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New Therapies for Melanoma Cancer Strategies

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Abstract

Objectives: Melanoma is the most common and severe type of skin cancer. Patients with melanoma may undergo surgery, chemotherapy, biological therapy (immunotherapy and cancer vaccine designation), radiation therapy, or a combination of these regimens. In addition, at any stage, they may attempt to relieve and control pain and other symptoms of malignancy, along with reducing the side effects of treatment approaches and emotional and practical problems that are treated by managing the symptoms of illness, supportive care, or so-called palliative care. Some therapeutic strategies are the application of immunotherapy and the design of a cancer vaccine which can activate active immune response by stimulating T-cells and reducing the tumor volume and size. Consequently, it creates the memory of immunity. One of the main causes of cancer immunotherapy is T lymphocyte cytotoxicity in cancer. The next strategy is to use immunotherapies (monoclonal antibodies), including antibodies against CD20, CD40, CTLA-4, and PD-1 antigens.

Methods: This study was reviewed by using a search of keywords including 'mRNA vaccination', 'monoclonal antibody', 'melanoma', and 'CD20 antigen' in Google Scholar, PubMed, and Elsevier databases. Approximately 60 articles were selected that were examined thoroughly.

Results: The results of our study revealed that vaccine cluster of differentiation, monoclonal antibodies are considered as treatments for melanoma cancer.

Conclusions: mRNA plays a key role as the biological treatment of melanoma. More precisely, it is a vector for tumor antigens and a stimulator for inducing antitumor immune responses. In this regard, the study groups use strategies based on the mRNA encoding of the entire mRNA tumor in both in vivo and in vitro environments. Finally, the use of anti-CD20 antibodies with the activator and the inhibitors of immunosuppressive agents have been successful, and surveys in this area have focused on this issue.

Keywords: Monoclonal antibody, Melanoma cancer, mRNA vaccines, Palliative care

Introduction

Melanoma is the most prevalent and severe type of skin cancer. Based on data, more than 63 000 people suffer from malignant skin cancer (melanoma) and 9000 cases die in the United States each year (1,2). Patients with melanoma cancer may be affected by surgery, chemotherapy, biological therapy, or radiation therapy, or a combination of these treatments, or at any stage, relieve and control pain and other symptoms (3). The side effects of treatment, along with emotional and practical problems are treated by managing the symptoms of illness, supportive care, or palliative care (4). Palliative medicine or care is one of the specialized health care branches which focuses on relieving pain, symptoms, and stress due to serious illnesses (3). The goal of palliative care is to improve the quality of the patient and his or her family's life. This type of care is used at any age and every stage of the disease, and it can be thoroughly combined with other treatments, reducing the symptoms, "occasionally discontinuing treatment" and improving the quality of treatment which completes the process (5,6).

The use of immunotherapy drugs and the design of a cancer vaccine are among the strategies for the treatment of this disease. The first strategy for the treatment of melanoma is the administration of cancer vaccines, which mainly aim to induce immune responses by activating tumor-specific T cells and thus reducing the size of the tumor, and subsequently securing memory (2). To achieve such goals, the vaccine should be effective in stimulating the immune system to kill cancer cells. The simultaneous activation of the inherent and acquired immune system is essential for the effectiveness of vaccination against cancer. Dual vaccine (antigen and adjuvant) can be used to treat cancers due to the ability to activate innate immunity by adjuvant and acquired immunity through cancer antigens (7). The strategies for immunotherapy (monoclonal antibodies), which have been approved since 2011, include ipilimumab and vemurafenib in 2011, dabrafenib and trametinib in 2013, and pembrolizumab with the Keytruda brand in 2014 (i.e., the newest drug

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treatment for Melanoma). These drugs block cellular pathways (PD-1) and CTLA-4, inhibiting the activity of the immune system to fight the cancer cells of melanoma (5,8).

Other Treatments

Melanoma is classified based on tumor thickness from zero to four stages. In stage 4, melanoma cells are spread to other parts of the body, lymph nodes, and the areas of the skin that are far from the primary tumor. In this case, melanoma is called metastasis. The treatment depends on the progression of the disease, age, and the general health of the patient, and other factors (9). At the zero stage of melanoma, patients undergo partial surgery to remove the tumor and part of the surrounding tissues.

In step 1, the surgical procedure is used to remove the tumor (about 2 cm out of the tissue around the tumor) and the patient undergoes skin grafting to cover the scar tissue. In stage 2 or 3, patients with melanoma usually undergo surgery to remove the tumor, and the surgeon, if necessary, removes about 3 cm of the tissue around the tumor, and skin grafting will be used to repair the skin lesions. Occasionally, lymph nodes may also be removed at this stage. Melanoma patients often receive palliative care in stage 4. Palliative care helps the patient to feel better both physically and emotionally. This type of treatment is used to relieve and control pain and other symptoms, and to reduce the side effects of the treatment (e.g., nausea) rather than the prolongation of life. Palliative care is often an important part of the treatment program. Many patients receive palliative care to reduce the symptoms of anemia during anticancer treatments for slowing the course of the disease. Some patients are just under palliative care to improve their quality of life by reducing pain, nausea, and other symptoms (9,10). All types of melanoma treatments, including biological therapy, surgery, chemotherapy, and radiation therapy have some complications. The undesirable effects, which will be resolved in the case of palliative care, vary depending on the type of treatment. These treatments may cause hair loss, swelling, swollen flu, or cold such as fever, joint pain, weakness, loss of appetite, nausea, vomiting, and diarrhea. In addition, radiotherapy often causes excessive fatigue. People with melanoma may also not be reluctant to eat, especially if they feel unwell or tired. Additionally, side effects such as cramps, nausea, or vomiting can be problematic. The taste of food may differ in their opinions. Palliative care, including the provision of healthy eating, is essential in all of these settings (11,12).

Agarwala et al showed that histamine dihydrochloride is effective in inhibiting reactive oxygen species. In fact, this combination plays a synergistic role in the activation of normal endodontic cells and T cells with cytokines. This study was designed to determine whether adding histamine to the interleukin-2 (IL-2) regimen improves the survival of metastatic patients with melanoma (13). The use of histamine in the IL-2 regimen is well-tolerated and its survival is significantly associated with those with melanoma metastasis who received IL-2 alone. More testing is needed to confirm and understand the role of histamine in this combination therapy (13).

Evidence Acquisition

In this study, a comprehensive overview was conducted regarding available resources in the field of introducing new therapies for melanoma in several databases including Google Scholar, PubMed, and Elsevier using keywords such as mRNA vaccination, monoclonal antibody, melanoma, and CD20 antigen. Nearly 60 articles were selected and reviewed thoroughly.

Results

Designing a Vaccine for Melanoma

Dendritic cells (DCs) are progenitor cells that play an important role in initiating and directing immune responses. DC has an ability for creating and directing a variety of immune responses based on environmental factors and messages and can answer to responses in terms of growth and puberty (14). Due to the highly important role of these cells, they can be used to vaccinate and perform immunotherapy for various diseases including cancers (15), allergies, and autoimmune diseases (16). Currently, many DC therapeutic applications rely on the use of DC autologous manipulated with peptide antigens in the lab and return them to the patient's own body (17). However, the development of strategies to increase the therapeutic potential of DC is necessary. To optimize such a viewpoint, several parameters should be carefully considered, including DC subgroup review, injection method, dose, and the number of injections. An important factor in determining the efficacy of DC-based immunotherapy is their ability to induce antitumor T cells, which depends on the state of maturation and DC activation. Meanwhile, the use of mRNA, as a new solution to eliminate the limitations of other nucleotide vaccines, is intended by researchers. The main causes of using mRNA in comparison with plasmid DNA or viral vectors are the absence of additional plasmids or viral sequences in the mRNA molecule, which facilitates their entry into DC cells. Non-interference with the genome of the cell, which reduces the risk of using mRNA, is also an easy and inexpensive process for producing, purifying, and manipulating mRNA from other factors (18). However, first, decomposition by extracellular agents and enzymes such as ribonucleases must be avoided for using an mRNA molecule as a vaccine. The mRNA formulation within nanoparticles such as DOTAP or DOPE or positive protein cations such as protamine is used for this purpose (19,20). The mRNA molecule should have a 5'cap and 3'Poly A tail. The presence of 5'cap and 3'Poly A tail is necessary for the greater stability of the mRNA molecule in the cytosol and an increase in the efficiency of the translation of the mRNA fragment, and therefore,

several processes have been proposed for optimizing these parts (21,22). In addition, many efforts have been made to identify potent stimulants for the production of DC by adding inflammatory cytokines or receptor-like TLR receptors to the culture medium, as well as DC maturation within the body (23). Bonehill et al reviewed the ability of DC cells to stimulate T cells in their previous studies using CD40 L, CD70 mRNA, and a CaTLR activated form, which transmitted them to DC. The combination of the CD40 L, CD70, and caTLR4 mRNA is termed TriMix and trans-mixed to TriMix DC transformed with the term TriMixDC. This study is the first one to introduce TriMix into DC, which ultimately results in the production of DC (24). The adult DC obtained by entering the antigen coding mRNA, which may be Tena-specific antigens or tumor-associated antigens are activated and can trigger T-cell responses against the tumor. Bonehill et al et al studied from 2008 through 2013 about the enhancement of DCs activity due to increasing their ability to stimulate CD4+ and specific CD8+ cells against tumor antigens for designing a DC-based vaccine and the evaluation of the vaccine efficacy in melanoma (24). The research team examined the expression of the mRNA entering the DC and the functionality of the expressed proteins, as well as the phenotypic, cytokine, and chemokine produced by the adult DC. Generally, in this study, TriMixDC was first developed for therapeutic purposes and the ability of this compound to develop immune responses (25).

In another study, Bonehill et al used the simultaneous entry of TriMix as an adjuvant and mRNA for total tumor antigens due to adult and DC activation instead of using the peptide antigen in separate steps. Subsequently, the ability of these cells to stimulate specific T cells and their application as a vaccine in advanced melanoma was investigated as well (26). First, the ability of DCs for stimulation of T- cells, creation, and comparison of mature phenotype and secretion of cytokines in the transfected state in associated with the mRNA encoding of entire MelanA antigen and TriMix was studied in separate step. Then, the ability of DC to induce CD8+ cells for other tumor-associated antigens, including the Mage-A3, Mage-C2, and tyrosinase antigens, was evaluated in the body of 2 patients with melanoma. CD137/CD107a markers showed that adult DC resulted in strong responses that did not exist in the samples before vaccination.

Likewise, Bonehil et al directly injected the mRNA of the tumor antigen and TriMix into the mouse lymph node (26). Following the intra-luminal injection of TriMix, the evaluation of phenotype results, cytokine secretion profile, and the stimulation of allogeneic T cells, it was found that DC cells were transmitted to the environment through the transfer of TriMix. These results indicated the ability to absorb DC mRNA from the lymphatic environment. To demonstrate the efficacy of DC (DC vaccine where TriMix and mRNA encoding the Trp2 antigen are transmitted to it and matured), producing the melanoma tumor MO4 in mice, DC vaccine was injected intravenously into mice and then the tumor was created in these mice. The results revealed that tumors of a high-rate stage have grown in mice in the control group (DC treated with NGFR) compared to vaccinated mice. Indeed, tumor growth and long-term survival were better in vaccinated mice in comparison with the control group (27).

Along with these studies, Bonehill et al evaluated the role of TriMixDC and its efficacy in pre-clinical studies, and a clinical trial of phase 1B to investigate the safety and effectiveness of the intravenous injection of TriMixDC-MEL in patients with advanced and underlying melanoma (28). In this study, 15 eligible patients (according to inclusion and exclusion criteria) and those with advanced treatment melanoma were collected during 2009-2011. Patients were classified into 4 consecutive groups. Then, the TriMixDC-MEL combination was transmitted using the simultaneous transmission of the TriMixDC encoding mRNA for one of the melanoma antigens (i.e., MAGE-A3, MAGE-C2, and tyrosinase), which is attached to the HLA Class II targeting signal. Following confirming this combination, these patients received TriMixDC-MEL increasing dosages. The goal of this trial was to investigate the safety and effectiveness of intravenous injection of TriMixDC-MEL. Therefore, the side effects associated with treatment were evaluated following injection. The results of this study showed that this combination was easily tolerated by patients and demonstrated no severe toxicity (above grade 3) in patients. Finally, all patients represented hypersensitivity reactions of type II (inflammation and swelling) in the injected areas.

Monoclonal Antibodies to the Treatment of Melanoma Cancer

Monoclonal antibodies are one of the most important biological agents in the targeted treatment of diseases such as cancer. Based on recent reports from the Antibody Society, over 40 monoclonal antibodies have been approved by the United States Food and Drug Administration (FDA) and several monoclonal antibodies are undergoing clinical trials (29,30). Antibodies have many applications in treatment because of their special ability to detect a large number of specific epitopes and high-affinity binding to various antigens. It is also possible to organize them into distinct structural and functional domains of sustainability and the possibility of engineering antibodies (31). Recently, monoclonal antibodies are used to treat melanoma cancer. The mechanism of action of these antibodies helps immune-stimulating agents or suppresses immune-suppressant factors including CTLA-4 and PD-1. The monoclonal antibodies of ipilimumab and tremelimumab to the CTLA-4 antigen, which are at the cell surface active T's are expressed. These two recombinant monoclonal antibodies are immunoglobulin that bind to the CTLA-4 antigen on T lymphocytes. CTLA4 inhibits the pathway of T-cell activity. CTLA4 inhibition allows T

cells to multiply and proliferate and indirectly regulates the response of the T cell to the tumors (32, 33). In their study, Enewold et al showed that the use of ipilimumab monoclonal antibody in patients with non-surgical metastatic melanoma increases the average survival of patients by 5 years (34). The main side effects of using this monoclonal antibody are fever and chills and skin rashes (35). According to Lordick et al (29), other monoclonal antibodies, whose mechanism of action contributes to the immune system for the destruction of tumor cells, are the pembrolizumab antibody entitled 'Keytruda' and the nivolumab antibody designed in 2014 against a planned cell death receptor (PD-1). Several studies reported that these two monoclonal antibodies against PD-1 have high efficacy in treating patients with metastatic melanoma (36-38).

Monoclonal Antibodies Against CD20 in the Treatment of Melanoma

The CD20 molecule is a 297 amino acid phosphate with the second-membrane passage of the membrane, part of the MS4A family of proteins encoding it on the long arm of chromosome 11. This marker was created on the surface of the precursor and mature B cells, and its expression disappears when B cells evolve into plasma cells. CD20 is a part of the Ca channel at the level of normal and malignant B cells that plays an important role in regulating the activity and proliferation of B cells in addition to producing antibody responses independent of T cells (39). Due to the large expression on the surface of B cells, CD20 is considered to be easy for attachment to the desired antibody while not to be transmitted to the cell for inhibiting antibody binding as an ideal target for non-conjugated therapeutic antibodies. These characteristics facilitate attracting efficient operating factors during CDC and ADCC processes. In addition to absorbing effective factors, when the antibody binds to CD20, binding antibodies to CD20 can generate signals which directly control growth and trigger cell death in tumor cells (40, 41). Payandeh et al investigated the three-dimensional structure of this antigen with detailed bioinformatics software (42). In melanoma, there are heterogeneous tumor cells that contain several types of genotypes and phenotypes. Melanoma, like all other malignancies, has separate cell subsets (43). Working on these subsets began with the introduction and description of cancer stem cells, which were initially identified in blood and brain tumors, and recently, many other tumors (44). In melanoma, several populations have been observed having a capacity for self-regeneration, differentiation, tumorigenicity, or resistance to drugs, including B cells that express the CD20 marker. These CD20 melanoma cells are tumor stem cells, in which there is a degree of self-renewal and differentiation into several cell-groups. Previous research confirmed the transplantation of melanoma cells. The CD20 positive to the living creature was a highly genetic tumor, indicating that these cells exhibited the ability to start the tumor (45).

Schmidt et al concluded that targeted eradication of CD20 positive melanoma cells eliminates melanoma lesions in a completely stable manner while targeting other cells fails in this regard. Thus, the growth and progression of melanoma depends on the presence of CD20 positive subunit cells, and selectively eliminating these cells effectively eliminates tumor lesions (46). Additionally, anti-CD20 antibodies have been found to have a potent therapeutic effect in 9 patients with melanoma with metastatic stage IV. The rapid and sustained reduction of CD20 positive B cells from the circulation using monoclonal antibiotic therapy with rituximab can be well-tolerated considering that CD20 is specifically expressed in B cells and B malignancy cells (47). Therefore, CD20 may be an appropriate target for early-onset melanoma (CIC) cells (48). Immunoliposomes (conjugated liposomes with antibodies) have been recently demonstrated a high potential for use as drug-delivery systems purposefully. Conjugate liposomes with an anti-CD20 antibody can be specifically targeted for CIC melanoma cells (49).

Doxorubicin immunoliposomes release anti-CD20 antibodies which have similar therapeutic efficacy about non-targeted liposomes, while VLM (immune-suppressants release anti-CD20 antibodies) releases drug more than atypical liposomes leading to improved therapeutic efficacy (50,51). The mechanism of action remains unclear. It is likely that the rapid release of vincristine (VCR) at the cell surface can compensate for releasing the drug far from the cells, thus enhancing the time taken to absorb VCR even when the immunosuppressive cells do not enter the cells. Therefore, the release of a conjugated VCR monoclonal antibody drug may need to be adjusted to improve the therapeutic efficacy (52). Johnston et al found that targeting tumor cells with conjugated microspores with antibodies is effective even when target cells are present in less than 0.1% of the total cell population. Therefore, the conjugated VCR immunomodulocytes with an anti-CD20 antibody may retain their targeting ability to CD20 positive cells in melanoma patients although these cells form only a small percentage of the total tumor cells (53).

Song et al conducted a study to obtain a robust drug delivery system for targeting specific CD20 positive melanoma cells in 2015. In this study, the immunoglobulin conjugated immunoglobulin group developed the anti-CD20 CD20 (Vincristine-Lip) antibody, optimized and improved the immunosuppression profile and the composition of antibodies to achieve specific targeting, and increased cell cytotoxicity to CD20 positive cells. Then, antitumor activity and mechanisms, including the ability to remove CD20 positive melanoma cells in vitro and in vivo (54). Another strategy to build the foot anti-tumor therapy after treatment Anti-CD20 mAb increases the immune function of T cells by stimulating the

pathways for activating DCs (55).

It has been well-documented that monoclonal anti-CD20 antibodies can significantly prolong the life span of patients with B-cell malignancy. Many patients survive several years after treatment with antibodies. However, some patients ultimately show resistance to the drug or recurrence of the disease. To solve this problem, scientists have recently suggested combined anti-CD20 mAb and immunosuppressive agents such as anti-PD-1/PD-L1 immune inhibitors. This combination is highly effective for the production and durability of immune responses. Studies have focused on investigating the combination of various factors and factors with the anti-CD20 mAb in the treatment of B-cell malignancy. Patient et al showed that target mutations in the variable ofatumumab antibody could have a higher binding tendency to the CD20 antigen (56). Pre-clinical models suggest that immunostimulatory antibodies (e.g., anti-CD40 antibodies), along with anti CD20 antibodies can create and improve antitumor immune responses. Therefore, in future studies, controlling B-cell malignancies is likely to include the simultaneous use of immunosuppressive agent in combination with strong anti-tumor monoclonal antibodies against CD20 (36, 57). Figure 1 depicts monoclonal antibodies against CD20 in the treatment of melanoma.

Discussion

Monoclonal antibodies and their derivatives, including nanobodies, are widely used in the detection of cancer markers and pathogens (58), and many studies have been done to determine their characteristics and improve their functions (59,60). Melanoma tumor cells are heterogeneous cells that contain several types of genotypes and phenotypes, thus they need new therapeutic strategies and strategies that are more effective compared to standard therapies. Given the key role of mRNA, this molecule is used as a vector for tumor antigens and stimulants for inducing antitumor immune responses. In this regard, study groups use strategies based on the use of mRNA encoding total mRNA tumors in both in vivo and in vitro environments (61) although the key issue that can be discussed in immunotherapy is that cancer tolerance proteins are insiders. Most tumor antigens are known in the immune system of the human body. Therefore, recent studies have focused on targeting tumors (62). A cancerous and normal cell profile is obtained to identify somatic mutations via next-generation sequencing (NGS) technology (63). In this regard, NGS plays an important role as a powerful tool for a better understanding of cancer. This technique can provide a comprehensive somatotropic map of individual tumors, which is called "mutant". In addition, NGS can be used to examine the entire exon exome for the gene expression profile, namely, RNA sequence (64). Based on the tumor expression profile, two strategies may be used for treatment. One way is to directly inject an mRNA vaccine, which combines TriMix and selected tumor antigens derived from the sequencing of a person's tumor into the individual. The next solution is a DC-based vaccine. In a laboratory environment, the mRNA encoding a specific tumor antigen is transmitted to DC cells with TriMix, then adult and active autologous DC cells are returned to the patient's body. The most important goal is to use these strategies and palliative care, along with the treatment that can improve the quality of life. In conclusion, the future of drug development for melanoma patients has a progressive trend and innovations in this cancer have a helpful impact on clinical trials.

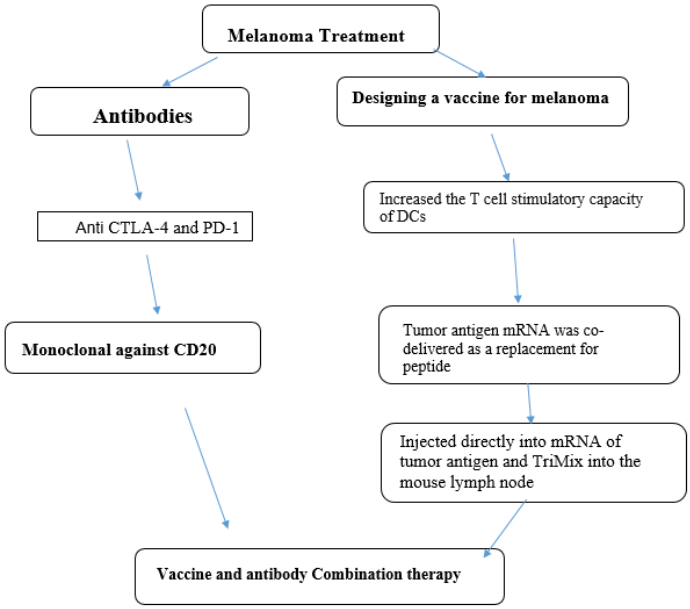

Figure 1. The Workflow for the Main Approach Regarding Melanoma Treatment.

Authors' Contribution

MDR, FM and ZP: concept and design. DA, HF and MM: performing of the study and writing of the draft. All authors read and approved the study.

Conflict of Interests

The authors declare that they have no conflict of interests.

Ethical Issues

Not applicable.

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