are often promising. Studies have investigated the effects of a DC vaccine targeting prostate cancer with metastatic disease. For example, in a stage one pilot

study on the MUC1-peptide DC vaccine in patients with metastatic prostate cancer, Rong et al. found that the vaccine increased the immune response to the cancer antigen MUC1 in patients who had metastatic prostate cancer without causing significant harm [45]. According to Mehrotra et al., a potential therapeutic approach to prevent prostate cancer metastases by activating CD8+ T cells involves immunization with peptide-pulsed DCs and the TLR-3 agonist poly-ICLC [46]. Liang et al. investigated the CTL responses induced by DC immunization for pancreatic cancer using longitudinal evaluation of therapy responses, and they demonstrated that DC vaccinations can significantly increase CTL response and inhibit the migration of cancer cells [47].

The following are some ICIs for pancreatic cancer.

1. **Ipilimumab:** A fully human anti-CTLA-4 IgG1 monoclonal antibody, received clinical authorization for use in the United States and Europe in 2011 [48]. However, the outcomes for patients with localized and advanced or metastatic pancreatic adenocarcinoma (PAC) have been poor. Ipilimumab therapy in PAC patients has also been associated with increased toxicity [49]. Interestingly, combination chemotherapy has shown more promising results. In fact, gemcitabine, a common therapy for advanced PAC, has demonstrated enhanced immunological response by boosting naive T cell activation [50].
2. **Tremelimumab:** In a stage II study (open label), the anti-CTLA-4 inhibitor tremelimumab, a human IgG2 mAb, was evaluated as a monotherapy (NCT02527434) [51]. Patients who met the eligibility criteria experienced tumor progression after receiving a 5-FU gemcitabine regimen in the cohort of patients with metastatic pancreatic carcinoma. Tremelimumab yielded unsatisfactory results, with disease progression observed in 18 out of 20 individuals. However, in a phase I trial (NCT00556023), Aglietta et al. assessed the efficacy of Tremelimumab in combination with gemcitabine [52].

Different vaccine therapy for pancreatic cancer:

1. **GVAX:** Granulocyte-macrophage colony stimulating factor (GM-CSF) is a protein expressed by pancreatic cancer cells in the irradiation allogeneic whole tumor cell vaccination known as GVAX [53]. This process stimulates T cell priming and APC antigen uptake [54]. In a phase I study, GVAX was evaluated in patients with localized PAC after resection but before adjuvant chemoradiotherapy.

The safety and efficacy of GVAX in promoting the development of anti-tumor immunity were assessed by examining enhanced delayed-type hypersensitivity reactions to autologous tumors after immunization [55].

2. **Survivin-2B 80-88 (SVN-2B) and KIF20A-66 Peptides:** These are derived from the tumor-associated antigens (TAAs) kinesin family member 20A (KIF20A), which are up-regulated in pancreatic adenocarcinoma (PAC). These peptides represent HLA-A24-restricted cytotoxic T cell epitopes. The epitope peptide KIF20A-66 was developed as a cancer vaccine. In a phase I/II clinical study, patients with metastatic pancreatic cancer who received KIF20A-66 peptides as second-line therapy showed a better prognosis compared to the control group receiving best supportive care after gemcitabine chemotherapy failure [56–58].

3. **Algenpantucel-L:** It is a whole-cell vaccine composed of two genetically modified allogeneic pancreatic cell lines (HAPa-1 and HAPa-2) that express the murine enzyme (1,3)-galactosyltransferase (GT), which is the main barrier to xenotransplantation and the cause of hyperacute rejection. Ongoing clinical trials are comparing algenpantucel-L to chemotherapy and chemoradiotherapy in individuals with borderline resectable and metastasized unresectable PAC [59, 60].

4. **Mucin 1 (MUC-1):** The type I transmembrane protein with significant O-linked glycosylation, known as mucin 1 (MUC-1), is essential for normal cell signal transduction as well as oncogenic signaling that promotes invasion, angiogenesis, and metastasis. In solid tumors, including PAC, it is highly expressed in cancerous cells [61, 62]. A phase I trial using the MUC-1 peptide in PAC found that mucin vaccination is safe and may enhance responses to tumor antigens (TAs). A MUC1 peptide-loaded DC immunization used as adjunct therapy was shown to be well tolerated and had no harmful side effects. Four out of 12 individuals who received the vaccine have been observed for more than four years and show no signs of recurrence [63].

5. **Dendritic Cell Vaccine (DC):** Antigen-presenting cells (APCs) known as DCs have the capability to activate immature T cells and enhance the immune system's ability to combat tumors [64]. *Ex-vivo*

DCs obtained from patients are cultured with a specific antigen to generate DC vaccines, which are then activated and matured before being reintroduced into the patient. In a recent study, researchers once again administered autologous DCs separated from the peripheral blood of HLA-A2 positive patients. Three different A2-restricted peptides, namely survivin, carcinoembryonic antigen (CEA), and human telomerase reverse transcriptase (hTERT, TERT572Y) (SRV.A2), were loaded into the DCs. Additionally, Poly-ICLC was administered intramuscularly (I.M) to the patients [64]. The most common side effects of the treatment were fatigue and flu-like symptoms. Early experiments have shown promising results with other synthetic peptides derived from pancreatic melanoma antigens that could potentially be loaded into DCs [65].

6. **K-Ras Vaccine:** According to the K-Ras vaccine, the K-Ras oncogene was found to be mutated in 95% of people with pancreatic cancer. A previous study conducted in 1995 discovered a temporary Ras-specific T-cell response in 2 out of 5 patients with PAC and a KRAS mutation who were vaccinated with a synthetic Ras peptide that matched the K-RAS mutation found in the tumor tissue. Surprisingly, individuals with advanced illness who responded to the peptide vaccine had a longer overall survival of 4.9 months compared to non-responders who had 2 months. Peptide-specific immunity was generated in 58% of patients. However, a more recent study included patients with localized PAC who had undergone adjuvant chemotherapy [66, 67].

8.5 PIPELINE OF CV

Patients, their families, medical professionals, researchers, and the general public can easily access information on officially and privately funded clinical studies for various illnesses and conditions through ClinicalTrials.gov, a web-based resource. Websites provide information on medical research involving human volunteers. Clinical trials are primarily documented on ClinicalTrials.gov as interventional studies. In a clinical trial, human volunteers are randomly assigned to interventions, such as medical products, behaviors, or surgeries, according to a protocol or plan. The results of these interventions on biomedical or healthcare outcomes are then evaluated. Information on the pipeline of many prostate and pancreatic CVs can be found on the webpage [68] (Tables 8.1 and Table 8.2).

TABLE 8.1 Clinical Trial Studies of Different Vaccines for Prostate Cancer, as per USFDA, from 2015 to 2022

NCT Number, Phase, and Status	Out Come	Start Date	Completion Date	References
NCT05104515 Phase I – R	NRA	01–11–2021	31–01–2023	[69]
NCT05010200 Phase I – R	NRA	05–01–2022	01–12–2027	[70]
NCT04701021 Phase I – R	NRA	07–02–2021	31–12–2023	[71]
NCT04090528 Phase II – R	NRA	21–10–2019	01–12–2025	[72]
NCT03532217 Phase I – C	NRA	14–09–2018	25–07–2022	[73]
NCT03493945 Phase I–II – R	NRA	01–05–2018	31–12–2023	[74]
NCT03481816 Phase I – C	• Castration-resistant metastatic prostate cancer (mCRPC) progresses due to low levels of testosterone. • Vaccinations are being developed to enhance the immune response in identifying and eliminating cancer cells. • The effectiveness of ETBX-071, ETBX-061, and ETBX-051 is currently being evaluated against mCRPC.	24–07–2018	09–03–2020	[75]
NCT03315871 Phase II – R	NRA	20–03–2018	01–12–2023	[76]

TABLE 8.1 *(Continued)*

NCT Number, Phase, and Status	Out Come	Start Date	Completion Date	References
NCT02485964 NA-C	• PSA is considered the ideal antigen for the treatment of prostate cancer. • These vaccines have the ability to enhance Langerhans cells, thereby promoting T-cell activity. They also exhibit antigenicity by containing a large number of T-cell aptamers and demonstrate high bioavailability in a single solution.	01–08–2015	01–12–2018	[77]
NCT01706458 Phase II – C	• For individuals who have not responded to hormone therapy, this treatment is intended to address issues within the body. • Vaccines may help the body mount a potent immune response to eradicate tumor cells.	20–05–2013	13–08–2020	[78]
NCT01696877 Phase I-II – C	• Men who will undergo a procedure to remove their cancerous prostate glands and who have previously received standard hormonal therapy are administered a single intravenous injection of the drug cyclophosphamide and the investigational prostate cancer vaccine GVAX.	18–01–2013	18–12–2018	[79]
NCT01487863 Phase II – C	• The study determines the effectiveness and safety of sipuleucel-T in males with castrate-resistant metastases of prostate cancer.	01–12–2011	01–06–2016	[80]

*R: Recruiting; **C: Completed; *** NRA: Non-recurring; and **** NA: Not applicable.

TABLE 8.2 Clinical Trial Studies of Different Vaccines for Pancreatic Cancer, as per USFDA, from 2015 to 2022

NCT Number	Outcome Received	Start	End	References
NCT05111353 Phase I – R	The safety of the vaccination was assessed by counting the number of patients who experienced each type of adverse event. Vaccine immunogenicity was assessed by the amount of neoantigen-specific T cells (both Arm 1 and Arm 2).	10–Oct–22	31–Dec–27	[81]
NCT03956056	Vaccine immunogenicity is determined by ELISPOT analysis.	13–Feb–20	21–Jun–23	[82]
Phase I – R	Vaccine immunogenicity is determined by multiparametric flow cytometry.			
NCT05013216 Phase I – R	The study evaluates the enhanced performance of transformation in interferon (IFN-Î3) generating mutant-KRAS-specific CD8 and CD4 T cells, the number of study participants who experienced adverse effects related to the study drug, and the fold change in interferon-producing mutant-KRAS-specific CD8 and CD4 T cells at week five.	11–Apr–22	01–May–26	[83]
NCT05116917 Phase II – R	The following statistics are calculated: overall survival (OS), progression-free survival (PFS), disease control rate (DCR), disease management rates (DCR), EORTC QLQ-C30, and adverse treatment events.	05–Nov–21	01–Dec–24	[84]
NCT04117087 Phase I – R	The percent change in interferon (IFN)-Î3-producing mutant-KRAS-specific CD8 and CD4 T cells, as well as the disease-free survival (DFS) rate, is calculated.	29–May–20	01–Jun–24	[85]
NCT04810910 Phase I – R	Participant numbers for adverse clinical and laboratory events (AEs), relapse-free survival (RFS), and overall survival (OS) are determined.	30–Mar–21	30–Mar–25	[86]
NCT04161755 Phase I – R	Substance-related toxicity.	11–Nov–19	11–Nov–23	[87]
NCT04799431 Phase I – R	The progression-free survival (PFS) according to RECIST 1.1, the disease control rate (DCR), and the overall survival rate (OS) are calculated.	01–Jan–23	01–Jan–28	[88]
NCT04157127 Phase I – R	MTD of DC vaccine, dose limiting toxicities (DLTs) reported, time to recurrence, and overall survival	03–Aug–20	Nov–25	[89]
NCT04627246	Analysis of the carbohydrate antigen 19-9, a tumor marker (CA19-9).	11–Sep–20	Sep–28	[90]
Phase I – R	Immunological response gene and cell analysis, and their link with both immune-related adverse reactions and recurrence, free existence.			

TABLE 8.2 *(Continued)*

NCT Number	Outcome Received	Start	End	References
NCT05438667 Early Phase I – R	Time to progression; overall survival; progression-free survival, peak plasma concentration; Perivascular TCR-T cell count, maximal plasma concentration (C_{max}), peak timing, and peak value of cytokines (T_{max}).	07–Jun–22	30–Jun–25	[91]
NCT04111172 Phase II – R	Adverse event (AE) frequency | guanylyl cyclase C-specific T-cell response (GCC).	10–Nov–20	Dec–24	[92]
NCT03953235 Phase I-II – R	Please determine how the immune system reacts to the neoantigens expressed by the GRT-C903 and GRT-R904 genes. Phase 1 – Objective response rate (ORR) with RECIST v1.1 Clinical benefit rate (CBR) using RECIST v1.1 and duration of response (DOR) using RECIST v1.	18–Jul–19	Dec–23	[93]
NCT04246671	Patients with toxic dose limitations.	10–Aug–20	Jan–26	[94]
NCT04807972 Phase II – R	The study includes the patients' global impressions of severity (PGIS) and the patient global impression of changes (PGIC) measures to assess participants' overall perception of the changes in their pancreatic cancer symptoms.	28–May–21	15–Jun–25	[95]
NCT03948763 Phase I – C	Dose-limiting side effects, frequency of adverse events, frequency of objective responses, mutant KRAS-specific T cells, and the proportion of patients who discontinued study therapy due to an adverse event.	26–Jun–19	–	[96]
NCT03767582 Phase I–II – R	Participants who experienced study drug-related side effects, and the proportion of patients who responded to immunotherapy.	12–Dec–19	Mar–23	[97]
NCT02757391 Phase I – Terminated	The level of tetramer-positive T-cell population that develops after T-cell infusion determines whether an immune response will last, the quantity of intracellular cytokines determines whether an immune response will last.	09–Aug–19	02–Oct–20	[98]
NCT04853017 Phase I – R	Evaluate the safety of ELI-002. Please find the decrease and clearance rate of circulating tumor DNA (ctDNA).	04–Oct–21	Dec–24	[99]

*R: Recruiting; **C: Completed; *** NRA; **** NA: Not applicable.

8.6 FUTURE OF CV

There are currently more treatment options available for prostate and pancreatic cancer patients compared to the past. However, the prognosis for these diseases remains poor and they are falling behind in a rapidly developing field. In order to address this, changes must be made to the design of clinical trials, and the participation of patients in these trials should always be considered. There are still unanswered questions regarding the role of radiotherapy in cancer treatment and the specific population that will benefit from it. Additionally, new concerns have been raised regarding resistance to immunotherapy and the effectiveness of focused therapy. Nevertheless, progress is being made, from innovative pharmacological approaches to increased studies involving elderly patients, those with poor performance status, and individuals with refractory diseases. These initiatives provide hope that more effective treatment strategies are within reach [42]. Numerous clinical trials are currently underway to evaluate the efficacy of immunotherapy in treating prostate and pancreatic cancer, including the use of immune checkpoint inhibitors, adoptive cell transfer, and combinations with chemotherapy, radiotherapy, or other targeted molecular treatments. It is well established that prostate and pancreatic cancer have a low tumor mutational load and low immunogenicity. Limited mutation rate hinders neoantigen formation and release, resulting in a low number of tumor-infiltrating lymphocytes (TILs). Moreover, the stroma of pancreatic cancer tissue exhibits a highly desmoplastic nature and harbors a significant population of immunosuppressive fibroblasts. Further research should focus on overcoming immunotherapy resistance in pancreatic cancer patients by addressing multiple immune vulnerabilities through combination immunotherapy and cytotoxic approaches. Additionally, the identification of reliable biomarkers will guide the selection of optimal treatment strategies to improve patient outcomes. The integration of diverse therapeutic modalities with immunotherapy holds promise for patients with pancreatic cancer [6].

Currently, this research is undergoing rigorous testing and evaluation in clinical studies conducted in Madison, Wisconsin, under the supervision of the U.S. FDA. McNeel and his colleagues will assess antigen-specific DNA vaccines and gain insights into the interaction between prostate cancer and the immune response through the clinical trial process. According to McNeel, immunotherapies are expected to expand rapidly in the coming years, potentially including vaccines for the majority of individuals with prostate cancer. “If we can develop tumor-specific immunizations, prostate

cancer may become similar to high blood pressure, a chronic condition," he said. This approach could potentially lead to earlier interventions and improved outcomes across the board [41].

KEYWORDS

- **autologous tumor cell-based vaccines**
- **chronic condition**
- **cytotoxic T-lymphocytes**
- **dendritic cell**
- **immunotherapy resistance**
- **pancreatic cancer**
- **prostate cancer**
- **tumor-infiltrating lymphocytes**

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CHAPTER 9

Combined Therapy of Cancer with Vaccines and Chemotherapeutic Agents

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ABSTRACT

Cancer is the most vulnerable group due to the potential of malignant cells to evade and exploit the host immune system. According to the WHO, cancer was the leading cause of death globally in 2020, accounting for approximately 10 million deaths. Cancer prevention is a crucial public health concern in the 21st century, as it plays a vital role in the fight against cancer. Despite advancements in oncogenic drug development, there are still limitations such as early blood clearance, resistance to cytotoxic drugs, toxicity associated with chemotherapy, and site-specific drug delivery. In recent years, therapeutic cancer vaccinations have gained significant popularity with the development of novel treatments for specific oncologic indications. While cancer immunizations are routinely administered to previously diagnosed cancer patients, resistance to single-agent immunotherapy often leads to treatment failure and toxicity, including neurotoxicity, immunotherapy resistance, and hyper-progressive disease. Only a small portion of patients experience long-term benefits. Due to these limitations, a paradigm shift is necessary in cancer therapy. The growing body of evidence from preclinical and clinical studies supports the potential of certain chemotherapy drugs to synergize with vaccines, offering a successful approach known as

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"chemoimmunotherapy" (CIT). The purpose of studying therapeutic cancer vaccines (CVs) in conjunction with chemotherapy is to improve immune response, treatment effectiveness, and reduce resistance to single-agent immunotherapy. In this review, we will highlight the combination of CVs and chemotherapeutic agents, focusing on the latest combination tactics, synergistic effects, related mechanisms, and associated processes.

9.1 INTRODUCTION

Despite significant advances in cancer detection and understanding, typical treatment options have remained largely unchanged. Surgical resection, chemotherapy, and radiation therapy are commonly used therapies. However, in many cases, these treatments prove ineffective and lead to tumor recurrence or metastasis, necessitating an alternative approach to treatment [1]. Conventional anticancer treatment is believed to work by selectively destroying tumor cells or permanently halting their proliferation. Cytotoxic medications either disrupt DNA synthesis or cause chemical damage to DNA, resulting in the death of tumor cells [2]. According to findings in cellular biology, most chemotherapy drugs currently in use eventually activate the proapoptotic suicide machinery of cancer cells [3]. Despite the increased effectiveness and improved survival rates provided by modern treatments, the side effects and long-term consequences of chemotherapy remain a major concern for both patients and clinicians. The most dreaded adverse effects for cancer patients undergoing therapy are chemotherapy-induced nausea and vomiting (CINV) [4]. Mucositis of the mouth and gastrointestinal tract can lead to local ulceration and pain, resulting in malnutrition, impaired absorption, weight loss, anemia, fatigue, and an increased risk of sepsis. Diarrhea and constipation are other common side effects caused by chemotherapy; severe diarrhea can be fatal due to dehydration and electrolyte imbalance [5]. Studies have shown that platinum-based chemotherapies can cause hypersensitivity reactions. Evidence suggests that chemotherapy can cause persistent, subclinical musculoskeletal muscle injury, as well as central and peripheral neurotoxicity that can persist for many years after treatment is completed, significantly impacting the functional ability and quality of life of cancer survivors. Chemotherapy-induced side effects include peripheral neuropathy with many anticancer drugs, such as proteasome and angiogenesis inhibitors, vinca alkaloids, taxanes, and platinum-based therapies. Long-term chemotherapy-induced peripheral neuropathy (CIPN) is associated

with a high rate of morbidity, including depression, ataxia, and sleeplessness. Chemotherapy-induced gastrointestinal neuropathy has been linked to severe gastrointestinal impairment in cancer survivors [5, 6]. Due to adverse drug reactions, hypersensitivity, toxicities, and relapse in cancer patients undergoing chemotherapy, new treatment strategies are needed. Recently, cancer immunotherapy has become a crucial component of cancer treatment. Immunization options include DNA, peptide, protein, viral, recombinant vector (including yeast- and bacterial-based), whole tumor cells, and DC. In summary, cancer immunotherapy is a therapeutic approach that enhances the immune system's response to cancer by increasing the number of highly active cancer T cells capable of lysing tumor cells and curing malignancies [1]. However, therapeutic efficacy, uncertainty in prognosis, toxicity, and cost are the primary concerns of immunotherapy. Recent evidence suggests that combined immunotherapies will be necessary for effective cancer vaccination programs to overcome tumor immune evasion [7].

9.2 CANCER VACCINES (CVS)

Several cancer medications do not induce long-term immune cells and instead treat the patient quickly, necessitating the need for this treatment to be improved so that it elicits robust responses that help prevent a recurrence. Cancer vaccinations could help to tackle this problem. Cancer therapeutic vaccinations may target a variety of cancer cell-produced antigens. The early CVs combined whole cell preparations with an adjuvant or virus to stimulate a more robust immune response. Numerous potential CV targets have been identified through recent research. Modern vaccines either target tumor-specific antigens (TSAs), which are only found in cancerous cells, or tumor-associated antigens (TAAs), which are present at low levels in both cancer cells and healthy cells. By employing recently found antigens, such as brachyury, a stimulator of the epithelial-mesenchymal transition, vaccines can also target tumor stem cells and interfere with the invasion and metastatic processes. Along with TAAs, patients can also be protected against their neoepitopes with tailored therapeutic CVs as a stand-alone treatment or in conjunction with other medicines (altered antigens generated by a specific tumor). Effector T cells may eventually get activated as a result and eliminate cancerous tissues. The benefit of CVs is that they can utilize a person's complete immune system, producing robust and long-lasting responses [8, 9]. In 1990, the Intravesical BCG vaccine (TheraCys), which was licensed

by the FDA for the efficacy and prophylaxis of initial or recurrent invasive non-muscle urothelial carcinoma after transurethral surgical removal, was the first CV ever compared to CIS DFS of four months and nine days and Ta/T1 DFS of 10 months and five days. TheraCys extended DFS to 30 months in bladder carcinoma *in situ* (CIS) patients and 22 months and five days in patients with urothelial carcinoma [10].

9.2.1 DNA IMMUNIZATION

Vaccines utilizing DNA are typically bacterial plasmids with tumor antigen (TA)-encoding genes attached to Cytomegalovirus promoters. These vaccines aim to elicit a non-specific immunogenic response that complements the adaptive immunogenic response to cancer containing these antigens. Consequently, DNA vaccines aim to enhance the generation of T lymphocytes, such as CD8+ and CD4+ cells, that specifically target tumors [11]. Another objective of DNA vaccines is to overcome immunological tolerance and induce immune memory, particularly when DNA immunization is employed to stimulate T cells against an antigen to enhance the efficacy of other therapeutic approaches [12].

9.2.2 PEPTIDE IMMUNIZATION

Vaccines made from peptides are not the genetic components of the disease being treated, such as a tumor or an infectious pathogen, and are designed to induce an immune response [13]. Peptide immunization does not overburden the immune system since only the epitope of interest is administered to the individual's immune system. These vaccinations are cost-effective and easy to manufacture. However, due to the presence of immunosuppressive chemicals in the tumor microenvironment (TME), they often fail to trigger an immune response [14], and the effectiveness of peptide-based vaccines when combined with adjuvants is unknown. Therefore, the safety of adjuvant use should be evaluated and ensured to guarantee the efficiency of peptide-based vaccines. IMLYGIC or T-VEC, an FDA-approved peptide-based vaccine that is not strictly peptide-based but can be considered as one, is a modified herpes simplex virus-1 that replicates in the tumors of recurrent melanoma patients, causing cellular injury and death. This virus is classified as a peptide vaccine because it is engineered to express a granulocyte-macrophage colony-stimulating factor, which enhances the anti-tumor

response and T-cell priming [15]. T-VEC was an FDA-approved genetically modified oncolytic viral melanoma therapy for metastatic disease in 2015 [16]. T-VEC, a type of therapeutic tumor vaccine, belongs to a novel class of medicines that utilizes a genetically engineered, GM-CSF-expressing live attenuated herpes virus. T-VEC has a dual mechanism of action, allowing it to induce both local and systemic immune responses. It is administered via injection into unresectable, nodal, subcutaneous, or cutaneous tumors in melanoma patients with recurrent disease, where it acts as a local cytotoxic agent by causing viral replication and cell death. GM-CSF produced during viral replication promotes T-cell priming by antigen-presenting cells (APCs) presenting tumor antigens (TAs) generated after tumor rupture caused by the virus. Tumor-antigen-loaded dendritic cells (DCs) then move systemically and elicit a distant immunogenic response, even though the immune reactions in the injected tumor are higher than those in distant metastases [17].

9.2.3 DC IMMUNIZATION

In 1973, Robert Steinman first described dendritic cells (DCs) due to their high MHC expression and diverse form. DC-based vaccines are produced by collecting patient DCs and *ex vivo* transfecting them with the target antigen (TA); these cells are then reintroduced into individuals with cancer, which can be done through various routes such as intralymphatic, intravenous, intranodal, and intradermal routes. DCs readily recognize and destroy tumor cells. DCs have the ability to influence adaptive immunological responses, making them effective stimulators of memory and immune cells. Additionally, DCs activate naive T cells. However, due to the limited number of DCs in peripheral blood and tissues, their population must be increased *in vivo* or *ex vivo* prior to vaccine administration [18]. The advantages of DC-based vaccines include their personalized nature, the ease of inducing an immune response as DCs are primarily responsible for initiating adaptive responses, the ability to tailor a response to a specific antigen or set of antigens, and their relative safety as they do not cause adverse effects [19]. Sipuleucel T, also known as PROVENGE, is an FDA-approved DC-based cancer vaccine used to treat castration-resistant prostate cancer that has spread beyond the prostate gland and is unresponsive to hormone therapy. While DC-based vaccines for other types of cancer have not yet been licensed, current efforts are focused on prostate cancer, metastatic melanoma, and breast cancer [20]. Sipuleucel-T (PROVENGE) cellular immunotherapy is utilized for the

treatment of asymptomatic or mildly symptomatic mCRPC. This therapy involves the extraction of autologous peripheral blood mononuclear cells, including APCs, through leukapheresis. These cells are then expanded using a fusion product of TA PAP called PA2024 and GM-CSF, which activates the APCs. During a 40-hour incubation period, the recombinant antigen is transformed into peptides by the APCs, affecting Major Histocompatibility Complex signaling and T-cell stimulation. Each dose of the vaccine contains at least 50 million activated autologous CD541 cells (also known as ICAM-1 cells) with PAP-GMCSF [15].

9.2.4 WHOLE CELL CANCER IMMUNIZATION

Whole-cell CVs are composed of whole tumor cells that have been modified *ex vivo*. Despite the lack of significant results in CVs, whole-cell CVs offer several advantages. They have the advantage of expressing both tumor-associated and tumor-specific antigens. Additionally, whole-cell CVs elicit both cellular and humoral immune responses [15].

9.3 FACTORS THAT INFLUENCE TUMOR IMMUNOTHERAPY

Many factors impact the effectiveness of tumor immunotherapy. First, it is linked to humoral immunity, which is connected to inheritances and internal microbiota [21, 22]. Second, it is related to malignant cells. Patients with a high number of clonal sources of neoantigens and minimal tumor neoantigen intra-tumor heterogeneity benefit more from treatment. Other factors that significantly impact therapeutic outcomes include tumor mutation burden and the target of tumor cell mutation. Third, environmental elements such as daily routine, food, bacterial illness, and medication usage are associated with it [23].

9.3.1 IMMUNOTHERAPY DRUGS RESISTANCE MECHANISMS

Cancer immunity encompasses the observation, balance, and evasion of immune cells [24]. Tumor cells have the ability to evade immune recognition and attack by interacting with immune regulatory cells, downregulating the expression of tumor antigens, producing immunosuppressive substances, and employing other mechanisms to evade the host immune response, allowing them to proliferate and eventually form visible tumor lesions. Furthermore, tumor development can be induced by deliberately upregulating proteins that prevent immune detection, such as Programmed death-ligand 1 (PD-L1),

arachidonic acid lipoxygenase, and Indoleamine 2,3-dioxygenases (IDO-1 and IDO-2) [23].

9.3.2 PRIMARY IMMUNOLOGICAL RESISTANCE MECHANISMS

Tumor-specific T cells produce interferon (IFN), which detects tumor cells and associated antibodies in cells that display antigens, having an anticancer impact on Antigen Presenting Cells (APCs). This result can directly obstruct the proliferation of tumor cells and cause tumor cell death. It can also attract additional immune cells, stimulate TA presentation, and increase the production of antigen presentation proteins, such as the major histocompatibility complex (MHC) molecule [25]. If the pathway is altered, programmed death-ligand one (PD-L1) expression is exposed, and the expression of PD-L1 in cancer cells increases, inhibiting PD-L1 or Programmed cell death protein 1 (PD-1) immunotherapy. Alternatively, cancerous cells directly express PD-L1, which interacts with PD-1 on T cell surfaces to inhibit active CTL, reduce the immunological response, and result in T cell reduction, leading to primary drug resistance [23].

9.3.3 THE ACQUIRED IMMUNE RESISTANCE MECHANISMS

Invading lymphocytes from tumors have the ability to unleash a significant number of IFN-γ mediated cancer cells that express PD-L1 when the body mounts an anti-cancer immune response. When combined with PD1 from CTL, PD-L1 can decrease the immunological killing action of effector T cells on malignancies [23].

9.3.4 INTERNAL AND EXTRINSIC IMMUNOTHERAPY RESISTANCE MECHANISMS

Internal and extrinsic mechanisms of immunotherapy resistance can be distinguished. Primary resistance can be caused by alterations in the antigen survival process mechanism, cancer-related gene expression and antigen mutagenesis, insufficient HLA expression, and more. Mechanism modifications in the antigen manufacturing process include MAPK pathway activation and PI3K pathway augmentation due to persistent WNT/-catenin signal pathway expression, loss of PTEN expression, or highly expressed mixed PD-L1 expression. Acquired resistance can result from the loss of IFN signaling, target antigens, HLA, and T-cell activity. The extrinsic route may

be influenced by alterations in the immunological checkpoints CTLA-4, PD-1, or others, T-lymphocyte letdown and phenotypic changes, immunosuppressive cell populations such as Regulatory T-cells, myeloid-derived suppressor cells (MDSCs), and macrophages-II, and tumor microenvironmental cytokines and metabolites like CSF-1 [26].

9.3.5 THE SELF-NEUTRALIZATION MECHANISM OF CANCER CELLS

Programmed cell death protein 1 is highly expressed in the cancerous cells of certain individuals. However, Immune Checkpoint Inhibitors PD-1/PD-L1 still have a limited effect. According to research, this condition arises because these tumor cells express both Programmed death ligand 1 (PDL-1) and Programmed cell death protein 1 (PD-1). Prior to the action of Immune Checkpoint Inhibitors (ICIs) PD-1/PD-L1, the interaction between tumor cells' Programmed Death Ligand 1 and its Programmed Cell Death Protein 1 leads to the loss of the ICIs PD-1/PD-L1 target. This process is known as self-neutralization [23].

9.3.6 ANTIGEN-PRESENTING CELLS SELF-NEUTRALIZATION MECHANISM

Antigen Presenting Cells decrease the capacity of T cells to transmit signals of Programmed cell death protein 1 by blocking the binding of Programmed cell death protein 1 and Programmed death ligand 1 to each other, resulting in reduced antitumor activity [23].

9.3.7 EXOSOME RELEASE FROM CANCER CELL SUPPRESS IMMUNITY

Exosomes, which contain the protein programmed death ligand-1 (PD-L1) and inhibit T cells from entering the tumor, are released by cancer cells to suppress the immune response [22]. Colorectal cancer, breast cancer, lung cancer, and metastatic melanoma release exosomes containing PD-L1. The immune system employs various mechanisms to inhibit Programmed death ligand 1 (PDL-1) and Programmed cell death protein 1 (PD-1), rendering them ineffective in adhering to the receptor or having any impact on the receptor site. In summary, a combination of intrinsic immune system features such as the degree of mutation, cytokine composition, and external environmental factors like gut flora and pathogenic

bacteria contribute to the total escape of the immune system, including programmed death ligand 1 (PDL-1) and programmed cell death protein 1 (PD-1) co-expression [23].

9.3.8 TREATMENT MEASURES AGAINST TUMOR RESISTANCE

Avoiding tumor cell resistance is challenging throughout the therapy process, but patients can achieve the most outstanding treatment results through proper approaches. Firstly, it is crucial to prioritize immunotherapy for the appropriate group prior to treatment. Factors such as the presence of PBRM1 gene deficiencies, intestinal flora, as well as the type and quantity of tumor markers, are all assessed to determine the patient's eligibility for therapy. Subsequently, personalized treatment strategies and combination therapy are designed for each patient to enhance cancer cell sensitivity to immunotherapy, accelerate tumor cell death, and minimize the development of drug resistance in tumor cells [23].

9.4 RATIONALE FOR COMBINING CHEMO- AND IMMUNOTHERAPIES

Full-dose chemotherapy is known to affect immune system cells that replicate. In fact, high-dose chemotherapy can induce immunosuppression, and medications developed for cancer treatment are now primarily used for autoimmune diseases or to prevent organ transplant rejection [3]. Increasing evidence suggests that certain chemotherapeutic drugs are more effective against tumors in immune-competent hosts compared to immunodeficient ones. Conventional chemotherapy has the potential to enhance the immune system. Targeted chemotherapeutic drugs, which are designed to target biochemical pathways essential for tumor cell survival and proliferation, also possess immunomodulatory properties. Chemotherapeutic agents enhance both the innate and adaptive arms of the immune system through various mechanisms [27]. Chemotherapy alone often falls short of curing cancer patients, and recurrence is common due to clinically undetected micrometastases. Therapies that can prevent recurrence are actively being explored in combination with chemotherapy to improve treatment outcomes. Cancer immunotherapy holds significant potential for treating various malignancies as it aims to enhance the body's inherent ability to fight tumors by improving the effectiveness of its own defense mechanisms. A successful anti-tumor immune response requires multiple crucial stages, starting from the production of antigens by cancer cells

and culminating in the destruction of cancer cells [28]. Combining immunotherapy and chemotherapy, known as chemoimmunotherapy (CIT), explores the concept that CIT may have a synergistic effect against certain malignancies, as depicted in Figure 9.1. However, CIT has not made significant progress in recent years due to the discovery that several chemotherapy agents often induce immunosuppression, especially at high doses. The notion that chemotherapy and immunotherapy are conflicting paradigms has been disproven by extensive research on anticancer immune responses, leading to the emergence of CIT as a new field of cancer research. The distinct immunological reactions induced by immunotherapy have a significant prognostic and predictive impact on cancer patients undergoing chemotherapy in these combination systems. By eliminating regulatory T cells (Tregs) and MDSCs, chemotherapy can reduce immunosuppression [29], while also increasing the susceptibility of cancer cells to the lethal action of T lymphocytes (CTLs), thereby enabling more effective immunotherapy. Recently, various immunotherapies such as immunization, TLR agonists, monoclonal antibodies (mAbs), adoptive immunotherapy, and immune checkpoint inhibitors have been proposed in conjunction with chemotherapy to combat cancer. These immunotherapies may target different stages of the cancer-immunity cycle. In this chapter, we will discuss the combined treatment of vaccines and chemotherapeutic agents [30].

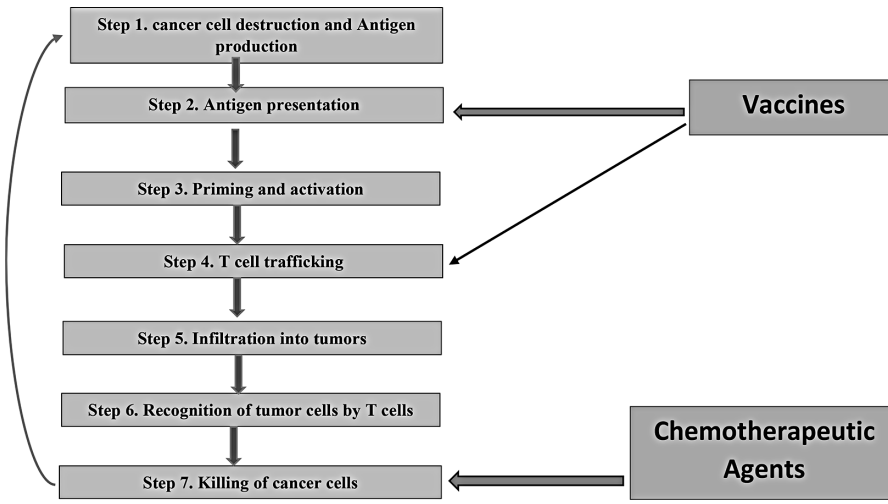

FIGURE 9.1 Combined effect of chemotherapy and vaccine on cancer-cell immunity cycle.

Note: Steps 1–7 shows the cancer immunity cycle. Chemotherapeutic agents act on Step 7 where it kills cancer cells, but the antigen that is released from it may cause relapse. Vaccine activates APCs and DCs to kill cancer cell but may not be effective alone. Combining the vaccine and chemotherapeutic agent has a synergistic effect as well as it prevents relapse.

9.4.1 EFFECTS OF CIT ON SYNERGY

One reason tumors grow is their ability to evade immune surveillance. Based on the results of preclinical and clinical studies, the combination of immunotherapy and chemotherapy has shown remarkable efficacy. This includes enhanced anti-cancer activity, inhibition of cancer infiltration and metastasis, reversal of drug resistance, and prevention of recurrence [31].

9.4.2 INCREASED ANTI-TUMOR ACTIVITY

Numerous research studies have validated the significant impact of synergistic chemotherapy and immunotherapy on tumor burden extension. As previously mentioned, Doxorubicin treatment increased the expression of CD80 on mature monocytic myeloid cells, granulocytic myeloid cells, and DCs. The combination of effective Doxorubicin dosages and cancer immunotherapies led to the maturation of tumor-infiltrating APCs, resulting in the formation of a co-stimulatory phenotype that induced anticancer T-cell activity in both CT26 and MCA205 tumor models [32].

CVs have been utilized in chemoimmunotherapy, demonstrating the extensive infiltration of vaccine-induced, multifunctional CD8+ T cells that synergize with chemotherapy drugs to enhance tumor cell killing. Instead of a single therapy, the combination treatment of long synthetic peptides (SLP) and chemotherapeutics effectively reduced tumor cell growth. SLP vaccinations only caused a temporary decrease in tumor growth in the majority of animals, with complete tumor elimination observed in 16% of the mice. Conversely, survival rates were improved by combining chemotherapy drugs such as topotecan, carboplatin, Gemcitabine, or Cisplatin. Particularly, Cisplatin exhibited strong synergy with SLP immunization in tumor elimination, without the need for a maximum tolerable dose (MTD). TUNEL analysis of living cells revealed that mice treated with the vaccine were heavily infiltrated with TNF-producing T cells, which may be responsible for the beneficial effect. This was further supported by the fact that the TNF inhibitor, etanercept, completely eliminated this synergy. Surprisingly, the combination of the two therapies did not cause

any additional systemic adverse effects. In subsequent research, Paclitaxel and a safe lipopolysaccharide (LPS) were co-encapsulated to create a chemo-immune therapeutic nanoparticle delivery system (TLNP) as the immunostimulant. TLNP-treated mice exhibited a significantly higher number of activated CD8+ T cells, CD4+ T cells, and DCs compared to mice treated with Paclitaxel and LPS alone, which correlated with improved tumor shrinkage. The presence of significant levels of TNF and IL-12, both critical cytokines released by active APCs, suggested that the combination treatment successfully promoted the infiltration of macrophages and DCs into the TME, activating an immunological stimulatory state. Seventy percentage of TLNP-treated mice with tumors survived 30 days after therapy, compared to 50% of Paclitaxel-treated mice and 30% of LPS-treated animals, indicating the restoration of a functionally active state in the immunosuppressive TME. The robust cancer-specific immunological response to LPS may be enhanced by paclitaxel-induced tumor-associated antigens (TAs) via Immunogenic cell death (ICD), thereby enhancing the destructive impact of paclitaxel [33].

9.4.3 PREVENTING INVASION AND SPREADING

More than 90% of cancer-related deaths are caused by tumor metastases, which are the most common type of cancer. Tumor metastases are a highly complex process characterized by significant tumor heterogeneity. Several significant risks associated with tumor heterogeneity, such as vascular endothelial growth factor A (VEGFA), Tumor Growth Factor, matrix metalloproteinase (MMP), cancer-associated fibroblasts (CAFs), and Tumor Associated Macrophages, contribute to tumor invasion and metastasis [34]. Bevacizumab, the initial licensed anti-angiogenic mAb, has shown improved patient survival when administered in combination with a chemotherapeutic agent for kidney, colorectal, and metastatic lung malignancies. Preclinical studies have proposed combined targeting of Tumor Associated Macrophages (TAMs) and cancer cells as an aggressive approach to reducing metastatic lung nodules. As previously mentioned, combination therapy has resulted in a significant reduction in mRNA expression of matrix metalloproteinase (MMP-9) and VEGFA compared to monotherapy equivalents. The concurrent delivery method has effectively limited tumor development and metastasis in various mouse breast and colon cancer models. Additionally, a prodrug hyaluronic acid-PLGLAGG-DOX (HA-Psi-DOX), which is MMP-2 sensitive,

has demonstrated increased tumor accumulation ability while causing fewer systemic adverse effects. PD-1 inhibition during HA-Psi-DOX therapy has led to the recruitment of tumor-infiltrating lymphocytes (TILs) to the cancer site, inhibiting cancer spread. The robust immune response and cytotoxicity induced by HA-Psi-DOX treatment are primarily responsible for its high anti-metastasis impact [25].

9.4.4 MEDICATION RESISTANCE REVERSAL

Chemotherapy alone may temporarily slow tumor development, but it can eventually eliminate the chemo-sensitive population. Cancer cells that develop resistance to chemotherapy drugs often lead to chemotherapy failure. The overexpression of the protein wingless-type MMTV integration site (Wnt) family, for instance, is believed to hinder cell lysis and the infiltration of cancer-promoting immune cells in CAFs [35]. Accidental exposure to cisplatin nanoparticles (NPs) increases Wnt16 production, resulting in stromal remodeling and resistance to tumor cell treatment. In this study, the research team [35] developed a quercetin phosphate prodrug and transformed it into lipid-coated calcium phosphate NPs. Intravenous delivery of lipid-coated calcium phosphate nanoparticles (LCP NPs) significantly downregulated Wnt16 expression, leading to a 50% reduction in collagen compared to the untreated control group. In a stroma-rich bladder cancer model, it exhibited a synergistic anti-tumor effect when combined with cisplatin NPs. P-glycoprotein (P-gp) and multidrug resistance-associated protein (MRP-1) are two examples of ATP-binding cassette (ABC) transporters that play a crucial role in the development of multidrug resistance (MDR) in cancer therapy. A preclinical study demonstrated that the sequential administration of TLR3 agonist polylysine-polycytidylic acid (PIC) and a limited dose of cisplatin inhibited the expression of P-glycoprotein (P-gp) and MRP-1, thereby enhancing the effectiveness of chemotherapy. CAFs, tumor-associated macrophages (TAMs), and MDSCs are examples of immunosuppression networks that were suppressed *in vivo* and were positively linked to the synergism of the combination treatment as well as a reduction in unwanted effects [36]. Cytotoxic chemicals react with the electrophilic properties of glutathione and become inactive as a result. Thus, the high levels of glutathione and cysteine in fibroblasts promote resistance to antibiotics. In a related development, it was found that fibroblasts reduce the buildup of cisplatin in ovarian carcinoma cells by releasing thiols and increasing

intracellular glutathione levels. Additionally, the scientists found that CD8+ T cells and IFN-γ particularly targeted stromal fibroblasts and altered their thiol metabolism, significantly affecting the cancer cells' response to chemotherapy. Therefore, exploring the interaction between immunotherapy and chemotherapy may undermine chemotherapeutic resistance and restore cancer sensitivity to chemotherapy medicines. Furthermore, the combination with ICIs highlighted the critical relevance of overcoming medication resistance in patients with mesothelioma in practical practice. The associated research has shown that treating gemcitabine with immune checkpoint blockers significantly increased the overall survival (OS) of patients who had previously been resistant to gemcitabine or anti-PD-1 monotherapy [31].

9.4.5 AVOIDING RECURRENCE

Due to the frequent occurrence of local recurrence or metastases elsewhere, cancer treatment is hindered by tumor reoccurrence after an initial decline following chemotherapy. Imbalances in co-stimulatory and inhibitory signals in metabolism are directly linked to tumor recurrence and are associated with a poor prognosis. Adoptive transfer (AT) of tumor-specific CD4+ T cells after cyclophosphamide (CTX) therapy resulted in a significant initial anti-tumor immune response, but it also led to an increase in inflammatory monocytes (CD11b+Ly6ChiCCR2hi) in mice with advanced lymphoma [37]. The programmed death protein 1 (PD-1) and its ligand PD-L1 pathway mediate the induction of functional toleration of anti-cancer CD4+ effector T cells. These immunosuppressive monocytes hinder long-term tumor control and enable recurrence. Due to the high rate of monocyte proliferation, it was predicted that reducing chemotherapy doses would favor an unrestricted anti-cancer immunogenic response. As a result, the combination therapy eliminated the suppressive role of inflammatory monocytes and prevented tumor recurrence. It was also observed that administering Dacarbazine the day before peptide immunization, along with IFNα, increased cancer cell death activity. The treatment demonstrated an increased anti-tumor activity due to the co-production of TNF, IFNα, and Granzyme B, which reduce late tumor recurrence following CIT [38]. Furthermore, the hypoxia-inducible factor (HIF)-regulated gene network influences several factors, including chemotherapeutic drug resistance, tumor metastasis, and recurrence. Based on this, bio-similar core-shell nanoplatforms were created to deliver catalase and Doxorubicin (mZCD)

for relieving tumor hypoxia. It has been proven that oxygen production is critical in downregulating the expression of HIF1 IF1α, which can enhance the therapeutic benefits of chemotherapy and reduce the expression of PD-L1. When combined with ICIs co-delivery, the dual suppression of the Programmed death protein 1 (PD-1) and its ligand PD-L1 induces a robust immunological reaction and has a superior effect on suppressing cancer spreading and extending cancer recurrence time [31].

9.5 COMBINING VACCINES AND CHEMOTHERAPEUTIC AGENTS

The therapeutic effectiveness of standard chemotherapy schedules primarily depends on the cytotoxicity of cancer cells. Until recently, it was commonly believed that chemotherapy would inevitably decrease vaccine-mediated immune responses and anticancer efficacy when used alongside cancer vaccination. However, there is mounting evidence that several chemotherapeutic medications possess immunomodulatory properties that can enhance immunization-mediated anticancer benefits. This synergy can be achieved through various mechanisms, depending on the specific chemotherapeutic medication, vaccine, and dosage regimen. Chemotherapeutic drugs can alter the transcription of tumor-associated antigens, major histocompatibility complex, and intracellular cell adhesion molecule-1, thereby modifying the phenotype of tumor cells and making them more susceptible to immune-mediated attack [39–41]. These drugs can also induce apoptosis in tumor cells, leading to IL-12-mediated stimulation of dendritic cells, antigen presentation, and cross-presentation to T cells. This results in the stimulation of cytotoxic T-lymphocytes with a more potent and effective cancer lytic capability. Chemotherapeutic drugs can also modify immunomodulatory cells such as Regulatory T (Treg) cells and MDSCs, induce leukopenia, and work in conjunction with vaccination to enhance effector immune responses to various TAAs. Table 9.1 demonstrates the immunomodulatory effects of chemotherapeutic agents. Recent research has shown that specific chemotherapy regimens, when combined with certain cancer vaccinations, may reduce tumor growth rates in cancer patients. Detailed analyses of the synergistic effects of cancer chemotherapy and immunotherapy treatments have already been conducted. Numerous preclinical studies have investigated the impacts of combining established vaccination platforms with chemotherapy, some of which have been implemented in clinical settings [42].

TABLE 9.1 Chemotherapeutic Agents and Their Immunomodulatory Mechanism

Drugs	Mechanism of Action	
	Activation/Enhancement	**Inhibition/Reduction**
Cisplatin	CD4+ T cell; effector T-cells; NK; CTL; APCs and macrophage; IL-1α; IL-2; TNF-α.	MDSC; Treg, IL-1; IL-6; IL-8; IL-10; IL-12; TNF-α; TNF-β.
Carboplatin	Macrophage; CTL, IL-1α; IL-2; TNF-α; IFN-γ, CD8+ T cell.	IL-10; VEGF; TGF-β; CCL2.
Paclitaxel	Macrophage; DC; effector T-cells, IL-2; IL-12.	Treg; MDSC, IL-1; IL-6; IL-8; IL-10; IL-12; TNF-α; TNF-β; TGF-β; VEGF; CCL2; CCL22.
Gemcitabine	NK; CTL; memory T-cells.	MDSC; Treg; macrophage, TGF-β1.
Docetaxel	CTL; NK; LAK; IFN-α; IL-6; GM-CSF; IFN-γ; TNF-α.	MDSC; Treg, TNF; TGF-β; IL-1.
Cyclophosphamide	CD41 T-cell development, DC activation.	Treg function
5-FU	IFN-γ production by intratumoral CD81 T cells.	MDSC

9.5.1 CHEMOTHERAPY PRIOR TO VACCINATION

Certain chemotherapeutic medications may exhibit enhanced efficacy when combined with vaccinations in specific sequences. The underlying cause of this phenomenon remains unknown. Antimetabolites such as 6-mercaptopurine, azathioprine, methotrexate, and 5-FU only inhibit proliferation after lymphocytes have been activated by the antigen. On the other hand, alkylating agents like CTX and busulfan can inhibit proliferation either before or after the host's exposure to an antigen. Clinical trials have demonstrated the effectiveness of administering CTX and paclitaxel prior to vaccination. There are several potential mechanisms that could explain this observation. Firstly, pre-immunotherapy chemotherapy may eliminate enough tumors to alleviate the inhibitory impact on immune effectors caused by the presence of tumors. Secondly, by reducing the tumor burden, the effector T cell to tumor ratio may be improved, enhancing the ability of effector T cells to eliminate the remaining tumor cells. Thirdly, a decrease in regulatory T cells may potentiate a more robust immune response upon activation. Lastly, chemotherapy may enhance the production of tumor antigens or modify their expression, thereby facilitating more effective recognition by immune effectors. Nowak et al. conducted a study involving gemcitabine in combination with

immunotherapies to investigate some of these processes [43]. As previously mentioned, gemcitabine significantly reduces the humoral immunological response to tumor antigens. However, it does not impede TA-specific cellular priming, as it promotes TA cross-presentation, T-cell proliferation, and tumor infiltration. Nowak and his team [43] hypothesized that because gemcitabine induces apoptosis in tumor cells, the immune system would be exposed to a large quantity of tumor antigens. While this exposure alone may not initiate a robust anti-tumor response, it could prime the host immune system for subsequent adjuvant immunotherapy. The timing of adjuvant treatment is crucial. Based on the understanding of gemcitabine's action, Nowak and his team investigated the synergy between gemcitabine and immunotherapy (CD40 ligation using FGK45). In mice with established tumors, pretreatment with gemcitabine followed by FGK45 resulted in tumor regression, while mice treated with FGK45 followed by gemcitabine experienced faster tumor regrowth compared to those treated with gemcitabine alone. Several trials using CTX have administered a single intravenous dose prior to vaccination, prioritizing the immunomodulatory impact, particularly the suppression of suppressor or regulatory T cells, over the anticancer effect. In these trials, patients who received CTX before immunization showed higher levels of peripheral blood cytotoxic T lymphocyte (CTL) precursor survival compared to those who received the vaccine alone. Intravenous CTX (or intraperitoneal in animal trials) appears to be more effective than oral medication, although not all researchers have observed a benefit from CTX pretreatment [44]. Studies with doxorubicin, melphalan, paclitaxel, and 5-FU have also demonstrated that administering the chemotherapeutic before immunization increases the immunogenicity (particularly CD8+ T-cell responses) of various vaccines, while others have found that doxorubicin is more effective when given after immunization. Chemotherapeutics are typically administered between 1 and 3 days before vaccination. The identification of the timing of medicine and immunotherapy poses a challenge when analyzing the function of CTX and potentially other chemotherapeutic drugs. In Berd's research, the first and second vaccinations were preceded by low-dose CTX therapy. This therapy utilized autologous tumor cells modified with the hapten dinitrophenyl (DNP) as a vaccine, which was administered with bacilli Calmette-Guérin as an adjuvant [45]. Prior to the research immunizations, a delayed tumor hypersensitivity skin test was conducted using autologous tumor cells modified with DNP. Notably, it was observed that patients who underwent baseline skin testing 3 to 8 days before CTX administration exhibited significantly larger DTH responses and had a

higher five-year overall survival rate compared to patients who underwent baseline skin testing on the day of or the day after CTX administration. The hypothesis was that administering the DTH test would stimulate the surge of regulatory T cells, which would then be eliminated by subsequent CTX administration. This elimination would allow for the expansion of effector T cells without inhibition by regulatory T cells three days later when immunized with the intended vaccine. In summary, our findings demonstrate that pretreatment with chemotherapy, particularly CTX and paclitaxel, enhances the immunogenicity of future immunizations. The activity of CTX is believed to be related to the regulation of suppressor or regulatory cells, although the mechanism of action for other drugs remains unknown. Additionally, it cannot be completely ruled out that tumor elimination by the chemotherapeutic agent may lead to antigen release and subsequent formation of an immune response against the released antigens. Nevertheless, the authors suggest that clinical studies combining chemotherapy and vaccinations should include immunizations administered in close proximity to chemotherapy treatment (1–3 days) [46].

9.5.2 VACCINE PLUS CYCLOPHOSPHAMIDE AND ITS COMBINATIONS

A variety of malignancies are treated using alkylating medications, such as CTX. These compounds have applications in cardiovascular-based treatment regimens due to their immunomodulatory properties in addition to their direct cytotoxic effects on malignant cells through DNA alkylation. Studies have demonstrated that CTX increases the expression of HLA and cytokine release in tumor cells, leading to enhanced maturation of dendritic cells (DCs) and improved cytotoxic T lymphocyte (CTL) killing. The direct effects of CTX on DCs and other components of the host immune system are well-documented. For instance, CTX treatment enhances the lytic capacity of CD8+ T cells. The role of regulatory T cells (Tregs) is crucial in tumor immune tolerance, as evidenced by emerging data, as they significantly impede the generation of effective anti-tumor immune responses in cancer patients. It has been discovered that CTX can reverse Treg suppression, enabling the stimulation of vaccine-induced solid immunity. In an experimental melanoma model, systemic CTX combined with DC vaccination resulted in increased anticancer effects. In the treatment of metastatic breast cancer, a study investigated the administration of 72 metronomic doses of CTX in conjunction with a sialyl-Tn-keyhole limpet hemocyanin vaccine

(THERATOPER, Biomira) [47]. CTX selectively abrogates Tregs, reduces residual Treg function, enhances DC maturation, and boosts memory T-cell responses, making it a promising option for cancer immunotherapy. Additionally, CTX is utilized as an angiogenesis inhibitor. These effects of CTX have been observed even at dosages lower than those required for cytotoxicity. In mouse models of melanoma, mesothelioma, and prostate cancer, low-dose CTX combined with DC vaccinations (GMCSF-secreting vaccine) significantly reduced tumor size compared to vaccines alone. Pretreatment with a lower dose of CTX has been well received in recent clinical studies. It has been observed that this approach enhances the immunogenicity of peptide CVs while significantly reducing the concentration of Treg cells [48]. Another study demonstrated that the immune response to whole tumor cell vaccination in a preclinical model of breast cancer can be enhanced by carefully timing the administration of CTX, doxorubicin, and paclitaxel. In this particular case, the combined anti-cancer effects of vaccination and chemotherapy were attributed to the improved effectiveness of the vaccine rather than the direct apoptotic impact of chemotherapy on cancer cells. A recent investigation focused on the interaction between CTX, doxorubicin, and a GM-CSF producing HER2/neu-expressing whole tumor cell vaccine in patients with metastatic breast cancer [42, 49].

9.5.3 VACCINES PLUS GEMCITABINE

Gemcitabine was observed to increase immune-supportive macrophages M1 and circulating CD4 and CD8 T cells while reducing MDSCs and Regulatory T-cells (MDSC and Tregs) in preclinical studies. These results were obtained from patients with ovarian cancer participating in a phase I and phase II study that included gemcitabine, Pegintron, and a p53 synthetic long peptide (SLP) vaccination. However, the effects of the vaccination in combination with gemcitabine on outcomes have been inconclusive. Algenpantucel-L combined with gemcitabine and 5-fluorouracil-based conventional adjuvant chemoradiotherapy resulted in phase II research with a 12-month Progressive Free Survival (PSF) of 62% and an OS rate of 86% for surgically removed pancreatic cancer [50]. Phase III research in metastatic pancreatic cancer that randomly assigned patients to either concurrent chemotherapy plus immunization, concurrent chemotherapy plus telomerase vaccination (GV1001), or chemotherapy alone in a 1:1:1 ratio did not achieve its primary OS goal, even though these results were favorable compared to historical data [10].

9.5.4 VACCINES PLUS PACLITAXEL AND DOCETAXEL

One of the most popular chemotherapy drugs for cancer is taxanes, which have been used to treat numerous cancers, such as lung, prostate, and breast carcinomas. It has been shown that docetaxel decreases MDSCs and Tregs while increasing CTL responses. Recombinant viral vaccines were administered to tumor-bearing mouse models, followed by docetaxel therapy, to enhance vaccine-induced T-cell responses to both vaccine-delivered antigens and tumor-derived antigens [49]. Docetaxel reduces tumor size in mouse models and is dependent on antigen-specific CD8 T-cells [51]. Additionally, other preclinical results indicate that docetaxel may increase calreticulin (CRT) production in tumor cells, thereby enhancing their susceptibility to CD8 T-cell-mediated cytotoxicity. Findings from a clinical trial on docetaxel and PANVAC vaccination as a combined treatment for breast cancer have demonstrated positive clinical outcomes. When combined with a DNA vaccination at a low dose, paclitaxel, another taxane medication, increased the CD8+ T cell/Treg ratio more than when the vaccine was used alone. Patients who received docetaxel alone also exhibited lymphocyte responsiveness to tumor-associated antigens, indicating the drug's proposed impact on immunity. Docetaxel and other vaccinations in prostate cancer are currently undergoing evaluation in multiple active studies. These include the treatment of castration-sensitive prostate cancer with PROSTVAC and docetaxel (phase II; NCT02649855), as well as the treatment of metastatic castration-resistant disease with docetaxel and DCVAC/PCa (phase III; NCT02111577) [10]. It has been demonstrated that paclitaxel increases the expression of antigen processing and presenting machinery (APM) proteins, such as calmodulin, low molecular mass polypeptides 2 and 7, and transporter 1. This raises the possibility of greater recognition by cytotoxic T lymphocytes (CTL) [52]. Similarly, treatment with sublethal doses of paclitaxel has been shown to elevate the expression of ICAM-1 on NK cells, thereby promoting NK cell-mediated lysis [53]. Furthermore, taxanes can directly influence various aspects of the host immune system, thereby impacting tumor immunogenicity. For instance, they can enhance macrophage activation and stimulate the intra-tumor production of inflammatory cytokines, leading to enhanced tumor lysis. It has been demonstrated that sublethal doses of paclitaxel improve IL-12-dependent antigen presentation by dendritic cells (DCs), accompanied by higher expression of APM components and improved costimulation. Additionally, prior treatment of paclitaxel in tumor cells exposed to DCs has been observed to result in the production of CD8+ T cells with a stronger lytic capacity [52]. Increasing data indicate that the short window of T-lymphocyte

recovery after lymphopenia associated with chemotherapy presents a unique opportunity to enhance the effectiveness of anti-tumor immunotherapy. For example, Docetaxel has been shown to modify the subsets of T, B, and NK cells, as well as to enhance CD8+ activity while eliminating Tregs. In preclinical experiments conducted on CEA-Tg mice implanted with CEA+ tumor cells, a combination of Docetaxel with an rV/F-CEA/TRICOM vaccination regimen demonstrated improved anti-tumor efficacy compared to the responses elicited by the vaccine or Docetaxel alone. Following immunization, Docetaxel significantly increased immune responses to recombinant viral vaccinations, including antigen-specific T cell responses to both the Tumor-associated antigen (TAA) supplied by the vaccination and to cascade antigens generated by the tumor. Docetaxel has been or is currently undergoing combined assessment with various platforms for cancer vaccination, such as – (i) a vaccination producing PSA and B7.1 (rV-PSA/B7.1; PROSTVACR); (ii) a prime/boost vaccination employing vaccinia and fowlpox viruses that express MUC-1, CEA, and TRICOM (PANVACR); and (iii) the DC vaccine Provenge R (Dendreon Corp.) [10, 42, 49].

9.5.5 VACCINE AND IRINOTECAN

Irinotecan inhibits DNA repair and induces a complex immune response involving the activation of tumor-suppressor proteins, as well as the enhancement of tumor immunosurveillance by Natural Killer (NK) cells and activated CD8 T-cells. The combination of G17DT (a vaccine that combines the N-terminus of the growth factor gastrin with a diphtheroid toxin to stimulate the production of anti-gastrin 17 antibodies) with irinotecan was investigated in a phase II multicenter study involving metastatic colorectal cancer patients who had progressed on irinotecan. Among the 161 patients, a partial response was observed in 3%, stable disease in 32%, and progressive disease in 65% of the treated individuals. Adverse reactions were comparable to those of irinotecan monotherapy, except for more severe injection site reactions reported by 52% of patients. Anti-gastrin 17 antibodies were detected in 62% of patients and were associated with a survival advantage [10].

9.5.6 VACCINE PLUS PLATINUM-BASED CHEMOTHERAPY

In preclinical investigations, the combination of carboplatin and paclitaxel demonstrates immunomodulatory anti-tumor effects. A recent study discovered that the combination of carboplatin, paclitaxel, and HPV16 peptide

vaccination improved survival in mouse tumor models, which was associated with a decrease in the frequency of myeloid cells in both the tumor and peripheral circulation. Additionally, carboplatin and paclitaxel together enhanced *ex vivo* T-cell activity for antigen recall. In a phase IIb/III study for stage IV NSCLC, TG4010 (modified Ankara vaccination expressing MUC-1 and IL-2) supplemented with platinum-based chemotherapy showed modest benefits. Patients were randomly assigned to either TG4010 plus chemotherapy or placebo plus chemotherapy. The combination group exhibited a longer median progression-free survival and more confirmed responses. Surprisingly, the TG4010 group experienced delayed reactions as well as longer-lasting responses [10]. When administered after vaccination, certain cytotoxic drugs such as doxorubicin and cisplatin enhance the cytotoxic impact of vaccine-generated CTLs, likely due to their modification of the immunogenic phenotypes of cancer cells. These medications render tumor-reactive T cells more susceptible to them and permeabilize cancer cell membranes to granzyme B, an enzyme produced by CTLs [49].

9.5.7 VACCINE PLUS DOXORUBICIN

DNA-intercalating medications known as anthracyclines, such as doxorubicin, have been associated with a decrease in the occurrence of various types of carcinomas, including lung, bladder, breast, and ovarian. Unlike other DNA-damaging medications, doxorubicin induces the rapid translocation of ERp57 and CRT to the cellular membrane, leading to caspase-dependent immunomodulatory cell death, effectively killing cancer cells when administered in lethal doses. Noncytotoxic doses of doxorubicin promote antigen presentation by DCs in an IL-12-dependent manner, which is linked to the regulation of APM components and increased activity of effector T-cells [42]. One study reported that durable CD8+ T-cells were enhanced by a combination of limited doxorubicin, INF-α, and autologous DC immunization treatment. Furthermore, it was observed that one out of the five animals that survived for 16 months without cancer after the recorded remission of recurrent illness had a median overall survival of 109 days [54].

9.5.8 VACCINE PLUS 5-FLUOROURACIL, GEMCITABINE, AND METHOTREXATE

A variety of malignancies, including pancreatic and colon cancers, as well as HNSCC, can be treated using antimetabolites. These medications have

demonstrated various immunomodulatory characteristics. Gemcitabine, for example, can enhance MHC-I expression in cancer cells, leading to increased susceptibility to cytotoxic T-cell-mediated lysis [42]. A unique therapeutic approach for advanced pancreatic cancer involves combining gemcitabine with an oncolytic measles vaccine virus (MeV), resulting in a mutual enhancement of each component's efficacy. In comparison to single-agent therapy, the combination of inadequate doses of both medications significantly increased cytotoxicity in three tumor cell lines [55]. Similarly, 5-FU therapy of colon cancer cell lines can enhance ICAM-1 and Fas expression, rendering them more susceptible to the lethal actions of CD8+ T lymphocytes [42]. Geary et al. [56] reported that mice treated with low-dose 5-fluorouracil and an adenoviral tumor vaccine together had a 95% survival rate, compared to 7% with Ad5-OVA alone and 30% with 5-fluorouracil alone. Furthermore, the presence of 5-fluorouracil resulted in increased amounts of OVA-specific CD8+ T cells in the spleens, improving the immunogenic response to cancer cells while also reducing tumor mass. It is possible that the ability of 5-FU to reduce MDSC populations is what has led to the observed therapeutic advantage [56]. Methotrexate, for instance, has been demonstrated to have direct effects on T-cell cytotoxicity. Antimetabolites can enhance DC performance both directly and indirectly. One study found that direct methotrexate treatment of DCs improved antigen presentation to T lymphocytes. Overexpression of interleukin-12 (IL-12) and antigen-processing machinery elements has been associated with stimulation of DC activity, and improved DC performance has been observed following contact with gemcitabine-treated cancer cells. Additionally, gemcitabine has been shown to reduce MDSCs in tumor-bearing rodents without affecting other immune cell subtypes [42].

9.6 CHEMO-IMMUNOTHERAPY – MECHANISMS OF ACTION IN GENERAL

9.6.1 DEBULKING OF TUMORS

Tumor debulking is one of the most significant advantages of cytotoxic treatment. The main source of immunosuppressive tumor microenvironment (TME) is tumor cells. Therefore, reducing the bulk of cancer cells decreases the production of immunosuppressive substances. Additionally, reducing the bulk of cancer cells decreases the number of cancerous cells that need to be eliminated by immune cells. This can have negative effects, especially in tumors with limited infiltration of immunological cells near the TME [27].

9.6.2 IMMUNOGENIC CELL DEATH (ICD)

Multiple studies have demonstrated that conventional chemotherapy enhances immunotherapy and promotes immunogenic cell death (ICD). Cytotoxic therapy leads to the production and translocation of damage-associated molecular patterns (DAMPs), which increases the adjuvanticity of cancer cells [57]. The release of intracellular chemicals such as ATP enhances antigen-presenting cell (APC) recruitment, while cytoplasmic annexin A1 from cancer cells interacts with formyl peptide receptor 1 to facilitate contact between dendritic cells (DCs) and injured cancer cells. Moreover, heat shock protein 70 (HSP70), heat shock protein 90 (HSP90), and calreticulin (CRT) exposed from the endoplasmic reticulum assist DCs in phagocytosing stressed cancer cells. Inflammatory cytokines such as type I interferon (IFN-I) are released from intracellular DNA and RNA through the Toll-like receptor 3 (TLR3), cyclic GMP-AMP synthase (cGAS)/stimulator of interferon genes (STING) pathway, and TLR9 receptor. C-C motif chemokine ligand 2 (CCL2), C-X-C motif chemokine ligand 1 (CXCL1), and CXCL10 promote T-cell recruitment, while IFN-I and other molecules like high mobility group box 1 (HMGB1), generated by stressed cancer cells, facilitate DC maturation and T-cell antigen presentation [27].

9.6.3 ENHANCEMENT IN THE ANTIGENICITY OF CANCER CELLS

Anthracyclines, CTX, platinum, and taxanes are just a few of the commonly used cytotoxic medications that target cell cycle progression and eliminate developing cells. Once tumor cells have perished, antigen-presenting cells (APCs) take up their contents and deliver tumor neoantigens to immune cells. Further research has demonstrated that cytotoxic drugs stimulate the antigen-presenting machinery. Gemcitabine can significantly enhance the expression of HLAs-A, -B, and -C and alter the repertoire of peptide antigens expressed in HLA class I by increasing β2-microglobulin production [58]. Topotecan, by activating the NF-κB/IFN-β/MHC-I signaling axis, also upregulates HLA class I expressions [27].

9.6.4 DEPLETION OF IMMUNOSUPPRESSIVE CELLS

Cytotoxic treatments, such as gemcitabine, 5-fluorouracil, CTX, and platinum, can reduce MDSCs in both humans and animals. Trabectedin induces caspase-8-dependent apoptosis to selectively decrease monocytes and macrophages [59]. Human Treg cells are more susceptible to the effects

of CTX compared to other immune cells due to their deficiency in the expression of the CTX generating transporter ABCB1. Additionally, chemotherapy alters the tumor microenvironment (TME) and promotes the development of immune cells, thereby enhancing anticancer immunity. For instance, CTX and doxorubicin facilitate the differentiation of tumor-associated macrophages into macrophage 1 [27].

9.6.5 MODULATION OF GENE EXPRESSION

DNA methylation, histone modification, chromatin remodeling, and the readout of these changes are examples of epigenetic modifications that have a significant impact on oncogenesis and are crucial events in various cancers, such as the elimination of tumor suppressor genes brought on by DNA methylation. Consequently, epigenetic modulators are a growing class of anti-tumor medications. In addition to the direct activation of ICD and enhancement of anti-tumor immunity reported with HDAC inhibitors vorinostat and Panobinostat [60], gene expression alteration is another important mechanism that contributes to the synergy between epigenetic modulators and immunotherapy. It has been shown that both HDAC and DNMT inhibitors increase the activity of the antigen processing and presentation system. Epigenetic modulators increase the expression of both HLA class molecules and tumor-associated antigens. Epigenetic modulators have also been demonstrated to have an immediate impact on the immune system, potentially increasing anticancer immunity. They can stimulate co-stimulatory molecules, including CD80, CD86, and ICAM-1, as well as immunological checkpoints like CTLA4, PD1, and PD-L1. Furthermore, cytokines can be generated, and epigenetic modulators can enhance immunotherapy response. Epigenetic modulators can also affect innate immunity. HDAC inhibitors can activate the activating receptor NKG2D on the surface of NK cells as well as the stress-inducing ligands MICA and MICB on cancer cells to promote NK cell destruction of cancer cells [27].

9.6.6 ENHANCEMENT AND RE-ESTABLISHMENT OF CHEMOTHERAPY RESPONSIVENESS

Several studies have found that immunotherapy and cytotoxic chemotherapy are mutually beneficial. Following cancer progression on anti-PD1 treatment, some patients with chemoresistant malignancies responded to the challenge

of chemotherapy. After disease progression on immune checkpoint inhibition, both non-small cell type cancers and Hodgkin's lymphoma showed enhanced responsiveness to salvage chemotherapy [27].

9.6.7 CHEMOTHERAPY'S DISADVANTAGES OF IMMUNOTHERAPY

Lymphodepletion is one of the most detrimental adverse effects of chemotherapy on the immune system, with the potential to induce immunosuppression. Certain immunosuppressive medications used in clinical settings for the treatment of autoimmune diseases are cytotoxic chemotherapeutic agents employed to target cancer cells, albeit with different dosages and regimens. The extent to which chemotherapy-induced lymphodepletion suppresses anticancer immunity remains a subject of debate. Typically, lymphocyte counts recover following lymphodepletion caused by cancer treatment, allowing the immune system to "reset." A study has shown varying degrees of recovery in different immune cell subpopulations with regards to anticancer immunity [61]. Additionally, chemotherapy may impact tertiary lymphoid structures (TLS). TLS are ectopic lymphoid aggregates that form in non-lymphoid tissues, including cancerous tissues, and resemble secondary lymphoid organs such as lymph nodes in terms of their anatomy. There is substantial evidence supporting the notion that TLS attract lymphocytes into tumors, similar to how lymph nodes do, thereby eliciting localized and systemic immune responses against malignancies. The presence and density of TLS in tumors are generally associated with prognosis across various cancer types, sometimes independent of pathogenic tumor-lymph node-metastasis (TNM) staging. The lymphodepletion effect of chemotherapy has the potential to impair TLS, either directly or through related treatments such as corticosteroids [27].

9.7 CONCLUSION

The research discussed here emphasizes advancements in CV development, as well as the synergies provided by combining cancer vaccinations with chemotherapy. The combination of chemotherapy and immunotherapy has been shown to promote cytolysis and reduce cancer cell growth through various mechanisms. Clinical investigations have demonstrated that combining chemotherapy and immunotherapy can be more beneficial than either therapy alone. Further analysis is still underway to evaluate the

findings of more extensive research on combined CIT. The innate immune condition of cancer patients will influence their response to chemotherapy; therefore, the dosage and sequence of combination therapies should be determined based on preclinical research and clinical viability. When developing rationale-based CIT pairings, these and other variables must be taken into account. Extensive investigations in preclinical studies and on patients have indicated that T cells can be trained to recognize tumor cells, supporting the idea that vaccination could be used for cancer prevention or recurrence. Vaccination has proven highly effective in reducing the incidence of malignancies with a microbial origin. However, vaccination against existing cancers has been largely ineffective. Until recently, it was widely believed that cytoreductive treatment, when combined with a CV, would inevitably impair vaccine-mediated immune responses and anticancer effectiveness. However, recent studies suggest that it may be possible to enhance vaccine-mediated anticancer effects by utilizing the immunomodulatory characteristics of cytoreductive regimens. This synergy can be achieved through various pathways, depending on the specific cytotoxic chemotherapy and vaccine used, the dosage regimen for each modality, and other factors. As a result, an increasing body of clinical and preclinical evidence supports the adoption of a combined strategy that incorporates immunotherapy and primary chemotherapeutic medications as the standard approach for treating various types of cancer. Clinical studies have demonstrated that therapeutic cancer vaccines (CVs) can be immunogenic, and some have shown efficacy in at least a small subset of patients. DC therapeutic CVs have been approved for use in clinical trials, and checkpoint inhibitors in combination with them are well advanced. Tumors can be heterogeneous and develop clinical resistance to monotherapies. Therefore, effective anticancer treatments need to target multiple sites simultaneously. The diversity of the host and the disease are often associated with limited options and poorer outcomes. Combination approaches must consider the specific genetic, epigenetic, and complex nature of cancer. By modifying inhibitory molecules, regulatory immune cells, and the metabolic resources and demands of T cells, vaccine-activated T cells can be promoted to be fully functional within the immunosuppressive tumor microenvironment (TME). Identifying reliable biomarkers for patient selection, standardized measures for toxicity monitoring, and a comprehensive understanding of the schedule and dosage of combination treatments will all be crucial for successful therapeutic development and decision-making.

KEYWORDS

- **cancer**
- **chemo-immunotherapy**
- **chemotherapeutic agents**
- **decision-making**
- **immunotherapy**
- **monotherapies**
- **therapeutic development**
- **vaccine**

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CHAPTER 10

Role of Various Cancer Vaccine Adjuvants in Cancer Management

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ABSTRACT

Basically, cancer is a group of diseases caused by abnormal cells that grow out of control, surpassing their normal boundaries and invading nearby body parts or spreading to other organs. Metastasis is one of the most significant reasons for death in cancer patients. The Food and Drug Administration (USFDA) has approved the first cancer vaccine (CV) along with other therapeutic benefits. Patients have indeed been tested for other CVs that enhance the immune system's response against cancer. Emerging vaccine technologies include adjuvants for DNA vaccines, cancer, and autoimmune vaccines, as well as mucosally administered vaccinations. Initially, alum was used as an adjuvant, but it had severe local and systemic adverse effects. However, it was able to generate a good antibody (Th2) response and was approved for human usage. Adjuvants are incorporated into vaccine formulations to facilitate potent and long-lasting immune

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responses. Several CV adjuvants exist, including Monophosphoryl lipid A (MPLA), CpG ODN (which increase the efficiency of antigen-presenting cells (APCs) and enhance the production of cell-mediated immune responses), toll-like receptor (TLR) cytokines (which enhance antitumor effects), and IL-4 and IL-13 (Interleukin) cytokines' receptors (which inhibit the development of functional Th1 immunity).

This chapter includes various adjuvants for cardiovascular diseases (CVs), such as Granulocyte-macrophage colony-stimulating factor (GM-CSF). The combination of adjuvants, such as a mixture of TLR agonist and α galactosylceramide, shows synergistic effects in CVs. Additionally, certain body cells, like Dendritic cells (DCs), play a role in boosting the immunity of CVs as antigen-presenting cells. Polymers like chitin, chitosan, and glycolated chitosan are also used as adjuvants in CVs to balance the immune response. Furthermore, the differentiation of adjuvants is related to their substances and mechanism of action.

10.1 INTRODUCTION OF ADJUVANTS

In the past, vaccines were primarily developed based on the detection of antigens (Ag) that induce specific immune responses. The objective of immunotherapeutic agents is to enhance the antitumor response while minimizing unintended adverse effects [1]. An immune response is triggered and activated by two components of vaccination: the adjuvant and the antigen, which are proteins and carbohydrates derived from the pathogen against which an adaptive immune system is required [2]. Immunology, including the understanding of T and B lymphocyte receptors and their ligands, conjugates, and even peptide vaccines, has been developed as a result of this strategy, which improves safety, increases effectiveness, and facilitates manufacturing. With the exception of the recently approved vaccine that prevents tumors caused by HPV infection, cancer vaccines (CVs) aim to treat severe disease rather than prevent it. Developing therapeutic CVs has been challenging because several early tumor vaccines showed promise in preclinical studies but were not effective in clinical trials. This chapter provides a list of adjuvants, delivery methods, and vaccination innovations that are currently being researched or authorized [1].

Adjuvants come from the Latin term "adjuvare," which means to improve or support. They are small chemicals used in vaccines that work

non-specifically to enhance the immune system's response to certain cell types. These adjuvants increase the immune response by inducing the immune system to react to them. There are various types of adjuvants, including cytokines, non-biological substances, synthesized small molecules, natural products derived from bacteria or other microorganisms, and biological products. Adjuvants are classified into two categories based on their predominant modes of action: immunopotentiatory adjuvants that boost innate immunity by activating pattern-like receptors (PRPs), specifically toll-like receptors (TLR), and delivery system adjuvants that promote the uptake of antigens by antigen-presenting cells (APCs) [3]. Emerging vaccine technologies include adjuvants for DNA vaccines, cancer vaccines, autoimmune vaccines, and mucosally administered vaccinations. When used alone, adjuvants have limited toxicity and elicit long-lasting immunological responses. However, they significantly enhance the size, breadth, quality, and durability of a specific immune response to antigens [4]. Table 10.1 provides the classification of adjuvants. The substances that have adjuvant activities include oil in water emulsion, natural and synthetic surfactants, mineral gel, and bacterial derivatives. In live vaccines, endogenous adjuvants are used to stimulate innate immunity by expressing molecular patterns connected to pathogens (PAMPs). For example, it has been proven that the innate immune system is activated by live attenuated yellow fever vaccines (YF-17D) through the signaling of numerous TLRs. Various mechanisms of adjuvant action are depicted in Figure 10.2. The incorporation of various TLR agonists such as TLR type-3, 4, 5, 7, and TLR type-9 has been the most important advancement in CV adjuvants. Multi-adjuvanted strategies that simultaneously promote immunity and prevent inhibition will be advantageous for future vaccines [5]. Macrophages, dendritic cells (DCs), cells generated from myeloid suppressor, as well as lymphocytes, are shown to be immune cell types that can either directly or indirectly promote or suppress gastric cancer by controlling immunological responses [6]. Finally, the researchers provide a brief overview of adjuvant safety before highlighting some noteworthy developments in various immunotherapy and a variety of additional treatments involving nanoadjuvants [7]. A list of various adjuvants, along with examples of adjuvants that have received FDA approval or not and are used to treat various cancers, their uses, and the manufacturers of each adjuvant, is shown in Table 10.1.

TABLE 10.1 List of Different Adjuvants, Including Examples of Adjuvants That Have Received FDA Approval or Not and are Utilized in the Treatment of Different Types of Cancers, Along with Their Respective Uses and Manufacturers

Type of Adjuvants	Adjuvant	Types of Cancers	Marketed Manufactured	FDA Approval Yes/No	Name of Inventor	Adverse Events	References
Dendritic-based	IL-12, cytosine phosphorothionate guanine and MPL.	Prostate cancer	Sipuleucel-T	Yes	Dendreon	Fatigue, fever, back pain, headache, vomiting, anemia, constipation, chills.	[63]
Cytokine-based	Granulocyte-macrophage stimulating factors.	Prostate cancer, gastric cancer	Sipuleucel-T	Yes	Dendreon	Fatigue, fever, back pain, headache, vomiting, anemia, constipation, chills.	[73]
Virus like particle	Major capsid (LI protein)	Cervical cancer, vulvar cancer, vaginal cancer.	Gardasil	Yes	Merck	Pain, swelling, redness, itching, bleeding, headache, fever, nausea, dizziness.	[13], www.gardasil.com
–	MPL	Cervical cancer, breast cancer	Cervarix	Yes	Glaxosmith Kline	Headache, myalgia, redness, swelling, fatigue.	[26, 95], European medicine agency.com
–	HPV	Cervical cancer	–	–	–	–	–
–	HPV + CpG oligodeoxynucleotides.	Genital cancer	–	–	–	–	[83]
Oil in water emulsion.	TRL	Not specific	QS-21, ISCOMS, ISCOMATRIX	No	–	Problems with stability and difficult degradation; concern over surfactant safety.	[18, 95]
CPG oligodeoxynucleotides.	TAL	Non-small cell lung cancer (NSCLC).	–	No	–	–	[13]

TABLE 10.1 *(Continued)*

Type of Adjuvants	Adjuvant	Types of Cancers	Marketed Manufactured	FDA Approval Yes/No	Name of Inventor	Adverse Events	References
mRNA-based	OVA	Non-small cell lung cancer (NSCLC).	–	No	–	–	[104]
–	TLR4	Breast cancer	Trimix	No	Sheck Exley	Pain or bruising, Temporary swelling of the penis skin.	[104], https://healthcare.utah.
Inorganic adjuvant	Silver, calcium phosphate, silica, aluminum compounds, zinc compounds, iron oxide, etc.	Not specific	–	–	–	*In vivo* degradation is difficult.	[18]
Liposomes	Phospholipids and cholesterol; nonphospholipid amphiphiles.	Not specific	–	–	Glenny	Problems with liquid stability and potential breakdown of unsaturated liposomal lipids.	[18]
Polymeric particle adjuvants	Chitosan, albumin, alginate, fibrinogen, collagen.	Breast cancer	–	–	–	Difficulties to creating polymeric particles with predictable and stable characteristics.	[18, 69]
Yeast-derived	GalCer (glycosphingolipids)	Lung cancer, liver cancer.	Heplisav-B, Engerix-B	–	–	–	[30, 82]

10.1.1 CHARACTERISTICS OF ADJUVANTS

There are different types of adjuvants that help boost the immunity of cancer vaccines to treat various types of cancers. It is important that these adjuvants are compatible with vaccination and do not affect the diagnostic or therapeutic effect. Table 10.2 describes the merits and demerits of adjuvants utilized in cancer vaccines to enhance the immune response.

TABLE 10.2 Merits and Demerits of Adjuvants Used in Various CVs

Merits	Demerits
Reduce the amount of Ag/number of vaccinations necessary to generate the optimal immunological response.	High cost for formulation.
Increase effectiveness.	Do not have FDA approval for use as standalone items.
Greater ease of manufacture.	Amyloidosis, autoimmune arthritis, anterior uveitis, and other conditions are caused by hypersensitization of the host tissue.
Improve safety.	Birth abnormalities (teratogenesis), cancer, and other genetic diseases.
–	Reduced effectiveness of immune response.

To minimize the risk of adjuvant and vaccine component degradation, it is necessary to assess the adjuvant effects and compatibility. One evaluation focuses on various aspects of mRNA cancer immunotherapy, including antigen and target selection, vector and adjuvant usage, different administration routes, and evaluation of clinical studies. This evaluation aims to identify the current status and challenges associated with these vaccines [8] (Figure 10.1).

Some of the main characteristics which are required for evaluation are:

- An effective group is essential for achieving optimal effect and stability. Factors such as oil in water, particle size, charge, etc., play a significant role in this regard.
- Should be safe when administered inside the body.
- Should be stable.
- Biodegradable and easy to remove once the desired effect has diminished.
- To promote Ag-specific immune response.
- Economic to produce.
- Must be economical.

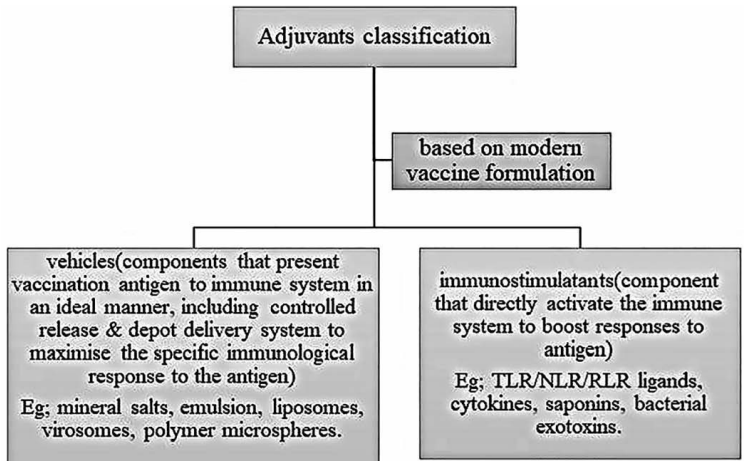

FIGURE 10.1 Adjuvant classifies as per used in modern vaccine formulation.

10.2 APCS (ANTIGEN PRESENTING CELLS) ROLE IN T-CELL ACTIVATION

Adjuvants primarily provide three functions in cancer vaccination: antigen provider, APC uptake/activation promoter, and cross-presentation activator [9]. The effective elicitation of immune responses by adjuvants involves three steps: antigen delivery, APC activation, and antigen cross-penetration. By concentrating on lymph nodes (LN) along the lymphatic circulation, the adjuvant demonstrates a considerable depot impact during the first stage of antigen delivery, enabling ongoing recruitment and stimulation of APCs at the site of injection [9]. In consideration, recent contributions to the development of effective T-cell and B-cell-based therapeutic cancer vaccines are emphasized. Researchers focus on adjuvants as the essential element for manageable APC activation and T-cell priming in T-cell-based vaccinations [10]. T-cell response can also be divided into CD4+ "helper" T-cell (Th) reactions and CD8+ cytotoxic T lymphocyte (CTL) reactions. CD4+ "helper" T-cell (Th) reactions are vital for secreting accessible immune regulators such as cytokines that support CD8+ T cell and B cell responses. Additionally, there are two types of helper T cells, Th1 and Th2, which promote B cell production, growth, and CTL-mediated destruction. Meanwhile, the secondary structure of B-cell receptors (BCRs) binds antigenic macromolecules such as proteins and polysaccharides (or, in some cases, synthetic materials) [11]. Natural killer cells (iNKT), due to their ability to stimulate various cell types, play a crucial role as a bridge between the innate

and adaptive immune systems. A potent iNKT cell agonist, the glycolipid-galactosyl ceramide (GC), induces the release of Th-1 and Th-2 type cytokines [12]. Enhancing the effectiveness of activated immune responses can be achieved through hydrogel/magnetic control. In the second step of APC activation, antigens from vaccines with a particulate adjuvant are more easily taken up by APCs. The third step, Ag cross penetration, involving TLR agonists, can promote DC activation, leading to increased exposure of foreign antigens by MHC I and MHC II molecules (major histocompatibility complex), thus promoting the development of robust cellular immunity [9]. Bone marrow (BM)-derived progenitor cells undergo rigorous positive and negative selection in the thymus before leaving the organ and acquiring specific T-cell receptors (TCR) capable of recognizing foreign peptides presented by MHC molecules on APCs, as shown in Figure 10.3. The three signals crucial for T-cell activation by MHC molecules, as shown in Figure 10.4, are also involved [13]. In addition to the cognate antigens, or signal 1, costimulatory cues from ligands on the surface of the APC, such as CD80, CD86, and CD40, as well as pro-inflammatory cytokines (PICs), or signal 2 and 3, are also essential for the effective activation of certain T cells. For the expression of these chemicals involved in signals 2–3, DCs must be fully mature (Figures 10.2 and 10.3).

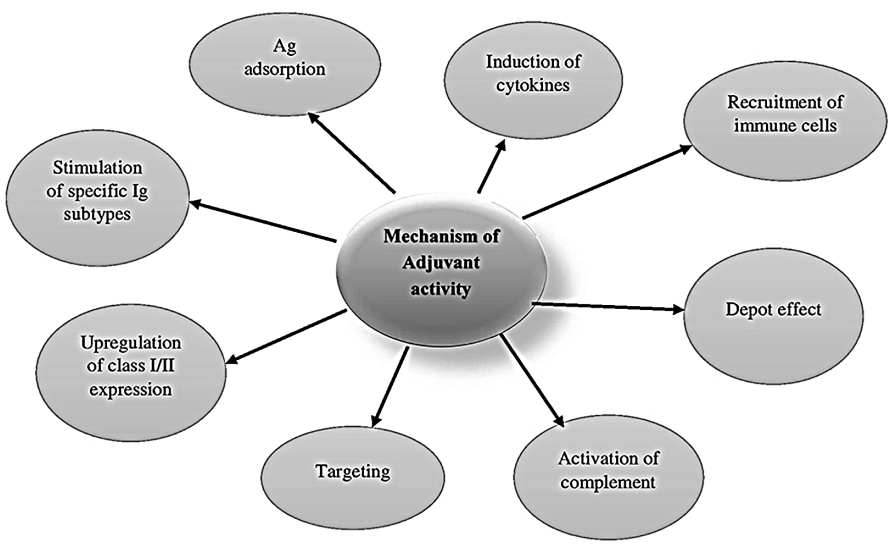

FIGURE 10.2 Various mechanisms of adjuvant action.

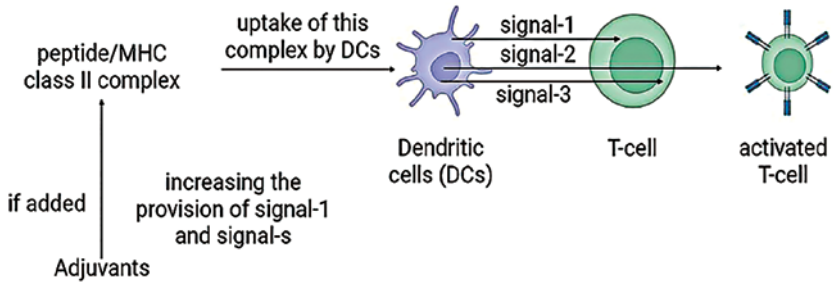

FIGURE 10.3 Signal 2 activates ligands for specific immune receptors that are gathered with Signal-1 to activate T-cells. Native CD8 T-cells require Signal-3 engagement to undergo clonal proliferation and establish signal transduction. The addition of adjuvants to the complex will increase the provision of both Signals 1 and 2.

Collectively, Signals 1–3 guarantee CD4+ T cell Type 1 immunological polarization and effective cytotoxic activities by CD8+ T cells (also known as cytolytic T lymphocytes, or CTLs). In contrast, signal 1 is activated when signal 2 is not present, and the presence of either immunosuppressive cytokine (Signal 3) or proinflammatory cytokines (Signal 1) induces type 2 immunity (through Th2 cells) or immunosuppression (via Tregs) [1].

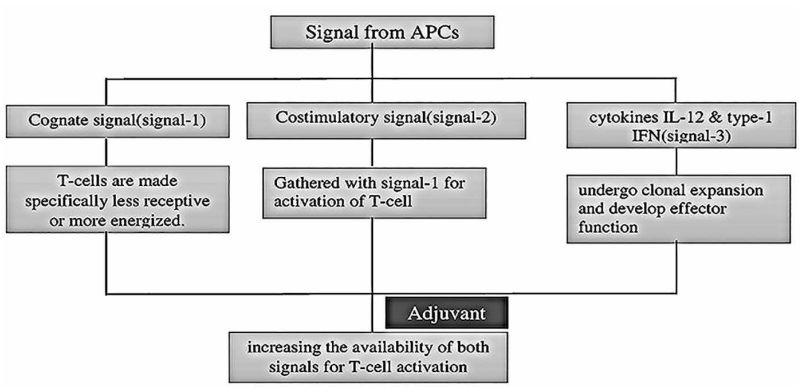

FIGURE 10.4 Distinct signals which come from APCs (Ag presenting cells) for activation of T-cell.

10.3 INTRODUCTION OF ALUM

Alum was initially used as an adjuvant due to its safety for human consumption, ability to generate a positive Ab (Th2) response, and lack of local and

systemic adverse effects [3]. Alum, a salt of aluminum, has a large surface area and high Ag capacity due to its hydroxyl (OH) and phosphate (PO_4) derivatives. It enhances Ab responses to an immunogen and stimulates T-helper type 2 responses. The structure, surface area, charge, and adsorptive characteristics of aluminum salts are influenced by various parameters, making their production challenging. For example, hemagglutinin from the influenza virus does not adhere well to alum [14].

In modern vaccines, antigens are added to pre-prepared aluminum salts, known as aluminum-adsorbed vaccines [15]. Alum is a special adjuvant that has been approved by the USFDA. It significantly enhances antigen persistence, absorption, and local immunostimulatory response [16]. Adjuvanted aluminum-based substances do not promote greater and more effective Th1/cytotoxic T-lymphocyte responses; instead, they induce lesser Th2 responses with the formation of IgM, IgE, and specific cytokines. While some alums directly act on MHC class-II for Ag presentation, others interact with Ag to form multimolecular Ag/adjuvant aggregates that facilitate uptake by APCs. When used in human vaccinations according to current immunization schedules, all aluminum salt adjuvants are considered safe [17].

Alum, which contains inorganic adjuvants such as aluminum hydroxide and aluminum phosphate/aluminum hydroxyl phosphate, was identified by Glenny in 1926 as having an excellent safety record for treating cancer. Nanoalum, including aloxide nanoparticles (NPs), aluminum oxyhydroxide particles, and metal oxide particles like calcium phosphate and zinc phosphate, are naturally found in the body. Calcium phosphate is used to treat influenza and herpes simplex virus [9]. Currently, TLR agonists such as Monophosphoryl lipid A (MPLA) and cytosine-phosphate-guanine (CpG)-1018 have been incorporated into licensed vaccines for their adjuvant activity. Additional TLR agonists are being developed for this purpose [18].

10.3.1 ALUM SUPPRESSES TUMOR GROWTH IN-VIVO

Aluminum adjuvants trigger the Nalp3 system, an intrinsic innate immune response pathway. *In-vitro* studies have demonstrated that aluminum particles from a variety of aluminum adjuvants can agglomerate transforming them into insoluble particles that are quickly phagocytosed by macrophages and cause the release of IL-1 and IL-18 [16]. One study was conducted to show that macrophages can still produce inflammasome-dependent IL-1 while being inhibited from actin/tubulin polymerization by cytochalasin and IL-1 synthesis by LPS (LPS alum) [19]. A novel approach for enhancing anti-tumor

immune responses uses aluminum salts (alum), *CpG* oligodeoxynucleotides (ODN), and the innate defense regulator peptide HH2 [16]. Systems based on NP present a promising approach to efficiently deliver various payloads, such as adjuvants to enhance therapeutic efficacy, in addition to mRNA. Adjuvant pulses could increase immune stimulation by restoring innate immune activation in the presence of these synthesized mRNAs in an anti-tumor vaccination [20]. The use of alum in human vaccinations has been licensed, however, it can have harmful local or systemic adverse effects, including myofascitis, abscesses, and sterile eosinophilia. Luteolin triggered TH1 immunity as opposed to the effects of the alum adjuvant, that also triggered TH2 immunity and may have a stronger tumor-killing effect. In contrast to alum, luteolin boosted the synthesis of IL-12, which reduces IgE expression [21].

10.3.2 GRAPHENE OXIDE NANOCOMPLEXES WITH ALUMINUM FUNCTIONALIZATION FOR EFFECTIVE CANCER VACCINATION

Applications of graphene oxide (GO) in biomedicine are extensive due to its high surface area, superior adsorption capacity, and biocompatible characteristics. GO can be employed as delivery systems for cytosolic drugs, thanks to its ability to disrupt lipid membranes. GO-Al (OH) nanocomplexes were prepared using the chemical precipitation method, and their surface morphology was determined using SEM and EDS. GO-Al (OH)/ovalbumin vaccine (OVA) formulations were created using a simple combining procedure. These formulations showed improved cellular uptake of Ag in DCs and a better ability to activate Th1 and Th2 responses [14].

FITC was covalently linked with OVA and subsequently loaded onto GO and GO-AlO to examine cellular absorption. However, without an adjuvant, these secure vaccination antigens often have poor immunogenicity. Therefore, there is a growing interest in creating effective and secure vaccine adjuvants. The combination of alum and CpG oligodeoxynucleotides successfully slowed the growth of tumors in mice when used as vaccine adjuvants [16]. A novel formulation containing sodium chloride and alum simultaneously improves cellular and humoral immunity [22].

10.3.3 MONOPHOSPHORYL LIPID A (MPLA) IS INTRODUCED AS AN ADJUVANT TO CANCER VACCINES

Monophosphoryl lipid (MPL) is an adjuvant made of aluminum that is used in approved prophylactic vaccinations for contagious diseases. MPL®, a

metabolite of Salmonella Minnesota lipopolysaccharide (LPS), is a potent activator of antibody and T-cell responses. MPL is the only TLR ligand used in approved human vaccinations. In Europe, MPL® is authorized for use as Pollinex Quattro® for the management of allergies due to its ability to reduce Th2 reactivity to allergens. GlaxoSmithKline (GSK) Biologicals is developing multiple MPL® formulations and mixtures (AS01, AS02, and AS04) as part of clinical studies with vaccines for cancer, HIV, vesicular stomatitis virus (VSV), leishmania, malaria, TB, and tuberculosis [23]. However, the hydrophobicity of MPLA poses significant challenges in its advancement as a prophylactic CV. The absence of the antigen in the APCs prevents MPLA alone from being sufficient to stimulate a unique adaptive immune system response to malignancy. To address this, a successful CV called "vacosome" was created by using a TLR 4 agonist such as phosphatidylcholine and reconstructing the cancer cell membrane. This vacosome promoted an antitumor reaction against breast cancer 4T1 cells *in vitro* and initiated and improved BM DC maturation [24]. Developing cancer immunotherapy vaccines that target tumor-associated carbohydrate antigens (TACAs) specifically can be beneficial. However, their low immunogenicity presents a significant challenge. To overcome this, the development of structurally specified completely synthetic glycoconjugate vaccines using a monophosphorylated version of Neisseria meningitides lipid A was investigated [25]. Novel therapeutic CVs were created by covalently joining a GM2 derivative to keyhole limpet hemocyanin (KLH) and MPLA. Furthermore, the self-adjuvant feature of the GM2-MPLA combination was demonstrated by its ability to trigger potent immune responses without the use of an adjuvant. The cancerous cell line that expresses GM2, MCF-7, was subjected to identical complement-dependent cytotoxicity by the specific antibodies of both conjugates, which demonstrated robust binding [26]. We determined whether co-administration of a DNA vaccine with a TLR-4 ligand, MPLA, and natural killer T (NKT) cell ligand – galactosylceramide (-GalCer) adjuvants might impact the effectiveness of DNA vaccines against tumors in order to increase the effectiveness of DNA vaccines targeted against human papillomavirus (HPV) tumors. In the current research, we proposed that NKT cell antigen-GalCer and MPL might enhance each other's anticancer effects in the field of cancer immunotherapy. It has been established that concurrent stimulation of several immunological targets by a mixture of adjuvants may result in producing an immune response that is more potent and lasts longer against tumors [27]. In order to examine the manufacture and immunological assessment

of an anti-CD205-tailored PLGA-based nanoparticulate CV, ovalbumin was employed as a model antigen and Monophosphoryl lipid was used. The adjuvant was enclosed within the NP using a double emulsification solvent evaporation process [28].

10.3.4 ALUM-CONTAINING APPROVED HUMAN VACCINES

There are several licensed human vaccines containing alum as shown in Figure 10.5. Despite their high reactogenicity, pertussis vaccinations that contained diphtheria and tetanus toxoid proteins along with inactivated Bordetella pertussis germs first became widely utilized in the 1940s [29]. According to a study on the adjuvant effects of aluminum in pertussis and HPV vaccines, an acellular form of the pertussis vaccine with lower reactogenicity was developed in Japan in 1981 after painstakingly identifying endotoxin-minimized protein fractions that conferred protective immunity. These were combined with the toxoids that cause diphtheria and tetanus and adsorbed on alum. The resulting subunit vaccine, known as diphtheria, tetanus, pertussis (DTP), was quickly accepted in Japan as a replacement for DTP, which ultimately reduced reactogenicity and also led to a significant decrease in whooping cough cases. MPL, developed by Edgar Rubi in the 1970s, is derived from the LPS portion of the gram-negative bacterial cell wall. MPL has been used to modify LPS through acid and base hydrolysis to create a detoxified version of "colay's toxin" for cancer therapy. Currently, MPL is used in combination with Alum and other adjuvants to enhance therapy by targeting TLR ligands, and this combination provides strong evidence in trials of the HPV vaccine [30]. The mechanism of alum involves the creation of a depot that allows for continuous release of Ag, the formation of particle structures that promote phagocytosis of Ag by APCs (DCs, macrophages, and B cells), an increase in MHC class-II expression, and Ag presentation. Alum enhances adaptive immunity by inducing tissue damage that activates inflammatory DCs through uric acid. Alum also exhibits a "depot effect," resulting in a gradual release of Ag from the vaccination site [31]. Two prophylactic CVs, Gardasil, and Cervarix, have been approved for specific types of cancer. These vaccines are produced through a fermentation process that utilizes salts, carbohydrates, vitamins, and amino acids as a medium for yeast growth, followed by further physical or chemical purification [3].

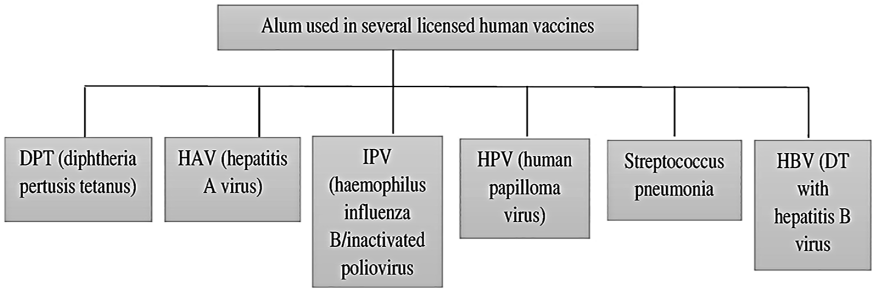

FIGURE 10.5 Alum is an ingredient in several approved human vaccines that protect against viral infections that might cause cancer.

10.3.4.1 DPT VACCINE

It is a monovalent vaccine. Vaccination for pertussis was approved in 1914. The first instance of alum-precipitated diphtheria toxoid was noted in 1926. In 1937, a tetanus toxoid with adsorption was first sold [32]. The long-lasting immune response observed in humans after a natural sickness is not produced by the current acellular pertussis immunizations [33]. Since 1981, Japan has employed vaccinations against pertussis, diphtheria, and tetanus toxoids, precipitated with alum (DTaP) including both initial and booster vaccinations. The acellular DTaP vaccine, in contrast to the whole cell DPT vaccination, contains an alum adjuvant to boost the immunogenicity of the pertussis toxin and filamentous hemagglutinin, as well as the diphtheria toxoid, tetanus toxoid, and other vaccine components [34]. Contrarily, TLR-independent adjuvants have been used empirically to boost antibody responses in alum-based vaccinations, such as the triple vaccine diphtheria, pertussis, and tetanus (DPT), HPV, and hepatitis vaccines. Aluminum salts (alum) and their gel forms are examples of such adjuvants [35].

10.3.4.2 HBV (HEPATITIS B VIRUS)

HBV is particularly important as an etiologic agent in regions with a high incidence of liver cancer. Chronic hepatitis, liver cirrhosis, and hepatocellular carcinoma (HCC) can all originate from HBV infection. Hepatitis B and C are the main causes of HCC. Differentiating between an adjuvant and an enhanced treatment can be challenging. The use of adjuvants can reduce the frequency of therapy in many lung cancer patients [29]. The adjuvant in the HBV vaccination is 3-o-desacyl-4-MPLA, aluminum phosphate. The addition of adjuvants can enhance immunogenicity [36]. In recent studies, a

new immunostimulatory adjuvant is used in the two-dose, inactivated, yeast-derived vaccination known as Heplisav-B. Another example of an adjuvant used for immunostimulation is a recombinant vaccination called Engerix-B, which contains aluminum [37]. Intravenously administered synthetic-GalCer (glycosphingolipids) and HBV protein antigen are combined to protect subjects from HBV [38].

10.3.4.3 HUMAN PAPILLOMAVIRUS (HPV)

Women who receive the AS04-HPV-16/18 vaccine prior to their first sexual encounter are afforded over 90% protection against cervical intraepithelial neoplasia grade 3 or higher, regardless of the HPV type, as indicated by data from clinical trials and real-world experience [39]. The vaccine does not have any impact on pathogen removal.

Adjuvant HPV vaccination has the potential to reduce the incidence of recurrent cervical cancer. Following surgery, a decrease in the occurrence of vaginal intraepithelial neoplasia (VaIN), vulvar intraepithelial neoplasia (VIN), and genital warts has been observed with adjuvant HPV vaccination [40]. Additionally, other adjuvant components include proteins derived from yeast (HPV L1 produced in yeast cells for large-scale protein production), polysorbate 80 (an emulsifier), and amorphous aluminum hydroxyphosphate sulfate (AAHS) [41]. Recent studies have demonstrated that subcutaneous injection of the human HPV peptide vaccine with CpG oligodeoxynucleotides as an adjuvant can prevent orthotopic genital cancer in mice [42]. Three distinct vaccines, Cervarix, Gardasil, and Gardasil 9, have shown efficacy in reducing the prevalence rates of cervical cancer [41].

10.4 CYTOSINE-PHOSPHODIESTER-GUANINE-OLIGONUCLEOTIDE (CPG-ODN)

Recombinant immunostimulatory oligonucleotides, known as CpG oligonucleotides (ODN), are specifically designed to activate Toll-like receptors (TLRs) 9. Subsequent research has identified TLR agonists with improved safety profiles, with the TLR9 agonist CpG ODN standing out for its ability to strongly stimulate Th1 immune responses. Furthermore, CpG activation promotes the expression of co-stimulatory molecules such as class II MHC, CD80, CD86, and the Fcy receptor in B cells [43]. The interaction of CpG with TLR9 (TRAF6) triggers a signaling pathway involving the activation

of MyD88, IL-1R-associated kinase (IRAK), and tumor necrosis factor receptor-associated factor 6. This signaling cascade leads to the activation of certain mitogen-activated kinases (MAPK) and transcription factors, including NF-kB and AP-1, resulting in the production of pro-inflammatory chemokines and cytokines. CpG ODN has been shown to enhance humoral and cellular immune responses, including Th1 cells and CTL, induced by vaccines against infections, allergies, and malignancies. Simultaneous administration of ODN and Ag to the same antigen-presenting cell (APC) accelerates production, increases the concentration, and prolongs the duration of the induced immune response [43]. CpG ODNs exhibit adjuvant characteristics that persist in immunosuppressed individuals when administered mucosally or systemically. Clinical studies have demonstrated the safety of CpG ODNs and their ability to enhance the effectiveness of concurrent immunizations [43].

10.4.1 DELIVERY OF CPG-ODN FROM A CRYOGEL CANCER VACCINE AT THE RIGHT TIME IS EXHIBITED BY ULTRASOUND-TRIGGERED RELEASE

For scaffold-based CVs, it remains unclear when to administer adjuvants, particularly the commonly used CpG-ODN. In order to investigate the hypothesis that timely administration of CpG-ODN can enhance immune responses, we developed a cryogel vaccination technique that enables on-demand release of CpG-ODN through ultrasound (US) stimulation. Upon US stimulation, a controlled burst release and sustained release of CpG-ODN were observed, with the latter continuing at an accelerated rate even after the US was turned off. Four days after vaccination, the cytotoxic T-lymphocyte (CTL) response to US stimulation in live mice was significantly higher compared to the control group. Furthermore, eight days after immunization, US stimulation resulted in a significantly higher IgG2a/c antibody titer compared to all other groups, except for those that received US stimulation. The peak accumulation of DCs in the cryogels coincided with the US-triggered release, which was optimal. This technology provides a promising platform for investigating the optimal timing of immunostimulatory drug delivery for tumor vaccination, as it allows for periodic control of vaccine components through on-demand delivery [44]. The development of a subcutaneous composite system, which utilizes on-demand medication administration repeatedly activated by ultrasound (US), is proposed. Model

drugs (dye) encapsulated in liposomes are in direct contact with gas-filled microbubbles, allowing for enhanced release triggered by US-induced events through the use of an *in-situ* cross-linking hydrogel. *In vitro* studies demonstrate negligible cytotoxicity of the composite when administered subcutaneously to rats, with only mild tissue reaction observed. Furthermore, *in vivo* experiments show that drug release can be triggered by the application of US two weeks after injection [45].

To facilitate *in situ* picture-guided melanoma vaccination, a novel blend of multipurpose nanoadjuvants (M-NA) was synthesized. The M-NA consists of an iron oxide/gold core and a cationic polymer shell, with CpG-ODN electrostatically fused on its surface through multilayer synthesis. Following intratumoral delivery, the M-NA can be absorbed by antigen-presenting cells (APCs) and retained in the dense extracellular matrix (ECM) of gliomas for an extended period. This method holds promise for imaging-guided locally tailored therapy [46].

10.5 TOLL-LIKE RECEPTOR (TLR)

Toll-like receptor (TLRs) are epidermal receptors that are primarily expressed by innate immune cells. They can be divided into TLRs that are expressed on endosomal membranes and those that are on the cell surface (TLR-1, 2, 4, 5, and TLR-6) and intracellular (TLR-3, 7, 8, and TLR-9). TLR agonists are gaining significance by employing them as adjuvants in vaccinations for severe and difficult diseases, including cancer, AIDS, and malaria, because they can connect the innate and adaptive immune system responses. TLR4 agonists have undergone testing as immunotherapy against cancer, anti-allergic medications, and adjuvants in vaccinations against pathogenic disorders. TLR9 agonists have also been researched for use in antitumor immunomodulation and as elements of vaccines against infectious illnesses. It may be easier to understand why TLR4 and TLR9 agonists underwent a more creative process as vaccine adjuvants if you consider that they were first identified as such [47]. Additionally, each TLR comprises a transmembrane domain, a leucine-rich repeat (LRR) portion for recognizing PAMP/DAMPs, and a TIR domain for subsequent signaling pathways and inducing an inflammatory response. TLRs are an excellent target for adjuvants as they can serve as a "danger" signal to initiate an immune response that promotes long-lasting defense [47]. TLR agonists can be combined with other adjuvants, either competitive or non-TLR, to create adjuvants with antitumor

activity or synergistic effects [48]. TLR3 activation has also gained recent attention [49]. Polyinosinic-polycytidylic acid stabilized by lysine and carboxymethylcellulose, known as poly-ICLC (Hiltonol), is one of the TLR agonists being investigated as a potential adjuvant for CVs. There are several other TLR agonists, such as Imiquimod and MPLA, a TLR4 agonist [1]. A three-cohort trial is noteworthy, in which ovarian cancer patients underwent frequent subcutaneous administrations of overlaying long peptides that could be delivered individually, mixed in Montanide, or combined with Montanide and Poly-ICLC. T cells had to be induced by Montanide, and Poly-ICLC made these responses stronger and more stable [49]. The development of a vaccine against the tumor-associated antigen mucin 1 (MUC1) is a significant challenge due to its poor immunogenicity. To enhance immune responses against MUC1, one study aims to create a three-component drug by linking a relatively low-molecular toll-like receptor 7 agonist (TLR7a) to carrier protein BSA via the MUC1 glycopeptide (BSA-MUC1-TLR7a). To investigate their synergistic effects, we additionally added an Alum adjuvant to the three-component mixture. According to immunological tests, anti-MUC1 antibody responses were synergistically boosted by the alum adjuvant and the built-in TLR7a, and immune responses were biased towards Th1. Meanwhile, the vaccine candidate's antibodies successfully detected tumor cells and led to complement-dependent cytotoxicity [50]. In addition to regulating infection and tissue healing and boosting antitumor effects through the stimulation and regulation of innate immune responses, mounting evidence suggests that TLRs also play a role in maintaining tissue homeostasis. TLR agonists are widely used to increase the effectiveness of numerous cancer medicines [51]. To demonstrate that TLRs can be used in conjunction with other adjuvants to enhance the effectiveness of DNA vaccines designed to prevent HPV infection, the anti-tumor effectiveness of DNA vaccination was tested in relation to the effects of monophosphoryl lipid A (MPLA), a TLR4 ligand, and the adjuvant galactosylceramide (-GalCer), an NKT cell ligand [27].

10.6 IL-4 AND IL-13 CYTOKINES' RECEPTORS

The Th1/Th2 paradigm has identified IL-4 and IL-13 as the archetypal Th2-expressed cytokines that counter-regulate Th1 responses necessary for the effective treatment of numerous intracellular infections. Interleukin (IL)-4 and IL-13 have been extensively studied for their roles in innate and

adaptive Th2-mediated immunity; nevertheless, it is still unclear how these cytokines alter the cellular connections required to prevent the development of functional Th1 immunity. HIV-specific vaccine efficacy can be significantly increased by IL-4/IL-13 suppressed vaccine methods. When the wealth of latest discoveries on IL-4 and IL-13 activity at the mucosae is taken together, IL-4/IL-13 is believed to be a cytokine. The capture vaccine technique to inhibit cytokine activities has significant potential to be used against numerous severe mucosal infectious diseases, particularly those that require protection from high-quality T and B cell immunity. As a result, cancer trials have suggested that targeting IL-13Ra2 may be an effective anti-cancer therapy. An immune toxin and an IL-13Ra2-targeted DNA vaccination have recently been evaluated in mouse tumor models as a potential treatment for advanced malignancies [52]. Immunosuppressive Th2 autophagy-restraining cytokines like IL-4 and IL-13 or protective Th1 autophagy-promoting cytokines like IFN are produced by today's vaccines and adjuvants. Another feature of TB infection is a low IFN-/IL-4 ratio and relatively high amounts of Th2 cytokines, which suppress Th1 responses and hinder efficient protective immunity. A secure and non-toxic immunization or adjuvant is necessary to reduce the pre-existing subversive Th2 autophagy-restraining cytokines and increase Th1 autophagy-promoting cytokine (IFN-) secretion (IL-4 and IL-13) [53]. Myeloid-lymphatic endothelial cell progenitors (M-LECP), a subset of BM-derived cells, display M2-type macrophage markers. We hypothesize that the immunosuppressive Th2 cytokines IL-4, IL-13, and IL-10 would promote pro-lymphatic specification of M-LECP during their formation from BM myeloid progenitors due to their ability to induce the M2 type in macrophages. By blocking Th2 pathways, immunosuppression may be reversed, and this may also prevent the development of pro-lymphatic progenitors, which eventually aid in the spread of cancer [54]. It is believed that IL-4 and IL-13 effectively function in pancreatic cancer cells through the IL-13R1/IL-4R signaling pathway [55].

It has become increasingly evident that the establishment of a perfect, long-lasting anti-tumor immune response relies on the generation of CD4+ T helper (TH) cells and cluster of differentiation (CD)8+ CTL cells. The production of TH1 (retinoic acid receptors) and gamma orphan receptors for IFN, IL-2, IL-12, and TNF (ROR-) is thought to be stimulated by peptide-based vaccines, while TH17 (IL-17) cytokine profiles are associated with this stimulation, as opposed to TH2 (IL-4, 6, 13, and IL-13, excluding IL-5) and forkhead box P3 (Foxp3) regulatory T cell (IL-10 and TGF-) production *in vivo* and *in vitro* [56].

10.7 GM-CSF (GRANULOCYTE-MACROPHAGE COLONY-STIMULATING FACTOR)

Granulocyte-macrophage colony-stimulating factor (GM-CSF) is used for the treatment of melanoma. Excision is the primary method of cancer treatment, but it is not effective for metastatic disease. Immunotherapy has shown promise in certain melanoma patients. Melanoma is a suitable target for immunotherapy due to the production of tumor-associated antigens by melanoma cells. Preclinical studies have been conducted to investigate the adjuvant properties of GM-CSF in limiting tumor formation and inducing growth inhibition. In October 2015, Ipilimumab was approved for adjuvant therapy in the USA for cutaneous melanoma patients who had undergone complete excision and total lymphadenectomy with pathologic involvement of regional lymph nodes larger than 1 mm in diameter [57]. GM-CSF can be delivered as an adjuvant by injecting it at the injection site, ingesting it systemically, or by injecting a viral or plasmid vector expressing GM-CSF. However, the results of this human trial have been inconsistent. When GM-CSF and IFA (incomplete Freund's adjuvant) were used as adjuvants for a vaccine containing 12 melanoma peptides, patients with surgical stage III/IIIB/IV melanoma showed a similar immune response. The development of other strategies utilizing GM-CSF continues, such as GM-CSF-expressing oncolytic vaccinia viruses, autologous DCs, allogeneic whole tumor cells, adjuvant GM-CSF following immunization with melanoma peptides or RNA, whole carcinoma cell vaccines, and GM-CSF DNA vaccines [58].

Early studies indicate that GM-CSF stimulates humoral and cellular anti-tumor responses and acts as an immune adjuvant. Patients who received the vaccine with QS-21 had a T-cell response rate of 44.4%, while those who received the vaccine with GM-CSF had a T-cell response rate of 50.0%. In independent investigations, only 26.7% (4/15) of patients who received GM-CSF as an adjuvant to a peptide vaccination showed a cytotoxic T-cell response [59]. A small increase in the number of natural killer (NK) cells and significant increases in CD4+ (P 0.001) and CD8+ (P = 0.007) cells from baseline were observed when GM-CSF was used as a chemotherapy adjuvant [59].

Patients with high-risk neuroblastoma (NB) have experienced success with adjuvant therapy using anti-GD2 monoclonal antibodies (mAbs) and GM-CSF [60]. The level and duration of gene expression in DCs increase when biomaterials and GM-CSF are utilized to create potential delivery vectors for genetically modified DCs or when host DCs are genetically modified *in situ* for immunization and the treatment of autoimmune disease [61].

10.7.1 GM-CSF USE AS AN ADJUVANT WITH CANCER VACCINES: USEFUL OR HAZARDOUS

The appeal of GM-multifactorial CSF's causes lies in its potential use as a vaccination adjuvant. Considering the numerous immune system consequences, GM-CSF has been utilized as a vaccination adjuvant or anticancer treatment in a significant trial conducted over the past decade. GM-CSF is employed as a standalone agent, as a side effect of whole-cell vaccinations using mutated tumor cells, as well as an adjuvant, safely and effectively in vaccinations composed of peptides and DCs that are used to treat various malignancies. A significant portion of current clinical research focuses on anticancer treatment using peptide-based vaccinations. However, when used as an immunological adjuvant, GM-CSF has the potential to inhibit rather than stimulate the immune system. Recent results from trials using GM-CSF as a therapy for melanoma have shown that immune adjuvants used in vaccines can have adverse and immunosuppressive effects. GM-CSF has also demonstrated potential as a vaccination adjuvant in studies involving peptide-based, DC-based, and whole-cell vaccines for the treatment of breast, prostate, pancreatic, renal cell, ovarian, and other malignancies [62]. In one of the phase II studies comparing median dose interferon Alpha 2B to adjuvant immunotherapy using the CSF-470 vaccination, bacillus Calmette-Guerin, and recombinant human (rh) GM-CSF in stages II B, II C, and III cutaneous melanoma patients, the CSF-470 vaccine plus BCG and rhGM-CSF given as adjuvant therapy to stages IIB, IIC, and III CM IFN-2b have been the subject of a randomized research (interferon). Treatment was less effective at delaying or stopping the development of distant metastases [58].

10.7.2 A ROLE FOR G-CSF AND GM-CSF IN NON-MYELOID CANCERS

However, the differentiation of progenitor cells in the bone marrow into differentiated granulocytes, macrophages, and T lymphocytes is regulated by G-CSF and GM-CSF, which stand for granulocyte and granulocyte-macrophage colony-stimulating factors. Our aim is to summarize current research on the adjuvant functions of G-/GM-CSFs in the pathophysiology of solid malignancies and provide insights into the complexity of their therapeutic applications due to the increasing usage of these agents in cancer treatment. The prospective mechanisms of progression are similar to those seen with the adjuvant use of recombinant cytokines, such as induction of

immunological tolerance and angiogenesis. However, caution should be exercised when using recombinant cytokines as an adjuvant treatment in patients with lung cancer, as G-/GM-CSF may accelerate cancer progression and distant metastases. Tumors that secrete GM-CSF include lung tumors, gliomas, bladder tumors, melanoma, and skin carcinomas, colorectal tumors, and bone metastases in prostate and breast tumors [62]. Although adjuvant IFN-2b does not improve overall survival (OS), it increases disease-free survival (DFS); however, it is accompanied by significant toxicity and is not always considered a gold standard therapy [58].

10.8 DENDRITIC CELLS (DCS) AS AN ADJUVANT-BASED THERAPEUTIC CANCER VACCINE

Dendritic cell (DC) activation is required for the initiation of T-cell responses to infections and malignancies [67]. Due to their ability to gather, analyze, and transport antigens to T cells, DCs play a vital role in vaccination. The cellular changes that accompany DC maturation include decreased antigen-capture activity, increased expression of costimulatory molecules and surface MHC class II molecules, the development of chemokine receptors (such as CCR7) that regulate their migration, and the capacity to secrete various cytokines (such as interleukin-12 [IL-12]) that regulate T cell differentiation. DCs can be utilized for cancer vaccination in a variety of methods, including *in vivo* non-targeted peptide, protein, and nucleic acid-based vaccines, vaccines consisting of antigens that selectively bind to DC antibodies, and vaccinations employing *ex-vivo* generated DCs that have been loaded with antigens. Adjuvants for vaccines work by stimulating DC maturation [63]. DCs are well-known for their special capacity to activate both innate and adaptive immune pathways [64]. Combinations of adjuvants that target several pathways may work in concert to increase the effectiveness of immune responses because they can synergistically activate DCs [23]. Adjuvant DC vaccine therapy is more effective than first-line therapy for primary solid tumors. The majority of DC-based vaccinations are now being developed in an adjuvant approach to work together with current cancer treatments. Clinical trials are now being conducted on some enhanced DC vaccines, some of which are anticipated to receive FDA approval. We also hope that DC vaccines will be created as a cancer-prevention vaccine. But the only DC vaccine available now is Dendreon's Provenge against Prostate Cancer, which obtained FDA approval in 2010 [65]. To achieve the highest level of

clinical success, new cancer vaccination techniques (Box 2) take into consideration adjuvants that stimulate cross-presenting DCs [66]. Tumor lysates or recombinant proteins are likely to function optimally in the context of relatively young DCs. However, preprocessed peptides that are MHC class I or MHC class II-restricted are less reliant on being engulfed by macrophages or DCs. Additionally, in the innovative realm of biotechnological, multiformat antibodies, such as bispecific antibodies, are being exploited to bring target cells into close proximity with DCs, thereby enhancing their reactivity or facilitating the delivery of antigenic or stimulatory payloads to CD40-expressing cells, such as DCs [67]. Recent studies have demonstrated positive clinical outcomes of DC vaccinations targeting hTERT and Survivin antigens in metastatic prostate carcinoma [68]. The efficacy of this antigen delivery technique in DC-based cell therapy was investigated in a study, along with the effects of antigen delivery using perfluoropropane gas-entrapping liposomes (Bubble liposomes, BLS) and ultrasound exposure on MHC class I presentation levels in DCs [69]. Other strategies, such as genetic vaccine strategies and peptide vaccine strategies, are also employed as adjuvants to enhance the effectiveness of DC-based cell vaccines [64]. All melanoma patients involved in the study exhibited distinct T cell responses following RNA-induced DC vaccinations. Glioblastoma can also benefit from the use of mRNA-transfected DCs in clinical trials [64].

10.9 COMBINATION OF ADJUVANT

Most vaccines currently available on the market only contain a single adjuvant. However, due to their inherent limitations, no single adjuvant can elicit all the necessary immune responses required for different vaccinations. As a result, researchers are exploring the potential of using vaccine formulations that incorporate multiple adjuvants. According to a new approach, carefully selecting combinations of adjuvants may enhance immune reactions to vaccines in complementary and even synergistic ways. This strategy is promising and provides numerous opportunities for vaccine scientists to tailor immune responses to specific vaccines [64]. The selection of the targeted antigen(s) and the need for effective adjuvants are two crucial factors in enhancing their innate immunogenicity [49]. A new theory suggests that the careful choice of adjuvant combinations can improve immune responses to vaccines in complementary and synergistic ways [64]. As observed so far, combining vaccination with effective adjuvant therapy should result in

heightened immune responses [70]. Therefore, current research combines peptides with adjuvants, aiming to achieve three main objectives: preventing rapid degradation and ensuring sustained release of the antigen, promoting efficient uptake of the antigen by native antigen-presenting cells (APCs), and stimulating full activation of APCs to initiate potent anti-Th1/CTL responses and long-term immunological memory. It is common practice to form an oil-in-water emulsion with Montanide ISATM51 (an incomplete Freund's adjuvant analog typically administered subcutaneously) to preserve the peptides and ensure their delayed release [71]. In individuals with melanoma, it has recently been shown that CpG has a potent adjuvant effect when paired with a long New York esophageal squamous cell carcinoma-1 (NY-ESO-1) peptide [72]. Imiquimod may be an extremely inefficient adjuvant on its own, but it could be highly useful when combined with other TLR ligands. Therefore, our objective is to identify a single preferred combination that can elicit potent anti-vaccine T cells and durable clinical responses in cancer patients by administering peptides (the antigen), montanide (for depot impact), and a TLR agonist (for APC activation). We believe that the most immunogenic combinations should be selected for larger studies to test for anti-tumor activity. Small-scale clinical studies intended to investigate peptide, adjuvant, and immunomodulatory combinations should assess the immunogenicity of these combinations. Synergistic combinations may lead to more unfavorable occurrences, so it is crucial to carefully monitor any resulting toxicities [49]. When the combined therapy was less effective, the addition of a tumor-antigen-based peptide, primarily linked to -GalCer, significantly enhanced the antitumor activity in tumor models [73].

10.9.1 TOLL-LIKE RECEPTOR (TLRS) AND ALPHA GALACTOSYLCERAMIDE (GALCER) COMBINATION THERAPY

Pathogen-associated molecular patterns are distinctive molecular markers for pathogens that Toll-like receptors (TLRs) can recognize. TLR3 agonists with varying degrees of efficiency have been previously utilized as adjuvants in cancer treatment to induce an IFN-mediated antitumor immune response [74]. TLRs play a crucial role in innate immune responses by detecting pathogenic antigens. Va14 natural killer T (iNKT) cells have been shown to recognize the glycolipid -GalCer as a ligand. TLR agonists can enhance the production of IFN-c in immune cells that have been administered GalCer injections to heighten their sensitivity. *In vitro*, GalCer-pre-treated

splenocytes were stimulated along with other TLR agonists including CpG ODN, Poly-inosinic acid: cytidylic acid (I:C), Lipopolysaccharides (TLR4 agonist), and Imiquimod (TLR7 agonist, TLR9 agonist), resulting in a significant increase in the production of IFN-c mRNA [75]. TLRs assist in the innate recognition of pathogen-associated molecular patterns (PAMPs) and the initiation of immune responses in dendritic cells (DCs) [76]. Overall, the treatment of NKT agonists, TLR ligands, and melanoma antigens via a multivalent nano-vaccine demonstrated synergistic anti-tumor innate immune efficacy in the B16F10 melanoma mouse model [77].

10.9.2 ALPHA GALACTOSYLCERAMIDE (GALCER) AND MONOPHOSPHORYL LIPID A (MLA) COMBINATION THERAPY

A lipid known as Monophosphoryl lipid A (MLA), derived from the LPS of Salmonella Minnesota R595 [78], is the first TLR4 agonist approved for use as a human immunization adjuvant. In this study, the effectiveness of the adjuvants GalCer and MPL in enhancing antitumor immune responses was examined when combined with an HPV-16 E7 DNA vaccination. The results showed that using a combination of adjuvants for the DNA vaccination was significantly more effective than using separate adjuvants in terms of lymphocyte proliferation, CTL activity, IFN-, IL-4, and IL-12 responses, as well as tumor protection against TC-1 cells [76]. The combination of -GalCer and MPL's powerful adjuvanticity may play a crucial role in the development of an effective and novel cancer immunotherapy. Researchers also predicted that the combination of NKT cell antigen GalCer and MPL could enhance the anticancer benefits for immunotherapy of cancer [27]. Two vaccines, Cervarix and Fendrix, already contain MPLA adjuvant and are used to prevent diseases such as HCC and cervical cancer, respectively [79].

10.9.3 ALPHA GALACTOSYLCERAMIDE (GALCER) AND CPG COMBINATION THERAPY

APC activation, cytokine release, and adaptive immunity can all be enhanced by intravenous infusion of α-GalCer and TLR ligands, as we have previously demonstrated. Furthermore, studies have indicated that administering the NKT cell agonist a few hours prior to the TLR agonist significantly enhances cytokine release. To investigate various intratumoral procedures, either a single dose of 2 g of α-GalCer was administered simultaneously

with the initial dose of CpG or six hours earlier (by delaying the first CpG dose by 6 h). Intratumoral administration of α-GalCer significantly improves intratumoral CpG-induced local and distant antitumor immune responses [73]. However, compared to each treatment alone, the combination treatment exhibited a lower rate of tumor growth, and the majority of the animals experienced complete rejections.

10.10 POLYMERS ADDED AS AN ADJUVANT TO CANCER VACCINES

Particulate adjuvants have shown promise in facilitating the delivery of antigens to lymphatic organs and enhancing immunogenicity. Examples of such adjuvants include inorganic particles, polymeric particles, liposomes, emulsions, and exosomes. A recent study by Sun and colleagues demonstrated the creation of AlO(OH)-polymer nanoparticles (APNs), which are nano-sized vaccine carriers with improved lymphatic motility. The research findings revealed that APNs effectively facilitated cytosolic transport and cross-presentation of antigens upon internalization by antigen-presenting cells (APCs), resulting in the development of adaptive cell-mediated immunity.

Polymeric particle adjuvants can be derived from natural or synthetic sources such as plants, animals, and microbes. Common examples include albumin, fibrinogen, alginate, chitosan, and collagen. These polymers offer advantages such as high loading capacity, efficient antigen protection *in vivo* or *in vitro*, affordability, biocompatibility, biodegradability, and ease of production. However, they also have limitations in terms of stability and controlled properties [9]. Biologically derived polymers with adjuvant properties can be directly obtained from biosources or chemically synthesized. These polymers can enhance antigen presentation, create depots, improve endocytosis, and bind to pattern recognition receptors (PRRs) to modulate the immune response when utilized in vaccines and immunotherapies [12]. Numerous methods, including conjugation, simple mixing, encapsulation, or adsorption based on charge or hydrophobic interactions, can be employed to produce antigens from polymeric particles [110]. Adsorption and encapsulation have traditionally been considered the two main strategies for loading antigens onto polymeric adjuvants [9]. While biologics and small compounds still dominate the adjuvant industry, recent research is beginning to support the use of immunomodulatory polymers in therapies. When studying the immunology of next-generation polymeric adjuvants that target PRRs, it is important to consider the polymers' ability to establish biophysical

connections with cells [11]. In order to generate potent and long-lasting T-cell responses in combined tumor immunotherapy, a pH-responsive multivesicular polymeric NPs are utilized to deliver the STING agonist cGAMP, as well as peptide oncoviral antigens and neoantigens [80]. The phospholipid bilayer shell structure is filled with a polymeric polyplex mRNA core. Dendritic cells (DCs) efficiently uptake the resulting lipopolyplex mRNA vaccine through macropinocytosis. DCs treated with lipopolyplex mRNA vaccination exhibit enhanced antigen presentation capabilities, effectively stimulating an anticancer immune response [81].

10.10.1 POLYMERIC NANOPARTICLES

The majority of these characteristics, such as cellular activity, higher bioavailability, protection of the antigen against deterioration, and controlled antigen release, appear to be present in nanoparticles (NPs). The most well-known type of polymeric NP used as an antigen delivery technique is poly(D, L-lactic-co-glycolide) nanoparticles (PLGA-NPs), sometimes referred to as PEG. Data from flow cytometry and confocal laser scanning microscopy (CLSM) demonstrate that human CD14+ monocytes and the mouse Hepa 1–6 hepatoma cell line are more receptive to PLGA/PEI (polyethyleneimine) NPs. In one study, it was shown that the therapeutic vaccination's anticancer effects are enhanced by the addition of an HPV E7 SLP to extremely tiny polymeric nanoparticles (NPs) [68]. Both melanoma cancer and HPV cancer are treated with the use of these polymeric NPs. In order to create a vaccine technology for acute myeloid leukemia (AML) cell membrane, the leukemic cell membrane material is coated on immune-stimulating adjuvant-loaded nanomaterials [83]. Toll-like receptor agonist (TLRa)-loaded NPs have the power to increase tumor suppression and destruction while reducing the danger of immune-related damage.

10.10.2 ALBUMIN AS AN ADJUVANT IN CANCER VACCINES

Pulmonary immunization using albumin-binding surfactant molecules adducts of peptide antigens and CpG adjuvant (amph-vaccines) elevated vaccination levels in the lungs and mediastinal lymph nodes (MLNs) [84]. We incorporated aluminum ions into NPs using bovine serum albumin (BSA), which has been shown to chelate inorganic ions like Au and Gd into NPs under benign conditions. The NPs' albumin may aid in supplying the quickly

expanding tumor with energy [85]. Al-BSA-Ce6 NPs is an innovative and distinctive alumina adjuvant delivery system that is useful in the treatment of cancer. An efficient vaccination adjuvant that boosts antigen-specific CD8+ T cell-mediated antitumor immunity is albumin and interferon-fusion protein. This composition lengthens the half-life of IFN *in vivo*, delivers it to LN, and boosts innate immunity to tumors [86]. Nanocomplexes made of albumin or AlbiVax are easily adaptable to immunogenic vaccination for individualized tumor immunotherapy. Exogenous neoantigen vaccines delivered by albumin/AlbiVax nanocomplexes can improve tumor therapy and neoantigen-specific immunity [87].

10.10.3 CHITOSAN AS AN ADJUVANT IN CANCER VACCINES

Due to their immunostimulatory effects and structural similarities to glucans, chitin, and chitosan have been the subject of several studies investigating their adjuvant properties. The preservation of the vaccine in the nasal cavity via mucoadhesion, as well as the opening of endothelial cell junctions for paracellular administration of the vaccine, is thought to be the mechanism of vaccine augmentation by chitosan through mucosal delivery. Small, phagocytosable chitin particles caused alveolar macrophages to release cytokines such as TNF, IL-12, and IL-18, which primarily induced NK cells to produce INF [88]. The complexation of a nucleic acid-based adjuvant with chitosan regulates immune adjuvant activity (CTS). Chitosan is negatively charged, making it more difficult for it to pass through cell membranes. The TLR3-type adjuvant action of the RNA adjuvant was significantly enhanced through complexation with CTS [89].

10.10.4 COLLAGEN AS AN ADJUVANT IN CANCER VACCINES

The benefit of collagen anchoring in extending the intratumoral retention of IL-2 has been observed. The addition of MSA improved the molecular weight of the design, resulting in reduced diffusive flux away from the tumor and ensuring electrostatic access of IL-2 receptors when bound to collagen fibrils. Clinical evidence has revealed the buildup of collagen type I along the aggressive margin of breast, colorectal, and skin cancers. This margin, in contrast to the tumor's core, typically contains a higher concentration of immune cells, particularly T cells [90]. Collagen is also utilized as a marker for detecting presurgical margins in the eradication of basal cell carcinoma

[91]. In human breast cancer, the presence of aligned collagen serves as a predictive indicator of survival [92].

10.11 NANO-ADJUVANT IN CANCER VACCINES

Because cancer vaccination utilizes delivery vehicles or adjuvants to induce potent cellular and humoral immunity, it is an effective technique for both preventing and treating cancers. Innovative delivery methods and vaccine adjuvants have been developed more frequently in immunotherapy as they can stimulate immune responses with fewer doses and adverse effects. Thanks to nanotechnology, investigators now have complete control over the production of gadgets at the molecular level. In this regard, pathogen-specific antigens are combined with artificial or natural nanostructures to create nano-vaccines, which have shown to elicit manageable immune responses. This approach recommends utilizing crucial pathogen sub-units such as peptides, proteins, membranes, polysaccharides, and capsules to produce vaccines that are more flexible and secure [93]. To enhance immunity, various metals can be employed as effective adjuvants in the creation of nano-vaccines. Additionally, antigens can be successfully delivered by metal-based nanoparticles to antigen-presenting immune cells [94]. Nano-based vaccines that are currently being developed to treat cancer include those based on proteins, peptides, nucleic acids, DNA, RNA, bacteria or viruses, liposomal vaccines, polymeric vaccines, and more.

10.11.1 IMMUNOSTIMULATORY NANO-ADJUVANT

CVs contain immune adjuvants that can promote the immune system, improve the immune responses evoked by antigens, and target specific immune responses. Small molecule antigens must possess these adjuvant qualities because they are typically not very immunogenic [95]. Immunotherapy is currently an effective clinical approach for treating cancer and infectious disorders. Potential immunostimulatory medicines for use in immunotherapy include small synthetic compounds that bind to TLR7 [93]. Examples of immunostimulatory nanoadjuvants include porous silicon microparticles (pSiMPs) and Selenium nanoparticles (SeNPs). It is now understood how immunomodulatory and nanotherapeutic approaches can be utilized to develop breast cancer vaccines.

10.12 DIFFERENT DELIVERY SYSTEMS OF ADJUVANTS

Significant advancements have been made in refining vaccination administration methods to overcome poor cellular absorption. DNA vaccines have proven to be more effective in both pre-clinical and clinical investigations due to advances in delivery and plasmid design. As shown in Figure 10.6, there are various vaccine delivery systems, but DNA vaccination holds promise for eliciting both cellular and humoral immune responses [96]. Some of the delivery systems include Electroporation and Gene gun vaccine delivery used in prostate cancer, NP vaccine delivery system, Self-assembly peptides used for gene therapy and bone regeneration *in vivo*, and genetic vaccines (DNA, RNA, viral vector, and peptide vaccine) as shown in Figure 10.7. Delivery devices that resemble viruses and virosomes are frequently utilized to treat infectious disorders. Examples of commercially available vaccinations that have been adjuvanted with virus-like or virosome delivery methods include the influenza vaccine, the HBV vaccine, and the HPV vaccine. Additional clinical research on VLP and virosome adjuvants is also underway at various stages, showing promising results in producing high antibody titers and inducing adaptive immunity [38].

10.12.1 GENE GUN DELIVERY

In 1990, the first study on a gene delivery method was published by laboratory researchers [5]. The epidermal Langerhans and keratinocytes can be directly transfected with DNA using a gene gun, which is a transgenic device. This delivery method utilizes transgenic vectors such as plasmid DNA (pDNA) [5]. Direct DNA transfer is advantageous for the expression of transgenic proteins using mammalian gene expression vectors. These instances promote the development of DCs and their migration to the local lymphoid tissue, where they serve as the starting point for T cells to initiate antigen-specific immune responses used for melanoma vaccination in the skin. In lung cancer, non-coated DNA exhibited the highest levels of cytotoxic T-lymphocyte activity and the most effective anti-tumor effects [96]. In one of the investigations, GM-CSF gene-transfected autologous tumor cells were used for vaccination to achieve optimal therapeutic outcomes [97]. The FDA has set the intensity level up to 0.3–3 w/cm^2, which raises the temperature by no more than 1°C [98]. In one of the studies, ultrasound microbubbles (USMB) were used for cardiovascular therapy. This technique

has been employed to treat various cancers such as breast cancer, brain cancer, pancreatic cancer, melanoma cancer, and HCC. Focused ultrasound breaks through the blood-brain barrier, improving the delivery of chemotherapy drugs for the treatment of glioblastoma [99]. A recent study has been carried out which states that, in the TME, US simultaneously stimulated LA and BMT, resulting in the production of singlet oxygen and NO gas. Additionally, after being exposed to US, cells showed strong CD80, CD86, and MHCII expression [100]. Another study titled "Focal Therapy with High-Intensity Focused US: Efficacy in 1379 Men Without Metastatic Prostate Cancer" was also conducted [101].

10.12.2 ULTRASOUND

By briefly rupturing cell membranes with ultrasound (US), DNA can be effectively and immediately transported into the cytosol and incorporated into the cells. This method is employed to introduce naked plasmid DNA (pDNA) into colon cancer cells [54]. The frequency of US ranges from kHz to MHz, depending on the model organism selected for the preclinical test and the tissue types. The frequency level is lower for therapeutic uses, which results in deeper penetration due to less attenuation, leading to the best therapeutic results. The FDA has set the intensity level up to 0.3–3 W/cm^2, which raises the temperature by not more than 1°C [8]. In one of the studies, USMB is used for a CV. This technique has been used to treat various cancers such as breast cancer, brain cancer, pancreatic cancer, melanoma cancer, and HCC. The Focused United States enhances the delivery of chemotherapeutic drugs for the treatment of Glioblastoma by disrupting the Blood-Brain Barrier [57]. A recent investigation has been conducted which affirms that, within the Tumor Microenvironment, US concurrently stimulated Local Anesthesia and Bone Marrow Transplantation, resulting in the generation of singlet oxygen and Nitric Oxide gas. Furthermore, cells exhibited robust expression of CD80, CD86, and MHCII after exposure to US [20]. The effectiveness of Focal Therapy with High-Intensity Focused US has been demonstrated in 1379 men with Non-Metastatic Prostate Cancer [53].

10.12.3 ELECTROPORATION

Nucleic acid molecules are delivered to the target cells using this technique. The technique involves electroporation of a plasmid containing an antigen

into a dendritic cell (DC). Due to the penetration of water molecules through the lipid bilayer, the cell membrane can be reorganized to orient the polar heads of the phospholipids towards the water. As a result, nanopores are formed in the cell membrane [102], allowing large compounds such as DNA or RNA, typically encoding human IL-12, to pass through and enter the cell's cytoplasm. This method is employed to electroporate muscles in living organisms, effectively stimulating anti-tumor immune responses against cancers expressing the prostate antigen. The vaccination is both safe and effective [96]. It represents a potential approach for gene delivery to enhance cardiovascular immunity [102]. In mouse cancer models, intratumoral electroporation of self-amplifying RNA, which produces IL-12, along with an adjuvant to boost vaccine immunity, has demonstrated anticancer effects [103].

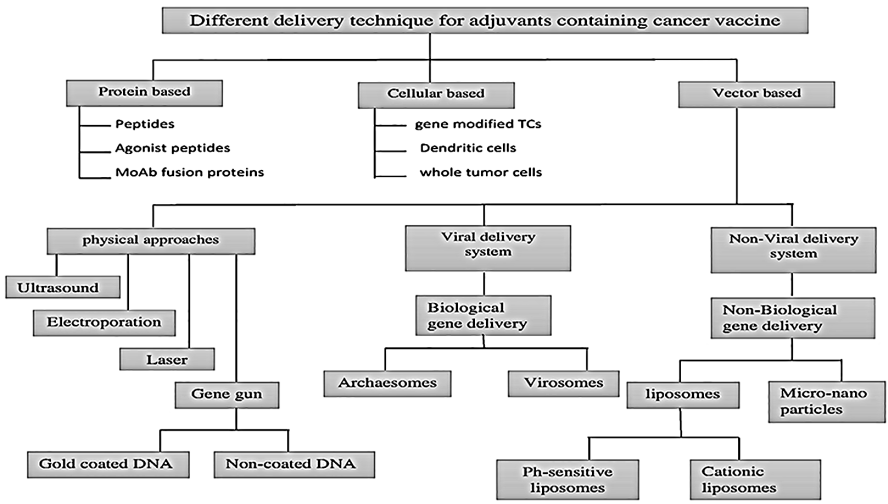

FIGURE 10.6 Classification of various vaccine delivery systems of adjuvant containing cancer vaccines.

10.12.4 LIPOSOMES DELIVERY SYSTEM

Gregoriadis and Allison were the first to describe the capacity of liposomes to elicit immunological responses to integrated or linked antigens [104]. Liposomes were one of several innovative novel drug delivery methods that demonstrated a breakthrough in the ability to transfer active compounds to the target of action. Currently, numerous formulations are being used in clinical settings [105]. Liposomes, which are used as vaccine delivery vehicles,

can have antigenic stimuli incorporated in the bilayer, adsorbed onto their surface, or engrafted onto them [104]. A liposomal antigen delivery method that has been altered with a pH-sensitive dextran alternative has been shown to improve antigen cytosolic delivery [106]. Vaccine delivery methods based on liposomes are very flexible and adaptable [107]. In 2014, cyclical di-GMP/YSK05 liposomes were identified as a new adjuvant delivery mechanism for cancer immunotherapy. In this study, CD80, CD86, and MHC class I expressions were found to be significantly higher [108]. It appears that liposomes have a significant advantage over other antigen delivery methods due to their capacity to be modified by a variety of ligands in order to effectively and specifically target APCs. A VADS made of liposomes has already been successfully used to create vaccines that have been licensed for clinical vaccination against a variety of illnesses, including those brought on by the most difficult pathogens, like shingles and malaria [109]. Liposomes are a type of carrier that fall under the first category of adjuvants; they have the advantage of carrying insoluble antigens and greatly enhancing the immunogenicity of synthetic peptides or weak protein antigens. Liposomes are now being researched as antigen delivery methods for tuberculosis, hepatitis A, malaria, influenza, and non-small cell lung cancer (NSCLC) [17].

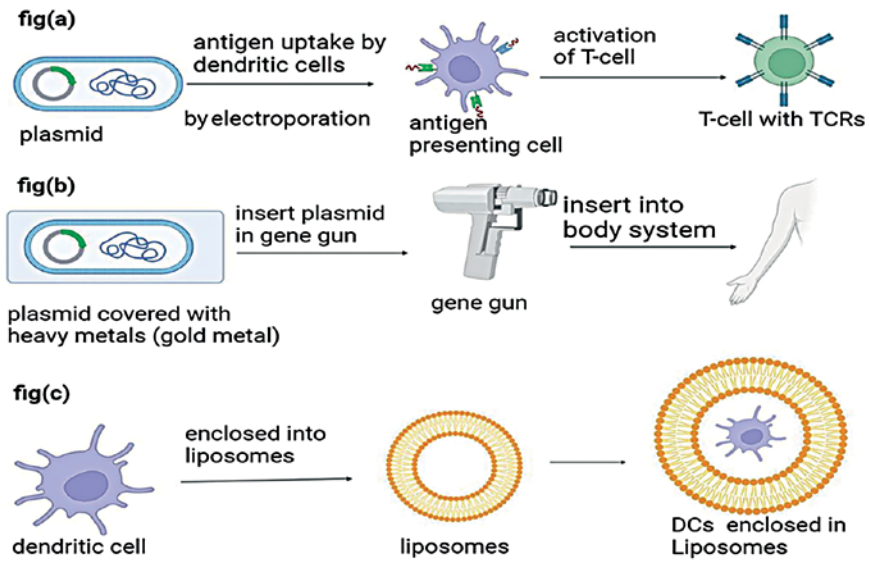

FIGURE 10.7 (a) Electroporation delivery system; (b) gene gun delivery system; and (c) liposomes delivery system, these all method are used to deliver Ag *in vivo*.

10.13 CONCLUSION

We continue to heavily rely on aluminum-based chemicals for human adjuvants, despite the abundance of information on immune function that has emerged in recent decades. The efficacy of these adjuvants was initially recognized over 80 years ago. While there are promising indications that new adjuvants could address some of the limitations associated with aluminum-based adjuvants, there is concern that many of these potential alternatives may not receive approval for human use due to logistical or financial considerations, rather than concerns for the greater good. The regulatory standards for human adjuvants have significantly increased since the introduction of alum as an adjuvant. In fact, it is likely that current products would not exist if alum had not been in use for such a long time and had not received regulatory approval. The recent discoveries regarding IL-4/IL-13 and their receptors raise additional questions, particularly regarding their impact on infection and immunity in the innate and adaptive compartments. For example, the primary cellular sources and catalysts for the production of IL-4 and IL-13 remain unknown. Several vaccines, such as Fendrix for hepatitis B, Shingrix for shingles, and Cervarix for cervical cancer, have been authorized with adjuvants containing TLR4 agonists. Similar to this, Heplisav-B, another Hepatitis B vaccination, contains a TLR9 agonist. The earlier discovery of TLR9 agonists may account for their early development as vaccine adjuvants. In conclusion, TLR agonists have become highly effective immunostimulatory agents against both infectious diseases and cancer, as well as vaccine adjuvants. To ensure effective anticancer action, DC vaccinations require a variety of molecular or immunological markers, depending on the context (e.g., cancer type, antigen heterogeneity/immunogenicity, tumor immune microenvironment, overall immunological fitness). Particularly for certain types of cancer, next-generation DC vaccines can be used in combination with adjuvant therapies. To make DC vaccines more accessible to patients, technological advancements that reduce the barriers to regulation-compliant manufacturing are also required, especially when combined with already expensive ICIs and the current financial pressures on healthcare systems. GM-CSF was used as an adjuvant in the design of Sipuleucel-T. In a phase III trial, the Montanide ISA-51 peptide vaccine with modified gp100 and incomplete Freund's adjuvant showed excellent results. Additionally, as the group of PRRs (Pattern Recognition Receptors) expands, more research is being conducted on their potential use as adjuvants in therapeutic cancer vaccines. There are now multiple clinical trials targeting TLR3 agonists and TLR9 agonists that focus on combining them with GM-CSF or other TLR agonists. Nanoparticles (NPs), self-assembling peptides, and

needle-free delivery methods have all been developed as a result of improvements in vaccine delivery methods. Electroporation delivery system, as it has been with previous cancer and infectious disease vaccines. Although these more contemporary delivery methods offer improved substitutes, their use has been constrained by the discomfort of electroporation administration and the requirement for specialized vaccine delivery equipment. Due to their flexibility, ability to store tiny drug candidates or antigens in the form of RNA or peptides, and high safety profile, liposomes are being exploited as delivery systems. Liposomes must be tuned to have the proper charge and particle size for incorporating their contents and transporting them across the cell membrane. To enhance local immune response and trafficking to LN, cancer antigens can be targeted through peptide-adjuvant linkage, antigen incorporation into NPs, or genetic vector modification, such as the ImmunoBody® platform.

KEYWORDS

- **alum**
- **antigens**
- **bovine serum albumin**
- **dendritic cells**
- **granulocyte-macrophage colony-stimulating factor**
- **graphene oxide**
- **lipopolysaccharide**
- **mediastinal lymph nodes**
- **toll-like receptor**
- **A-galactosylceramide**

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CHAPTER 11

Cancer Vaccine and Chemotherapeutic Agent: Advances in Cancer Management

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ABSTRACT

Carcinogenesis is a multifactorial chronic process that encompasses a variety of factors. Monotherapy of chemotherapy would not be sufficient to eradicate resistant tumor cells and cancer stem cells (CSCs). As it solely relies on cytotoxicity and apoptosis induction, the potential of the immune system as a weapon against cancer remains untapped. Vaccine development has played a crucial role in saving mankind from deadly infections. The fundamental mechanism of any vaccine is based on activating specific immune cells in the short term while leaving memory cells to prevent disease recurrence. Each type of cancer has its specific antigen on tumor cells, such as carcinoembryonic antigen (CEA) and MUC1 present in colorectal cancer

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tumors. The FDA has endorsed the HPV vaccine to prevent vaginal, cervical, and anal cancer caused by human papillomavirus (HPV). The success of the Hepatitis B vaccine for liver cancer and sipuleucel-T (Provenge) for metastatic prostate cancer has sparked interest among immunologists and cancer biologists to develop vaccines for many other types of cancer. Cancer vaccines (CVs) can be considered as part of immunotherapy-based anticancer treatment. However, there are several challenges that need to be addressed for the proper efficacy of anticancer vaccines. These challenges include the systemic immunosuppressive environment, loss of antigen from tumor cells or deficits in major histocompatibility complex (MHC), the presence of glycocalyx on tumor cells, larger tumor size at advanced stages, and the application of vaccines in immunocompromised patients. So, it would be considered as synergism if we can combine chemotherapy with vaccine treatment. The combined strategy may appear like abscopal effect mediated by radiation therapy. Preclinical study of cyclophosphamide (CTX) and doxorubicin delayed the growth of breast cancer in mice when combined with GM-CSF/HER2/neu complete-tumor cell. The application of therapeutic CVs in conjunction with chemotherapy and the utilization of the immunomodulatory effects of chemotherapy to enhance the anticancer effects of vaccinations are the main foci of this review. The immunomodulatory effects include the induction of cancer cell death, which stimulates antigen-presenting cells (APCs) like macrophages and dendritic cells (DCs), increases tumor antigen (TA) expression, adhesion molecules, and leukopenia, and favors the development of T cells that are specific for the tumor.

11.1 INTRODUCTION

According to a report from the Centers for Disease Control and Prevention, cancer ranks as the second leading cause of mortality. Cancer can be considered an important source of premature death worldwide [1]. It becomes crucial to diagnose cancer at an early stage, as the number of cases rises drastically, and advanced stage patients have a high mortality rate. The GLOBOCAN 2020 assessment reported 10 million cancer-related deaths globally in 2020, with approximately 19.3 million new cases diagnosed. By 2040, it is projected that there will be 28.4 million new cancer cases worldwide, representing a 47% increase compared to 2020. This increase can be attributed to lifestyle modifications, environmental pollution, and demographic changes [2]. The escalating risk factors associated with

globalization and expanding wealth may further exacerbate this situation [3]. The highest mortality rates due to cancer are found in lung cancer, followed by colorectal, liver, stomach, and female breast cancer [4]. Cancer occurs as a result of multiple factors that chronically disturb the body's homeostasis. It is characterized by abnormal high cell proliferation, disturbed cellular differentiation, high nuclear to cytoplasmic ratio, mitochondrial defects, angiogenesis, chronic inflammation, immune evasion, and apoptosis evasion [5]. All of these abnormalities are caused by multiple defective genetic mutations [6] and epigenetic changes [7]. Early diagnosis remains a significant challenge due to lack of public awareness and socioeconomic factors. A majority of cases are detected at advanced stages of the disease when they reach the clinics. Nowadays, there are various therapeutic modalities available for cancer treatment. These include chemotherapy, hormonal therapy, radiation, surgical ablation, and immunotherapy [8]. Additionally, complementary medicine such as naturopathy and acupuncture are used as palliative support during cancer treatment [9].

The origins of chemotherapeutic agents can be traced back to World War II. Nitrogen mustard, initially used as a chemical weapon, was found to cause a significant decrease in leukocyte counts. In 1940, two renowned scientists, Louis Goodman and Alfred Gilman, explored the potential of this agent in lymphoma treatment. Since then, numerous alkylating agents, cytotoxic drugs, and antimetabolites have been discovered to save the lives of cancer patients [10]. The main mechanisms of chemotherapy involve halting the cell cycle, DNA alkylation, disrupting nucleotide synthesis, deforming microtubules, interfering with topoisomerase function, and inducing apoptosis in rapidly dividing tumor cells. However, the application of chemotherapy is limited by its toxicities and the emergence of resistance in many cases. Dose-limiting toxicities include mucositis, neuropathy, damage to vital organs, diarrhea, myelosuppression, and the development of secondary tumors as late side effects [11].

There are reports available on the failure of chemotherapy as monotherapy in cancer treatment. Following the use of platinum-based chemotherapy for metastatic cervical cancer, the patient experienced recurrence [12]. Even the treatment of advanced stages of cancer requires high doses of chemotherapy, which increases the vulnerability to irreversible toxicities. The use of anthracyclines and doxorubicin for childhood cancer causes cardiotoxicity at a late onset and raises the risk of mortality due to the disruption of the cardioprotective network of Vascular Endothelial Growth Factor A (VEGFA) and Signal Transducer and Activator of Transcription 3 (STAT3) signaling [13]. Myelosuppression is one

of the main concerns of chemotherapy, further promoting anemia, immunosuppression, and hemorrhage [14]. Immunosuppression is an essential element for the initiation and spread of cancer, responsible for tumor aggression and resistance. Numerous cancer microenvironments release immunosuppressive cytokines such as tumor necrosis factor alpha (TNF-α), interleukin (IL)-8, and IL-6, exacerbating clinical conditions and leading to resistance [15]. Transforming growth factor (TGF-), an essential immunoregulatory cytokine secreted by tumor cells and stromal fibroblast cells, causes the formation of cancer-associated fibroblasts (CAFs). These cells further promote cancer development, invasion, cancer stemness, and resistance [16]. All these indications lead to the suppression of antitumor immunity, which is the main key player in halting tumor growth. While chemotherapy acts on other hallmarks of cancer to prevent its growth, the unnecessary suppression of immunity may flourish the tumor and make it a more stubborn, heterogenous resistance cell factory day by day [17]. Therefore, there is a need for combined treatment with different therapeutic agents that curb tumor growth through immunomodulation and maintain vigilance through natural body defense mechanisms [18]. However, the use of immune-based therapy alone would not be sufficient to effectively control cancer progression.

Despite the use of monoclonal antibody (mAb) conjugates, in the case of HER2-positive breast cancer, more than 33% of patients with metastasis develop resistance [19]. The surface of most tumors shows the presence of different neoantigens, which can be explored to target the tumor more specifically and precisely through immune modalities [20]. Vaccine development can be considered the greatest lifesaving regimen for mankind. It has protected humans against numerous deadly infections, from smallpox to SARS-CoV-2. Sipuleucel-T (Provenge) received FDA approval in 2010 as the first therapeutic cancer vaccination to treat metastatic castrate-resistant prostate tumors. Subsequently, many vaccines have been tested in clinical trials as monotherapy or in combination to improve treatment outcomes and prevent cancer recurrence [21]. However, vaccines have their limitations and need to be used rationally and in combination with chemotherapy to achieve synergistic effects.

11.2 CARCINOGENESIS – ITS HALLMARKS AND IMMUNE DYSFUNCTION

The emergence of genetically abnormal and overproliferation of cells in the tissue, with dysregulation at multiple biological processes such as energy,

metabolism, respiration, cellular death, and differentiation, is referred to as carcinogenesis, which is the development of cancer. Carcinogenesis can be initiated by the transfer of hereditary mutated genes such as BRCA1 and BRCA2 in breast cancer [22], APC for colorectal cancer [23], KRAS in many cancers [24], and BCR-ABL for leukemia [25]. Environmental factors and inappropriate lifestyle choices often trigger the conversion of proto-oncogenes to oncogenes or defects in DNA mismatch repair genes and the deactivation of tumor suppressor genes, leading to the development of cancer over time. Carcinogenesis can be caused by physical, chemical, or biological carcinogens (cancer-causing agents).

11.2.1 GENETIC AND EPIGENETIC MANIPULATION AT THE ROOT OF THE DISEASE

The genetic insult caused by hereditary or environmental factors promotes the overactivation of oncogenes, which disrupt cell cycle regulators and allow for excessive cell proliferation. Genetic mutations act as drivers and confer significant advantages to tumor cells compared to normal cells, including enhanced proliferation, high metabolism, respiration, immune evasion, and apoptosis evasion [26]. Integrative OncoGenomics (IntOGen), developed by a group of researchers, provides a comprehensive identification of mutated genes in different tumors [27]. FMS-like epidermal growth factor receptor (EGFR), KRAS, tyrosine kinase 3 (FLT3), platelet-derived growth factor (PDGF) receptor, MYC, and BRAF are among the several oncogenes that undergo alterations. Adenomatous polyposis coli (APC), BRCA1 and 2, PTEN, TP53, and transforming growth factor receptor-2 (TGFRB2) are among the tumor-suppressor genes involved. Certain alterations such as translocation, amplification, and deletion lead to genetic mutations that initiate chronic carcinogenesis [28]. The knowledge of genomics can help in the development of personalized medicine for cancer patients. Recent theory also supports the involvement of epigenetics, in addition to genetics, in initiating and promoting tumorigenesis. Epigenetic changes do not involve alterations in the DNA sequence. The regulation of genetic expression through epigenetic modifications includes processes such as methylation, acetylation, and histone modification. Abnormal epigenetic modifications sustain tumor growth by promoting autophagy, which further enhances the survival of cancer stem cells (CSCs). These modifications activate cellular signaling systems such as Hedgehog, Notch, and Wnt-catenin, which maintain CSC

dormancy and contribute to resistance and recurrence [29]. Additionally, the role of micro-RNA (miRNA) has been reported in controlling transcription, apoptosis, and cell proliferation [30].

11.2.2 HALLMARKS OF THE CANCER – RECENT UPDATES

During carcinogenesis, the mutated cells acquire changes in functional characteristics. These capabilities are required for the transformation of normal to neoplastic state, known as hallmarks of cancer [31]. The vital hallmarks involve immune evasion, immortality, chronic inflammation, metastasis, invasion, angiogenesis, apoptosis resistance, hypoxia, metabolism dysregulation, and sustained proliferative signaling pathways [32]. The prevalent genetic mutations and epigenetic reprogramming during tumorigenesis support the cancer hallmarks that eventually develop tumors and promote metastasis through epithelial mesenchymal transition [33]. The presence of microbiomes can affect tissue homeostasis. The inhabitants of colon, lungs, skin, and urogenital mucosal microbiomes can halt or promote inflammatory changes based on chemicals released by a variety of populations. This carries a potential role in the development of the cancer phenotype [34].

11.2.3 STRONG CORRELATION OF CANCER OCCURRENCE AND IMMUNE ABNORMALITIES

At the initial stages of cancer evolution, the innate and adaptive immune systems work together to distinguish and eradicate tumor cells [35]. The immune system receives warning signals for the manifestation of cancer cells, including type I interferons (INF), damage-associated molecular patterns (DAMP), and altered expression of stress-induced ligands such as ULBP1, MICA/B, and NKG2D [36]. Innate immune cells such as γδ T cells, natural killer (NK) cells, natural killer T (NKT) cells, and macrophages are activated in inflammatory situations and identify and eliminate tumor cells through various pathways. These pathways include antibody-dependent cell cytotoxicity, generation of pro-inflammatory cytokines (PICs), phagocytosis, TRAIL-induced and FasL-induced apoptosis, and cytotoxic molecules [37]. Dendritic cells (DCs) carrying tumor fragments migrate to lymph nodes (LN) where they activate tumor-specific CD8+ T and CD4+ Th1 cells. Upon arrival at the tumor site, the activated CD4+ and CD8+ T lymphocytes work together to effectively eliminate tumor cells [38]. Tumor Necrosis Factor (TNF), IFN, and IL-12 are examples of Th1 effector cytokines that

CD4+ T helper cells produce together with their innate complements ILC1s, enhancing the actions of macrophages and cytotoxic cells [39]. Important functions in the elimination of tumors are also played by tumor-infiltrating B lymphocytes. They can develop the effector phenotype (Be1), which is demonstrated by the production of Th1 cytokines and cytotoxic chemicals and serving as tumor-specific antigens [40]. When B cells differentiate into antibody-secreting plasma cells, tumor cells that express antigens are susceptible to cytotoxicity and phagocytosis facilitated by antibodies [41].

The residual tumor and immune cells enter a phase of dynamic equilibrium if the tumor is not completely destroyed during the elimination phase. Novel tumor variants persist and develop during this phase, but they are eventually eradicated. This period continues until the tumor expands and develops mutations that allow it to evade immune recognition and eradication, leading to tumor advancement known as the escape phase [42]. Immune escape can be attributed to various factors, including down-regulation of MHC class I molecules, loss of tumor antigen (TA) expression, chronic activation-induced immune cell exhaustion, and the emergence of ligands for co-inhibitory receptors (PD-L1/PD-1, CD80/CTLA-4, HLA-E/NKG2A-CD94, MHC class I/KIR, HVEM/BTL) [43–45]. Additionally, the expression of CD47 by tumor cells can block the process of phagocytosis, as CD47 acts as a 'do not eat me' signaling molecule [14]. The role of T and B lymphocytes has been exemplified in Figures 11.1 and 11.2.

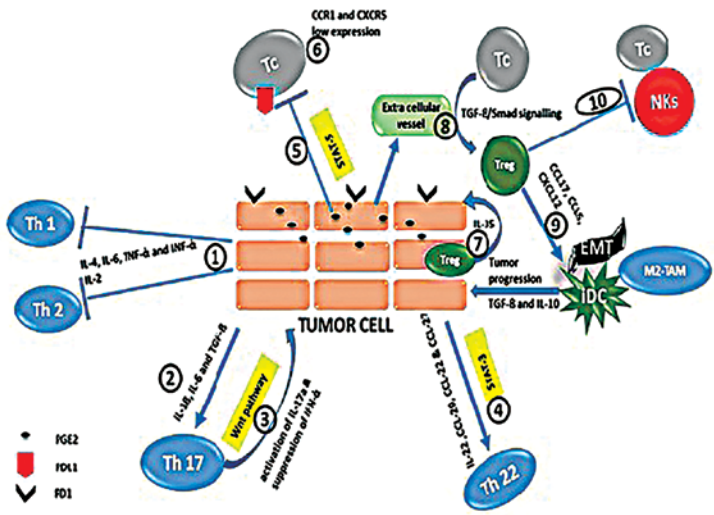

FIGURE 11.1 Role of tumor associated with T-lymphocyte.

Tumor associated T-lymphocytes in tumor micro-environment of colorectal cancer:

- The TH1 and TH2 CD4+ T cell subsets become dysfunctional as a result of upregulation of IL-4, IL-6, TNF-α, and INF-α, and down-regulation of IL-2.
- Tumor-derived IL-6, IL-1, and TGF-β contribute to the development of Th17 in the tumor microenvironment (TME).
- TH-17 activates the Wnt pathway by activating IL-17A and suppressing IFN-α in tumors, which leads to enhanced proliferation and inhibited apoptosis in colorectal cancer.
- IL-22 produced by TH22 involves increasing proliferation and anti-apoptotic activity in colorectal cancer that is suppressed by STAT-3 inhibitor.
- PGE2 tumor is responsible for suppressing T cells by downregulating T cell receptors (TCRs) such as CD3 and SLP63, as well as IL2R. It induces activation of the STAT-5 pathway in colorectal cancer.
- PD-L1 is present in the tumor microenvironment (TME) and binds to PD-1 on cytotoxic T-cells, resulting in evasion of the immune response.
- Through the stimulation of the STAT1 and STAT3 signaling pathways, IL-35 generated by tumor cells can evade the immune response by enhancing the number of Treg cells and suppressing the proliferation of CD4+CD25-T effector cells within the tumor.
- Extra cellular vesicles derived from colorectal cancer cells alter T cells into Treg cells via TGF-β/Smad, which suppresses the immune system.
- The generation of CCL17, CCL5, and CXCl12 by Treg cells induces epithelial mesenchymal transition. The secretion of IL-10 and TGF-β by accumulated TAM, DCs, and MDSCs leads to cancer progression.
- Treg prevents the activation of CD8+ T cells, CD4+ T cells, NK cells, NKT cells, B cells, and APC cells.

Tumor associated B-lymphocytes:

- The B-regulatory cells CD19LOW and CD27HIGH secrete high levels of IL-10, inhibit INF-gamma and TNF-alpha secreted by T cells, and reduce TH17 activity, all of which contribute to the development and spread of colorectal cancer.

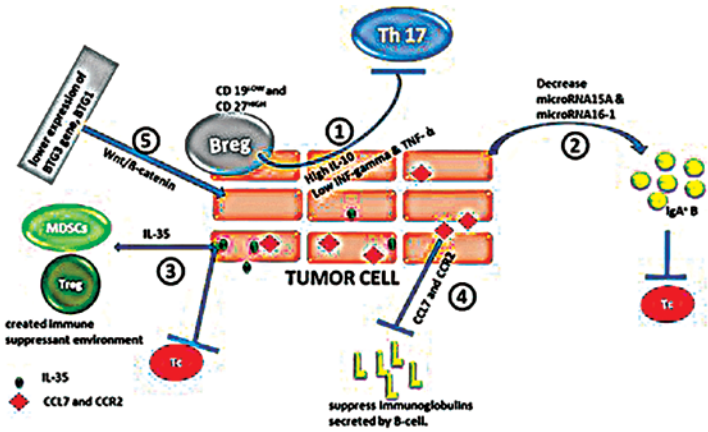

FIGURE 11.2 Role of tumor associated with B-lymphocyte.

- Production of IgA+ B cells increases by decreasing the level of microRNA15A and microRNA16-1, which suppress the immune response by evading the proliferation and activation of cytotoxic T cells.
- The immune-suppressive environment was formed by the tumor cells' production of IL-35, which had a positive correlation with Treg and MDSC cells and a negative correlation with CD3+ T cells.
- Chemokine CCL7 and its receptor CCR2 are produced in high amounts in colorectal cancer, which suppresses immunoglobulins secreted by B-cells.
- The Wnt/β-catenin pathway, which is responsible for progression, invasion, and EMT, is activated by the decreased expression of BTG3 and BTG1 genes. The diverse mechanisms through which tumorous cells bypass immune surveillance pave the way for the establishment of immunotherapy approaches. Immunotherapy aims to manipulate T-cell function and block tumor metabolism [46]. It also involves targeting immunosuppressive checkpoints (PD-1 and CTLA-4 axes), immunosuppressive modulators TGF-β and IDO, CD47 signaling, and CCL2-mediated recruitment of immunosuppressive cells [47].

11.3 CHEMOTHERAPY – THE CONTROVERSIAL GOLD STANDARD

The tumor cells are much more stubborn compared to normal cells. They have advantages in terms of proliferation, respiration, metabolism, telomerase

enzymes, and apoptosis inhibition. Except for mitochondrial defects, they possess all the characteristics that sustain tumor growth. It would be inappropriate to consider cancer treatment without the cytotoxic potential of chemotherapy. However, the failure of the therapy to discriminate between rapidly dividing normal cells and tumor cells leads to life-threatening toxicities [35, 48–50].

11.3.1 MECHANISMS OF TUMOR CELLS DEATH BY CHEMOTHERAPEUTIC AGENTS

Chemotherapy targets tumor cells mostly based on their rapid cell proliferation feature. The different mechanisms of chemotherapy involve the formation of DNA lesions by interacting with specific nucleotides [51], suppression of the Wnt/-catenin, MEK/ERK, and PI3K/Akt signaling pathways that promote cancer stemness [52], activation of apoptotic pathways [53], microtubule disaggregation [54], and impairment or intervention in nucleotide synthesis or integration [55]. The mechanisms, along with the therapeutic application and toxicities of first-line chemotherapeutic agents for the most prevalent cancer, are given in Table 11.1.

11.3.2 TUMOR MICROENVIRONMENT AND RESISTANCE TO CHEMOTHERAPY

Tumors consist not only of malignant cells, but also various non-malignant cells. These include adipocytes, dendritic cells (DCs), fibroblasts, components of the vasculature, and tumor-infiltrating lymphocytes. The tumor microenvironment (TME) plays a crucial role in the initiation and progression of tumors through continuous communication with the surrounding tissue [106]. By manipulating the TME, tumor cells can evade immune responses [85]. Elevated levels of VEGF-A contribute to abnormal angiogenesis, characterized by disorganized and leaky blood vessels that result in hypoxia [107]. Conversely, increased expression of VEGF-C/D promotes lymphangiogenesis, which stimulates tumor progression and dissemination [108]. Moreover, tumors induce stromal transformation by releasing VEGF, TGF-β, and PDGF-BB, leading to the conversion of healthy fibroblasts into cancer-associated fibroblasts (CAFs), lymphatic endothelial cells, pericyte detachment from blood vessels, and alterations in blood vessel structure [109]. The aberrant stromal components release immunosuppressive molecules

TABLE 11.1 The Use of Chemotherapy is Common in the Treatment of the Most Prevalent Types of Cancer, as well as Some That Have Immunomodulatory Properties

Chemotherapeutic Agent	Mechanism of Action	Dose Limiting Toxicities	Therapeutic Application
5-Fluorouracil [56–61]	By inhibiting the transformation of deoxyuridylic acid to thymidylic acid through the action of the enzyme thymidylate synthetase, it interferes with DNA synthesis.	Hepatotoxicity (serum aminotransferase elevations, coma with hyperammonemia, lactic acid elevations, and respiratory alkalosis), gastrointestinal (diarrhea, stomatitis), myelosuppression.	In the treatment of basal cell carcinomas as well as palliative cancer care, it is administered as an injection.
Doxorubicin [62–64]	To bind to nucleic acids, it selectively intercalates the planar anthracycline nucleus with the DNA double helix. This prevents DNA replication and transcription.	Cardiac toxicity includes reversible myopericarditis, left ventricular dysfunction, arrhythmias, and congestive heart failure (CHF). BM can cause myelosuppression, while dermatology poses an extravasation hazard as a vesicant.	Used to produce degeneration in proliferative cancers – acute lymphoblastic and myeloblastic, Hodgkin's disease, Wilms' tumor, neuroblastoma, sarcomas of bone as well as soft tissues, carcinomas (ovarian, transitional cell bladder, gastric, breast, thyroid, bronchogenic), malignant lymphoma.
Paclitaxel [65–67]	Paclitaxel attaches itself to the β subunit of tubulin, thus preventing the normal development and functioning of microtubules. Additionally, it causes cancer cells to undergo programmed cell death (apoptosis) by attaching to the inhibitor of apoptosis Bcl-2 (B-cell leukemia 2) and halting its activity.	Cardiotoxicity, nervous system (peripheral neuropathy), hematologic (neutropenia), bone marrow (myelosuppression), musculoskeletal, and connective tissue (arthralgia/myalgia).	It is used in the treatment of breast, lung, and ovarian cancer, along with its additional use in Kaposi's sarcoma.

TABLE 11.1 *(Continued)*

Chemotherapeutic Agent	Mechanism of Action	Dose Limiting Toxicities	Therapeutic Application
Cyclophosphamide [68–70]	Three mechanisms of action: (i) Alkyl groups bind to DNA bases, causing DNA fragmentation. This prevents the damaged DNA from being used for DNA synthesis or RNA transcription; (ii) cross-linking triggers DNA injury by preventing DNA from being divided for synthesis or transcription; and (iii) the process of generating nucleotide mismatches that result in mutations.	Bone marrow (myelosuppression), cardiac (cardiac necrosis or hemorrhagic myocarditis, pericardial tamponade), gastrointestinal (nausea, vomiting), Renal (hemorrhagic cystitis).	It is used to treat mycosis fungoides, lymphomas, myelomas, leukemia, ovarian adenocarcinoma, neuroblastoma, breast carcinoma, and retinoblastoma.
Methotrexate [71–76]	Cell division is prevented by inhibiting the nucleotide synthesis enzymes. This leads to increased levels of adenosine and adenosine triphosphate in the extracellular space, resulting in an anti-inflammatory effect on rheumatoid arthritis.	Hepatotoxicity (fibrosis, cirrhosis, enzyme elevations), gastrointestinal (diarrhea, ulcerative stomatitis), methotrexate-induced lung disease, malignant lymphomas, blood (neutropenia, thrombocytopenia), bone marrow (myelosuppression), renal (azotemia), Pulmonary toxicity	It is used in the treatment of several cancers, severe psoriasis, juvenile rheumatoid arthritis, and severe rheumatoid arthritis.
Etoposide [77, 78]	Etoposide inhibits DNA topoisomerase II, hindering DNA re-ligation that results in essential DNA synthesis errors during the prophase of cellular division and ultimately leading to the death of malignant cells through apoptosis.	Bone marrow (myelosuppression), hematologic (leukopenia, neutropenia), and mucosal toxicity.	It is applied to the management of testicular and small cell lung cancers.
Irinotecan [79–81]	Prevents replication and induces death by inhibiting the activity of topoisomerase I.	Hematologic (neutropenia), gastrointestinal (diarrhea, nausea, vomiting), constitutional symptoms (asthenia).	Used in the treatment of metastatic carcinoma (rectum or colon).

TABLE 11.1 *(Continued)*

Chemotherapeutic Agent	Mechanism of Action	Dose Limiting Toxicities	Therapeutic Application
Cisplatin [82–85]	Three mechanisms: (i) Alkyl groups bind to DNA bases, causing DNA breakage. This prevents the damaged DNA from being used to create new DNA or produce RNA; (ii) cross-linking induces DNA damage by inhibiting DNA division for synthesis or transcription; and (iii) the formation of nucleotide mispairing leads to mutations.	Renal (nephrotoxicity), neurology (neurotoxicity), auditory (ototoxicity), Bone marrow (myelosuppression)	It is used to treat various, carcinomas, sarcomas, germ cell tumors like ovarian and testicular cancers, lymphomas.
Levamisole [86–88]	Levamisole's ability to inhibit parasite growth may be attributed to its agonistic effect at nicotinic receptors in nematode muscle, which causes spastic paralysis. Levamisole improves T-cell response by promoting the induction and progression of T-cells, boosting the activities of monocytes and macrophages, and recovering impaired immunological function.	Bone marrow (myelosuppression), neurotoxicity, vertigo.	It is used to treat some skin and helminth infections and as an adjuvant in treatment of colon cancer.
Prednisolone [89–91]	Prednisone impedes polymorphonuclear leukocyte movement and diminishes the increased capillary permeability to lessen inflammation. It suppresses NF-Kappa B and other inflammatory transcription factors, impedes neutrophil death and demargination, restrains phospholipase A2, which lessens the production of arachidonic acid derivatives, thereby promoting anti-inflammatory genes such as interleukin-10.	Hematologic (neutropenia), skeletal wasting.	It is used to treat some cancers, inflammatory conditions, and adrenocortical insufficiency.
Lenalidomide [92–94]	Lenalidomide affects cytokine generation, controls co-stimulation of T-cells, and increases NK cell-mediated cytotoxicity to manifest its immunomodulatory effects. By preventing the growth and promoting death in tumor cells, lenalidomide directly combats tumors. By preventing the release of angiogenic growth factors by tumor cells, it has anti-angiogenic effects.	Hematologic (anemia, neutropenia, thrombocytopenia), bone marrow (myelosuppression), thyroid (hypothyroidism), non-neutropenic sepsis.	In low to intermediate risk myelodysplastic syndrome, it is used to treat multiple myeloma and anemia.

TABLE 11.1 *(Continued)*

Chemotherapeutic Agent	Mechanism of Action	Dose Limiting Toxicities	Therapeutic Application
Gemcitabine [95–97]	Following tumoral transformation into bioactive triphosphorylated nucleotides that interfere with DNA synthesis and target ribonucleotide reductase, gemcitabine exerts its antiproliferative effect. Additionally, it has self-potentiating pharmacological effects that improve the chances of gemcitabine triphosphate successfully binding to the DNA chain.	Bone marrow (myelosuppression), hematologic (thrombocytopenia), asthenia.	It is used as the only medication to treat pancreatic cancer. Several types of cancer, such as non-small cell lung cancer, metastatic breast cancer, and ovarian cancer, benefit from its usage as adjuvant therapy.
Vincristine [98, 99]	The antitumor activity of vincristine is primarily attributed to its suppression of mitosis metaphase through its association with tubulin. Additionally, vincristine may impact various processes, including cellular respiration, ATPase activity, calmodulin-dependent Ca2+-transport, glutathione metabolism, cyclic AMP, as well as amino acid, nucleic acid, and lipid biosynthesis.	Neurology (neurotoxicity, peripheral neuropathy), bone marrow (myelotoxicity).	Used in treatment of acute erythremia, acute panmyelosis, Hodgkin's disease, malignant lymphoma, and acute leukemia.
Docetaxel [100–102]	Docetaxel hinders the normal function of microtubule growth by attaching to the β-subunit of tubulin. Additionally, it attaches to the apoptosis inhibitor protein Bcl-2, causing neoplastic cells to undergo apoptosis.	Bone marrow suppression, hematologic (neutropenia), skin and neurosensory toxicity.	It can treat several malignancies, such as gastric adenocarcinoma, head and neck cancer, locally advanced breast cancer, metastatic (breast and prostate).
Oxaliplatin [103–105]	Oxaliplatin is converted non-enzymatically by displacement of the labile oxalate ligand to its active metabolites. After activation, oxaliplatin predominantly binds to the guanine and cytosine moieties of DNA, causing cross-linking and inhibiting the synthesis of new DNA as well as the transcription of existing DNA.	Nervous system (neurotoxicity, peripheral sensory neuropathy).	It is used to treat stage III colon cancer as well as rectum or colon carcinoma.

such as prostaglandin (PG) E2, IL-6, and TGF-β, which significantly hinder cell cytotoxicity. Additionally, they recruit and activate immunosuppressive cells through chemokines like CCL2, CCL5, and CCL22 secreted by tumor cells [110]. The transition from a pro-inflammatory Th1 response to an immunosuppressive Th2 response is brought on by infiltrating regulatory cells (Treg, Breg, and Treg cells), M2-like tumor-associated macrophages, immature DCs, CD4+ Th2 and ILC2s cells, and M2-like tumor-associated macrophages [111], facilitated through the discharge of IL-4, IL-5, IL-10, IL-35, IL-13, and TGF-β [112]. Also, tumor cells hamper cytotoxic T lymphocyte (CTL) because of metabolic exhaustion [113]. Tumor cells outrun T-cells to acquire nutrients like amino acids and glucose as well as oxygen [114]. Immature DCs or MDSCs and Treg cells, expression of ectonucleotidases (CD39 and CD73), Indoleamine 2,3-dioxygenase (IDO), arginase-1 by tumor cells, further attribute to T-cell nutritional deficiency and exhaustion by diminishing the tryptophan levels as well as rising its immunosuppressive catabolite kynurenine concentration, through restricting arginine's accessibility and by hydrolyzing ATP to an extremely immunosuppressive adenosine [115]. Additionally, Treg cells defend IL-2, which is essential for T-cell viability and function, by upregulating the expression of the CD25, an IL-2 receptor alpha-chain [116].

11.3.3 RESISTANCE OF CANCER STEM CELLS (CSC) TO CHEMOTHERAPY

According to two distinct hypotheses, tumors are constituted by heterogeneous cell subpopulations. One hypothesis is the stochastic or clonal evolution type, while the other is the CSC model or hierarchical [117, 118]. In the hierarchical model, tumor growth is initiated by a small cell subpopulation known as tumor initiating cells (TICs) [119]. According to the hierarchical assumption, CSCs are a minor subpopulation of cells within tumors that possess assets such as multipotency, self-renewal, and differentiation capabilities. Furthermore, cues from the stromal milieu, pre-existing somatic mutations, cell type source, and stage of malignant progression can all influence CSC-like properties [120]. Resistance to chemotherapy [121], immunotherapy [122], and radiotherapy [123] are displayed by these cells and their TICs [124].

The chemotherapeutic resistance of CSCs/TICs, which have undergone an epithelial to mesenchymal transformation, appears to be higher [125].

An increase in aldehyde dehydrogenase (ALDH) activity in these cells seems to mediate resistance to certain chemotherapeutic agents [126]. The ability of CSCs/TICs to present tumor antigens (TAs) to T cells for immune detection or immune response is determined by the expression of antigen presentation molecules such as Programmed death receptor 1 (PD-1)/-1L], coinhibitory molecules [e.g., cytotoxic T-lymphocyte antigen 4 (CTLA4), B7-H2, and B7-H3], co-stimulatory molecules (e.g., CD80, CD86), as well as MHC-I [127]. Resistance to conventional therapies is significantly associated with the intrinsic mechanisms involved in CSC/TIC resistance to chemotherapy or radiation therapy. Immunosuppressive chemicals produced by CSCs and TICs can impair the effectiveness of the immune system [128].

11.4 PARTIALLY USED CRITICAL WEAPON AGAINST CANCER

Although chemotherapy acts on multiple hallmarks of cancer, the immunosuppression induced by cytotoxicity to the bone marrow (BM) makes patients more vulnerable to cancer recurrence and chemo resistance. This situation also increases the susceptibility of immunocompromised cancer patients to other infectious diseases.

11.4.1 IMMUNOTHERAPY

The outcomes achieved in terms of robust positive response through immune-based therapies surpassed the expectations. For their groundbreaking breakthroughs in the field of immunotherapy, two outstanding scientists, namely James Allison and Tasuku Honjo, have just been awarded the 2018 Nobel Prize in medicine. Their findings shared the same approach of utilizing the immune system to combat cancer. Since 2011, the FDA has approved several medications based on the suppression of immunological checkpoints [129]. Most of these treatments have utilized antibodies that target the Programmed cell death 1 (PD-1) or CTL-associated protein 4 (CTLA-4) pathways, either singly or in combination [130]. Based on the expression of the tumor-associated antigen, which indicates the type of "stemness" of a cell, T cells can recognize CSCs/TICs. Because somatic point mutations in tumor cells might result in the creation of completely new epitopes, the immune system is able to identify altered antigens [131].

11.4.2 CAR T-CELL THERAPY

Research on the transfer of chimeric antigen receptor (CAR) T cells is currently being conducted [132] and has fantastic potential for the management of both liquid and solid tumors [133]. A single chain fragment on the antigen receptor of CAR T cells links it to an intracellular region, and it is typically complemented by a co-stimulatory protein. Due to their capacity to differentiate any antigen present on the surface of CSCs, CAR T cells provide a good substrate for the creation of selective CSC therapies [134].

11.4.3 IMMUNE-BASED MONOCLONAL ANTIBODIES AND CHECK POINTS INHIBITORS

The presence of immune-based abnormalities in cancer presents an opportunity to develop therapy aimed at resolving it and utilizing it as a natural defense against cancer. Monoclonal antibodies (mAbs) derived from plasma cells bind to the epitope part of the antigens, activating tumoricidal effects simultaneously with other cellular immune and complement mechanisms. The use of mAbs offers the advantage of long-term memory, which is lacking in chemotherapy [135]. Among the five types of natural antibodies used to produce mAbs, IgG is the most commonly employed due to its extensive interactions with monocytes, NK cells, neutrophils, and DCs [136]. Targeted tumor-associated antigens (TAs) are typically overexpressed on the surfaces of cancer cells. For example, cetuximab suppresses the growth factor receptor EGFR, leading to the death of tumor cells [137]. Trastuzumab prevents the overexpression of Human Epidermal Growth Factor Receptor 2 (HER2) from internalizing and heterodimerizing [138]. Rituximab, used to treat non-lymphoma Hodgkin's and myeloma, demonstrates more efficacy and less toxicity compared to conventional therapy by specifically targeting CD20 on tumor cells [139]. Immune checkpoints are proteins that regulate costimulatory and coinhibitory immune responses, acting as brakes. They are present on immune cells to prevent the development of autoimmune conditions. When counter proteins are present on opposing cells, immune activation is inhibited, making immune checkpoints function as inhibitory receptors [140]. Different proteins with checkpoint functions include Programmed death receptor-1 (PD-1), which is linked to apoptosis of T cells; Cytotoxic T lymphocyte antigen-4 (CTLA-4), expressed by Treg cells, NK cells, and Treg cells; and lymphocyte activation gene 3 (LAG3), responsible for T

cell activation and secretion of immune response-related cytokines. Other less explored checkpoints are T cell immunoglobulin and immunoreceptor tyrosine-based inhibitory motif domain (TIGIT), V-domain Ig suppressor of T cell activation (VISTA), as well as T cell immunoglobulin and mucin domain-containing 3 (TIM3) [141]. FDA-approved checkpoint inhibitors include Ipilimumab, Pembrolizumab, Nivolumab, Cemiplimab, Atezolizumab, and Opdualag. Recent developments in the field of drug discovery have led to the invention of antibody drug conjugates (ADC), which can be considered as an amalgamation of two different therapies. ADCs utilize the specificity of monoclonal antibodies and the toxicity of chemotherapeutic agents [142] (Figure 11.3).

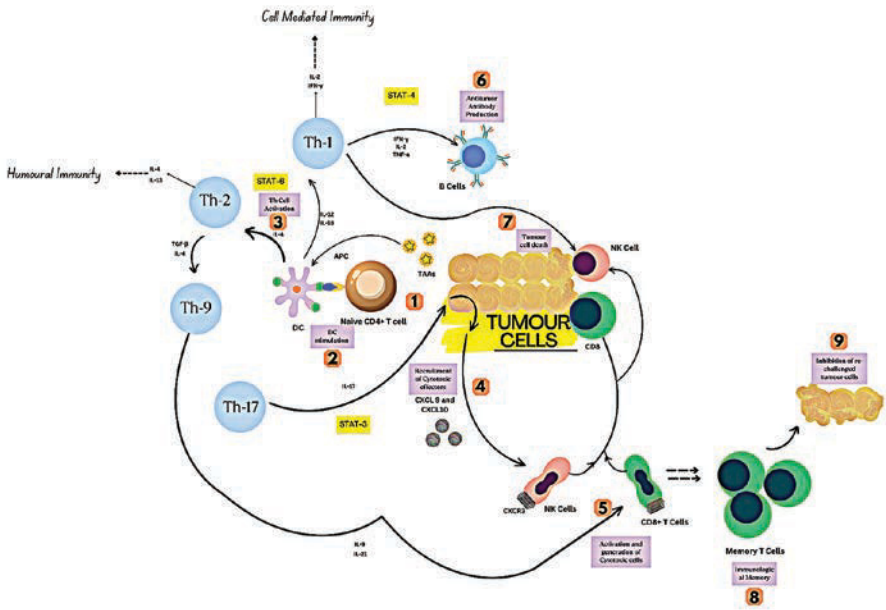

FIGURE 11.3 Different mechanisms of antitumor immunity.

Pathways to tumoricidal destruction by immunity:

- Generation of tumor associated antigens (TAAs) from tumor cells.
- Stimulation of DC by TAAs.
- DCs and the major histocompatibility complex (MHC) [MHC-I, MHC-II] complex function as antigen-presenting cells (APCs) to activate helper T (Th) cells and cytotoxic lymphocytes (CTLs).

- Activation of Th-17 cells leads to the release of interleukin 17 (IL-17), which induces tumor cells to secrete chemotactic factors – CXCL9 and CXCL10, followed by the subsequent recruitment of cytotoxic effectors – NK cells and CD8+ cells.
- Stimulation of Th-9 cells results in the release of Interleukin 21 (IL-21) along with Interleukin 9 (IL-9), which also leads to the activation and generation of cytotoxic cells.
- Interferon (INF-γ), IL-2, and tumor necrosis factor (TNF-α) are released upon activation of Th-1 cells, triggering the STAT-3 pathway to produce antitumor antibodies.
- Cytotoxic cells, together with B cells, exert their antitumor effect and induce apoptosis.
- Immunological memory aids in the generation of cytotoxic cells during tumor remission.
- Memory T cells contribute to the development of an immune response against re-challenged tumor cells.

To date, the FDA has approved a total of 12 antibody-drug conjugates (ADCs) for various types of cancer. Despite significant advancements in monoclonal antibody (mAb) therapy, the issue of resistance persists, similar to chemotherapy. The development of resistance can be considered as a form of therapeutic resistance. This can be attributed to factors such as the emergence of new mutations in the targets (e.g., CD20 in the case of rituximab, S492R in the case of cetuximab), the generation of new variants, immunoediting of the tumor, epithelial to mesenchymal transition (EMT), immune selection processes, and the activation of secondary growth signaling pathways by tumors [143, 144].

11.5 THE BOON OF MEDICAL PRE-INTERVENTION TO THE MANKIND-VACCINE

The term chemotherapy is used to describe the elimination of cancerous or infectious cells, which have different surface proteins compared to normal cells. A British physician demonstrated the effectiveness of vaccines by discovering the smallpox vaccine. According to the CDC, more than 18 deadly and highly disabling diseases are now under control thanks to the invention of vaccines. Recent reports have shown positive outcomes in reducing fatality rates for COVID-19 vaccinations [145].

11.5.1 VACCINE FOR CHRONIC DISEASES THAT INCREASE THE RISK OF CANCER DEVELOPMENT

As per the National Cancer Institute (NCI), many infectious agents chronically alter the tissue milieu and provoke cancer development. The infectious agents, such as bacteria and parasites, secrete certain inflammatory molecules, while viruses can modify the genotype of the host DNA. Even the secretion of immunosuppressive chemicals also favors the initiation of cancer chronically. Many virus infections chronically lead to carcinogenesis, such as Epstein-Barr Virus (EBV) linked to lymphoma, Hepatitis B and C linked to liver cancer, Human Immunodeficiency Virus (HIV) linked to Kaposi sarcoma, liver and lung cancer, and Human Papillomaviruses (HPVs) responsible for reproductive tract, head and neck cancer. Additionally, infection with Helicobacter pylori increases the occurrence of gastric cancer [146]. A list of specific vaccines that not only prevent the occurrence of infectious diseases but also halt carcinogenesis in the long run are given in Table 11.2.

11.5.2 AVAILABILITY OF SPECIFIC ANTIGENS ON THE TUMOR CELLS

Opportunities to develop cancer vaccines (CVs) are increased by the presence of specific tumor-associated antigens (TAs). The mutated genes transcribe mRNA that encodes peptides or proteins, which can be detected in blood or cancerous tissues. A single amino acid change in the peptide or frameshift mutation can be considered abnormal and create a neoantigen. Immune cells can recognize these altered patterns. Aberrant proteins (antigens) are present due to abnormal genetic mutations. The causative genes include *CDK4*, *CTNNB1*, *CASP8*, *KRAS*, *BCR-ABL*, and *P53* [197]. The proteins encoded by these mutated genes confer uncontrolled cell proliferation, resistance to apoptosis, and sometimes immune evasion. All of the aforementioned genes encode highly specific TAs. Other low-specific TAs include prostate-specific antigen (PSA) and carcinoembryonic antigen (CEA) [198]. Overexpression of certain proteins on the tumor surface beyond a threshold level also promotes recognition by cytotoxic T lymphocytes (CTL). Different genes responsible for overexpression include *RAGE-1*, *PRAME*, and *ERBB2* (HER2/NEU) [199]. There are antigens like Mucin-associated sialyl-Tn (sTn) antigens that interact with immune subsets and create an immunosuppressive

TABLE 11.2 The Potential of Vaccines in the Prevention of Dangerous Diseases That can Chronically Transform into Cancer Development

FDA Approved Brand of Vaccine	Types of Vaccine	Mechanism	Immunization Potential	Application
ENGERIX-B [147–150]	Hepatitis B vaccine (recombinant).	Produces targeted humoral antibodies against the hepatitis B viral surface antigen (HBsAg).	Antibody levels against HBsAg of less than 10 mIU/mL are recognized as providing protection from hepatitis B virus infection.	Active immunization against all subtypes of hepatitis B virus infection.
GARDASIL 9 [148, 151, 152]	Human papillomavirus 9-valent vaccine (recombinant).	Produces highly pure virus-like particles (VLPs) of the primary capsid L1 protein that initiate the humoral immune response.	It was found to be >90% effective and generated antibody response against 9 subtypes of HPV.	Indicated for the inhibition of oropharyngeal, genital, as well as head and neck cancers, precancerous or dysplastic lesions caused by HPV subtypes.
TICE BCG [154–157]	BCG live	Generates antibodies and antigen-specific T-cell response against tuberculosis and acts as an immunotherapy against bladder cancer.	The effective success rate of TICE BCG was found to be 90% for bladder cancer.	For the management and prevention of urinary bladder carcinoma in situ (CIS), as well as for the prevention of initial or recurring Ta and/or T1 papillary tumors following transurethral resection (TUR).
PREHEVBRIO [158, 159]	Hepatitis B vaccine (recombinant).	Produces targeted humoral antibodies against the hepatitis B viral surface antigen (HBsAg).	It is known that antibodies against HBsAg at concentrations of less than 10 mIU/mL offer protection from hepatitis B virus infection.	To prevent infection induced by all known hepatitis B virus strains.
CERVARIX [160–163]	Human papillomavirus bivalent (types 16 and 18) vaccine (recombinant)	Stimulates the production of antibodies against HPV types 16 and 18.	Provides 80% protection against HPV subtypes known to cause female genital oncogenic cancer.	It is recommended for the prevention of conditions such as anal, vulval, cervical, and vaginal cancers caused by carcinogenic HPV types 16 and 18.

TABLE 11.2 *(Continued)*

FDA Approved Brand of Vaccine	Types of Vaccine	Mechanism	Immunization Potential	Application
VAXELIS [164–167]	Diphtheria, tetanus, and polio toxoids, inactivated poliovirus, adsorbed acellular pertussis vaccine, haemophilus B conjugate, and hepatitis B vaccine.	Induces the production of specific antibodies against influenzae type b, diphtheria, haemophilus, pertussis, hepatitis B, tetanus.	Antibody levels of ≥1.0 μg/mL against polyribosylribitol phosphate (PRP) are considered as a safeguard against haemophilus influenzae type b, ≥0.1 IU/mL against diphtheria, ≥0.1 IU/mL against tetanus, and ≥10 mIU/mL against HBsAg are considered as a safeguard for hepatitis B virus infection.	It offers active immunization against haemophilus influenzae type b and diphtheria-related invasive infections (Hib), tetanus, pertussis, poliomyelitis, and hepatitis B.
RECOMBIVAX HB [168, 169]	Hepatitis B vaccine (recombinant)	Produces specialized humoral antibodies against the hepatitis B surface antigen (HBsAg).	Antibody levels of ≥10 mIU/mL against HBsAg are known to offer protection against hepatitis B virus infection.	Vaccination is intended to prevent infection caused by established subtypes of the hepatitis B virus.
GARDASIL [170, 171]	Human papillomavirus quadrivalent (types 6, 16, 11, as well as 18) vaccine (recombinant)	Produces humoral immune response initiated from highly purified virus-like particles (VLPs) of the main capsid L1 protein.	It was found to be >90% effective and generated antibody response against 4 subtypes of HPV.	This is used to provide active immunity against anal, vaginal, vulvar, cervical cancer, as well as precancerous or dysplastic lesions and genital warts.
HEPLISAV-B [172, 173]	Hepatitis B vaccine (recombinant), adjuvanted.	Creates specific humoral antibodies to the hepatitis B virus surface antigen (HBsAg).	Antibody levels of ≥10 mIU/mL against HBsAg are regarded as offering safeguard against hepatitis B virus infection.	For preventing infection brought on by any known hepatitis B virus subtype.
TWINRIX [174, 175]	Hepatitis A inactivated and hepatitis B (recombinant) vaccine	Generates antibodies to hepatitis A virus (anti-HAV) and antibodies to fight against hepatitis B virus surface antigen (HBsAg).	Antibody titer values for hepatitis A depend upon the assay used, whereas antibody concentration against HBsAg of ≥10 mIU/mL is known to provide protection against hepatitis B virus infection.	Provides active immunization of people aged 18 and older against infection with all known hepatitis B virus subtypes and diseases brought on by the hepatitis A virus.

TABLE 11.2 *(Continued)*

FDA Approved Brand of Vaccine	Types of Vaccine	Mechanism	Immunization Potential	Application
Liquid PedvaxHIB [176–179]	Haemophilus B conjugate vaccine (meningococcal protein conjugate).	Stimulates production of antibodies against polyribosyl ribitol phosphate (PRP) antigen of haemophilus B capsule.	The antibody concentration of >0.15 to >1.0 g/mL against PRP of the haemophilus B capsule is regarded as a safeguard to provide protection against haemophilus influenzae type B.	Infants and children between the ages of 2 and 71 months are recommended to receive routine vaccination of liquid PedvaxHIB against invasive disease caused by haemophilus influenzae type b.
ActHIB [180, 181]	Haemophilus b conjugate vaccine (tetanus toxoid conjugate).	Causes the development of antibodies against the haemophilus B capsule's polyribosyl ribitol phosphate (PRP) antigen.	To provide protection against haemophilus influenzae type B, an antibody concentration of >0.15 to >1.0 g/mL against the PRP of the haemophilus B capsule is considered to be a safeguard.	Vaccination for preventing haemophilus influenzae type b-induced invasive diseases.
HIBERIX [182–190]	Haemophilus b conjugate vaccine (tetanus toxoid conjugate).	Promotes the production of antibodies against the haemophilus B capsule's polyribosyl ribitol phosphate (PRP) antigen.	Haemophilus influenzae type B protection is provided by antibodies with concentrations of >0.15 to >1.0 g/mL against the PRP of the haemophilus B capsule.	For the purpose of actively vaccinating against invasive infection caused by haemophilus influenzae type b.
PENTACEL [191–194]	Tetanus toxoids, diphtheria toxoids, acellular pertussis adsorbed, inactivated poliovirus and haemophilus b conjugate (tetanus toxoid conjugate) vaccine.	The synthesis of specific antibodies that are effective against the following diseases is increased: Diphtheria, tetanus, haemophilus influenzae type B, hepatitis B, pertussis, and poliovirus.	The antibody concentration of ≥1.0 μg/mL against PRP is considered as a safeguard against haemophilus influenzae type b. A concentration of ≥0.1 IU/mL against diphtheria and tetanus, and ≥10 mIU/mL against HBsAg are considered as safeguards for hepatitis B virus infection. Additionally, an antibody concentration of 0.075–0.180 IU/ml is required to provide protection against poliovirus.	For effective protection against invasive infections brought on by diphtheria, tetanus, pertussis, poliomyelitis, and haemophilus influenzae type b.

TABLE 11.2 *(Continued)*

FDA Approved Brand of Vaccine	Types of Vaccine	Mechanism	Immunization Potential	Application
PEDIARIX [195, 196]	Diphtheria toxoids, acellular pertussis adsorbed, tetanus toxoids, hepatitis B (recombinant) and inactivated poliovirus vaccine combined.	Boosts the synthesis of specific antibodies that are effective against diphtheria, tetanus, haemophilus influenzae type B, pertussis, hepatitis B, and poliovirus.	An antibody concentration of 0.075–0.180 IU/ml is required to provide protection against poliovirus. Concentrations of ≥1.0 μg/mL against PRP are considered as a safeguard against haemophilus influenzae type b. Concentrations of ≥0.1 IU/mL against tetanus and ≥10 mIU/mL against HBsAg are considered as safeguards for hepatitis B virus infection. Lastly, a concentration of ≥0.1 IU/mL is required to provide protection against diphtheria.	PEDIARIX is recommended for active vaccination against tetanus, poliomyelitis, diphtheria, pertussis, and infections brought on by all recognized subtypes of the hepatitis B virus.

environment, thereby weakening the cytolytic capacity of these cells and even promoting metastasis [200]. These antigens can be used as diagnostic and prognostic markers for multiple cancers. CEA and CA 15-3 can be used to monitor breast cancer treatment efficacy and predict progression to an advanced stage [201].

11.5.3 FDA APPROVED CVS

The first experiment on the use of therapeutic vaccines was conducted by Dr. William Coley in 1890 to treat patients suffering from sarcoma. Due to the potential failure of existing therapies and the involvement of immune components in cancer development, numerous attempts have been made to develop cancer vaccines (CVs). CV is part of Immunotherapy [202]. CVs encompass various modalities, including peptide-based, whole cell-based, protein-based, DC-based, mRNA-based, and DNA-based approaches [19]. The world is in need of prophylactic personalized and therapeutic vaccines to reduce cancer-related morbidity and mortality. The FDA has approved four preventive CVs. Cervarix is used to prevent cervical, head, and neck cancer caused by HPV 16 and 18 infections. Gardasil and Gardasil-9 target other strains of HPV and provide protection against throat cancer and other cancers. HEPLISAV-B offers protection against hepatitis B virus (HBV)-induced liver cancer. Sipuleucel-T, approved by the FDA, is a vaccine for advanced stage prostate cancer based on the presence of prostatic acid phosphatase (PAP). Bacillus Calmette-Guérin (BCG) has FDA approval for the treatment of early-stage bladder cancer by activating general immunity [156]. Numerous CVs are currently in clinical trials, including Mammaglobin-A (NCT02204098), QUILT-3.013 (NCT02751528) for breast cancer, and Granulocyte Macrophage Colony-Stimulating Factor (GM-CSF) with Folate Receptor Alpha Peptide for Triple Negative Breast Cancer (NCT02593227). These trials are currently in progress [204].

11.5.4 CHALLENGES AHEAD

Although the success achieved for marketed CVs and many that are under clinical trials, there is concern regarding the complexity related to antigens and immunity. Tumors with low immunogenicity, changes in the heterogenous population of cancer cells, the presence of glycocalyx covering the surface antigen, the immunocompromised state of many diseases such as

acquired immunodeficiency syndrome (AIDS) and tuberculosis, changes in major histocompatibility complex (MHC) interaction with antigens, the systemic prevalence of immunosuppression, and the development of large tumor size at advanced stages of malignancy all pose great challenges that need to be resolved. There is evidence available that indicates a complex correlation between vaccine-stimulated immunity and therapeutic benefits [205]. The ever-changing heterogenous population of tumors and the unsteady tumor microenvironment create difficulties in drawing conclusions about therapeutic vaccines. To overcome all these obstacles of immune tolerance and evasion, adjuvant therapy is needed with CVs [206]. The preclinical screening model cannot be exactly extrapolated to the clinical situation, making it difficult to predict vaccine efficacy [207]. If an antigen has low binding capacity to MHC, it should be administered at a high dose to properly stimulate the lymphatic system of defense [208]. Improving the biopharmaceutical properties of vaccines is difficult due to inadequate data on vaccine ADME (absorption, distribution, metabolism, and excretion) [209]. Sometimes, commercialization pressure urges the design of trials with insufficient dosing schedules [210, 211].

11.5.5 THERAPEUTIC CV WITH CHEMO AGENTS – CONCEPT OF SYNERGISM

The concern of multiple hallmarks in cancer development and progression makes it necessary to suppress it from multiple sites to achieve sustainable progression-free life with an increase in overall survival (OS). The abscopal effect induced by radiation was first observed in 1950 during a preclinical experiment. It demonstrated that non-irradiated tumors shrink after the irradiation of the main tumor. The abscopal effects occur due to the awakening of systemic immunity. The exposed tumor releases certain cytokines that signal the immune system to recognize and eliminate similar tumors throughout the body. Thus, the exposed tumor could act as a live vaccine [212]. A more beneficial synergism can be achieved in the case of chemotherapy and cancer therapeutic vaccine. Cyclophosphamide (CTX) induces immunogenic tumor cell death, releasing many tumor-associated antigens and activating systemic immune response. Previous studies provide clues for the success of chemotherapy with immunomodulators. Chemotherapy not only causes cytotoxicity but also modulates host immunity. The positive changes include exaggerated immunogenicity of cancer cells, activation of tumoricidal cells

(CD8+ T lymphocytes, NK cells), and secretion of cytokines like TNF-α and interferon gamma [213]. The combination of granulocyte colony-stimulating factors (CSFs) with CTX has shown clear clinical benefits for advanced pancreatic cancer with reduced toxicities [214]. Treatment for hepatocellular carcinoma (HCC) is challenging with chemotherapy and shows a poor five-year survival rate. The development of resistance due to an immune suppressive milieu and patients with cirrhosis condition show poor response and resistance to chemotherapy alone [215]. The combined approaches improve the therapeutic outcomes due to tumor cell death by antibodies, stimulation of NK cells and T lymphocytes, while suppressing Treg cells. Chemotherapy overcomes immunosuppression, enhances the interaction of tumor cells with immunological components, and alters TME subpopulations [216, 217]. CTX-induced immunomodulation enhances the HER-2 specific monoclonal antibody vaccine-induced anticancer response in *neu* mice and breast cancer patients [218]. The inoculation of CTX-loaded hydrogel with tumor lysate in CT26 colorectal tumor-bearing mice shows promising outcomes in terms of tumor growth inhibition, increased survival rate, and sustained immune-based memory to prevent recurrence [219]. Pancreatic cancer has a low cure rate with chemotherapy alone, but combination with peptide-based CV shows great hope for the patients [220].

11.6 NOVELTY, NEW POSSIBILITIES, AND FUTURE PERSPECTIVES

Currently, various antigens or proteins are being screened for the development of cancer vaccines (CVs). These include mesothelin (targeting metastasis), WT1 (for Wilms' tumor), telomerase, and survivin (targeting the immortality of tumor cells). Virus-infected tumor cells express virus-derived proteins that can be used as targets for vaccine-based therapy [221]. Neoantigen-based vaccines may be available, sparing normal cells and targeting tumor cells more aggressively. Currently, researchers are developing personalized CVs based on massive parallel sequencing of epitopes and neoantigens from individual patients [222]. The use of preparative chemotherapy with CTX and fludarabine supports the treatment of autologous tumor-infiltrating lymphocytes for melanoma patients by increasing the level of IL-15, which dampens the activity of Treg cells [223]. Nano-technology-based formulations need to be developed to gain more advantages in targeted drug delivery to cancer cells and dose reduction [224]. In the future, the use of multiple antigens targeting vaccines may achieve a robust response against malignancy from

various immune cells [203]. Resistance development after discontinuation of chemotherapy can be managed by vaccination at certain time intervals [153].

11.7 CONCLUSION

The multifactorial nature of cancer must be suppressed with the combined edge of chemotherapy and vaccines. Monotherapy with either of these cannot fulfill the goal of cancer treatment. The chances of cure rate, disease progression-free life, and overall survival are improved when using combination therapy, while the development of resistance and recurrence is minimized. The ever-changing nature of cancer may necessitate the use of specific chemotherapy based on the prevailing cancer, along with the use of personalized vaccines. Many more studies are indeed required to find hope for the deadliest cancers, such as lung, colorectal, stomach, and pancreas.

KEYWORDS

- **cancer vaccine**
- **chemotherapeutic agents**
- **chemotherapy**
- **combination therapy**
- **immunomodulation**
- **immunotherapy**
- **monotherapy**
- **recurrence**

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