Emerging role of vaccines in glioblastoma treatment

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Abstract. One of the most devastating brain tumors is glioblastoma multiforme (GBM). Chemotherapy and radiotherapy are the current treatments offered. They were unable to eliminate all the cancerous cells since doing so might have harmed other healthy, functional cells. Vaccines used for therapeutic applications are examples of immunotherapy. This review discusses TRAIL mRNA vaccines, DC-mRNA vaccines, and VLP vaccinations. Regarding the process of VLP, tumor antigens on the surface are first endocytosed by dendritic cells, where they are then presented with MHC-I and MHC-II, activating CD4+ and CD8+ T cells, respectively. Malignant cells are eliminated when CD8+ T cells are developed into cytotoxic T cells and are responding to cells. To activate more cytotoxic T cells and produce antibodies, which are humoral responses, CD4+ T cells differentiate into Helper T cells and release cytokines. Also displayed is the pre-clinical examination of thymidine kinase genes carried by virus-like particles. The effectiveness of tumor cell death led by the genes carried by VLPs strengthened with the results on U87-MG cells and nude mice tests, and the tumor size was significantly reduced.

Keywords: Glioblastoma, Virus-Like-Particles Vaccine, mRNA Vaccine.

1. Introduction

Glioblastoma multiforme (GBM), which is well-known for its high mortality, recurrence, and morbidity rate, is classified by the WHO as a grade IV glioma of the central nervous system (CNS) [1]. It also inevitably contributes to numerous other health issues associated with CNS cancer. The glioblastoma (GBM) microenvironment and immunosuppressive central nervous system prevent immunization, and the poor treatment response proves to the GBM's extreme difficulty in recovering. The three aspects of the immune response inhibitors are the blood-brain barriers (BBB), neurons and resident microglia, and monocyte-derived macrophages. The bulk of the BBB consists of non-fenestrated endothelial cells (ECs), which are associated by tight junctions. As a physical and chemical barrier, the BBB actively regulates and controls the entry and exit of pathogens and immune cells [2]. Primary glioblastoma and secondary glioblastoma have different molecular pathways. In initial glioblastoma, the epidermal growth factor receptor (EGFR) is amplified and overexpressed, and these genetic changes are more prevalent than mutations in secondary glioblastoma. Currently, the major purpose of vaccinations is the prevention of infectious diseases. After the FDA authorized the first therapeutic vaccine, targeting prostate cancer in 2010, more focus was placed on the therapeutic vaccine side [3].

Gene therapy has always been considered as an insightful aspect as a novel cancer immunotherapy. Its goal is to change the suppressive tumor microenvironment and activate the host anti-tumor immunity, which will reduce tumor development and boost survival rates [4]. The nucleic acid vaccines (including DNA-based and mRNA-based vaccines) (Rhoads) are among the most alluring fields because they are not contagious and do not include any contamination from viruses during synthesis [5]. Antigen-presenting cells (APCs) can present full-length tumor antigens based on its ability to encode them, and at the same time increasing the likelihood of inducing a more extensive T cell response [6]. In comparison, messenger RNA (mRNA) vaccines have very significant advantages over DNA vaccines. mRNA-based delivery prevents insertional mutations, which is obtained in a higher possibility by DNA-based delivery, regardless of dividing or non-dividing cells. It is also

economical in its efficient production and in vitro synthesis, and the application of various mRNA vaccines have shown promising efficiency in other tumor therapies. The mRNA vaccines are designed to target tumor-associated (TAAs) or tumor-specific antigens (TSAs), both are assessed in vivo experiments and proved to be overexpressed in malignant cells. Modification of mRNA vaccines have developed through the years, and it'll be introduced in the newest application on GBM animal trials.

The viral protein that makes up particles, which are nanoscale structures, lack viral genetic material, which prevents them from replicating and infecting host cells [7]. They are primarily employed to deliver medications, imaging agents, and genetic information [8]. Many different types of cells, including prokaryotic cells [9], yeast [10], plant [11], and mammalian cell lines, can manufacture VLPs [12]. In addition, viruses like the HIV, HBV, andHCV can create VLPs by taking advantage of their structural proteins. These modified VLPs can trigger cellular and humoral reactions. This review analyzes JCPyV viruses that carry thymidine kinase genes to cause apoptosis.

2. mRNA vaccines

Among all potent DCs (Dendritic cells) electroporated with synthesized mRNA for use in immunotherapy is now the primary reagent chosen to deliver transfection of mRNA as it shows safety in patients with malignancy [13]. The mRNA gene fragment can function on both stationary and proliferating cells without endangering the host cells through translation towards targeting proteins or peptides in the cytoplasm rather than the nuclei. Synthetic mRNA is also simpler to transfect into target cells due to its lower construct size than matching DNA plasmid, demonstrating that it would be practical when passing through specific biological characteristics of GBM such as the brain-blood barrier (BBB) or brain-tumor cell barrier (BTB) [14]. Also because of the potential advantage of its fast and efficient production, the in vitro synthetic mRNA-based vaccine has great prospects in cancer related therapy. Still, there are several concerns about mRNA vaccines with one of them being relatively short life span inside the cell. mRNA vaccine can be classified into the following categories based on antigen located in as well as role of mRNA vaccine as a therapeutic support.

2.1. TRAIL mRNA vaccine

The fact that TNF-related apoptosis-inducing ligand (TRAIL) is specifically targeting towards the death receptors 4 (DR4) and death receptors 5 (DR5) makes it one of the best targets of all antigens (DR5), triggering apoptosis on the cellular surface of GBM without harming normal cells or being blocked by BBB [15]. Designing an intracranial injection of synthetic mRNA that can deliver anticancer genes, in this case TRAIL, towards the tumor cell was the goal of both preclinical and clinical trials. An essential cell lines the vaccine is targeting towards is the Denver Brain Tumor Research Group (DBTRG) glioma tissue, and by means of the Real-Time Cell Analyzer to assess the effect of TRAIL-mRNA produced artificially on the survival of DBTRG-Luc glioma cells (RTCA). The flow cytometric analysis can display if a TRAIL-induced apoptosis was occurring. Much of the current research, however, have been unsuccessful due to the high drug resistance, toxicities that are off-target, and the insufficient absorption of the TRAIL genes by the cancer cells.

2.1.1. Clinical trial from HuBei Medical university on murine model

A murine intracranial delivery of synthetic mRNA was carried out by Hubei University of Medicine applied the TRAIL vaccine, and their first approach is to find an appropriate transfection reagent for mRNA delivery. The in vitro test on 293 T cells assessed the toxicity of transfection reagents carrying luciferase mRNA (Luc-mRNA) as detection of the supplied mRNA's expression level, which could be monitored by a small animal imaging system.

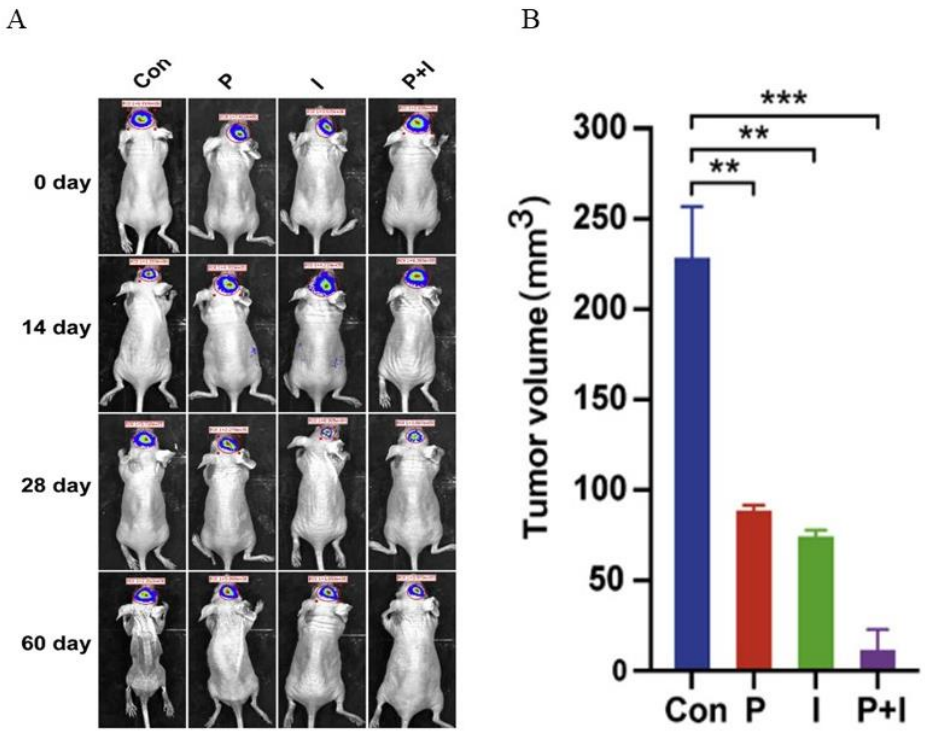

Figure 1. mRNA vaccines inhibit tumor growth on its size and weight in mice [16]

The evaluation of in vitro test from the present study showed TransIT-mRNA injection obtained a longer in vivo half-life and caused little inflammation response than the other, and the optimized dose concentration of 1 μg/20 μL (mRNA/TransIT-mRNA). Additionally, the synthetic red fluorescent protein (RFP) mRNA served as the brain localizer for precise mRNA delivery inside the brain. The therapeutic use of synthetic TRAIL-mRNA administered intracranially was examined in a mouse model of xenografted glioma derived from DBTRG-Luc cells. For cerebral xenografts, mice were given 3*10^5 resuspended cells in 4 L of complete medium while they were anesthetized and mounted on a stereotaxic platform. The injection site was 2 mm below the skull, 1 mm anterior and lateral to the bregma. Bioluminescence of tumors were monitored at D0, D14, D28 and D60 after injection through In Vivo Imaging System (IVIS) , and the results showed a significant inhibit of tumor growth on its size and weight (Figure 1). The duration of the mRNA transfection, however, was insufficient to trigger cell apoptosis. Still, the experiment discovered that the combination of pretreatment, which would be the pre-transfection of TRAIL for surgical removal of the tumor and intracranial injection of TRAIL-mRNA would perform a strong potent anticancer effect.

2.2. DC vaccines

CD133 mRNA-Loaded Dendritic Cell Vaccination against Cancer stem cells.

Another novel approach to trigger a long-lasting immune response from mRNA vaccine against GMB is through DC towards CD133, a pentaspan membrane glycoprotein that is highly expressed on brain tumor stem cells(BTSCs). It is also regarded as one of the best-characterized indicators for obtaining cancer stem cells (CSCs) [17]. CSC acts as one of the major sources for GBM tumoral reversal and resilience [18]. Specifically, CD133 interacts with multiple signaling pathways such as upregulating the expression of FLICE-like inhibitory protein (FLIP) to inhibit further apoptosis on targeting tumor cells. It also activates the Vascular endothelial growth factor-A (VEGF-A) expressions and interleukin (IL)-8 for further tumoral proliferation and growth [17]. Therefore, CD133 cells can be considered as an ideal candidate for targeted immunotherapy.

A CD133 mRNA-Loaded DC vaccination was applied on a humanized glioblastoma mouse model conducted by the Department of Neurosurgery, Cedars-Sinai Medical Center, Los Angeles, CA, USA in 2020with positive feedback. In this study, DCs were chosen to release interleukin (IL)-2 and cause a cellular immune response that is anti-tumor [18,19]. The trial was conducted both in vitro and in

vivo. Featured BTSCs (murine GL261 and human BTSC5 are cultured in stem cell media resulting in neutrosphere formation. Comparatively to DCs without RNA transfection, those transfected with modified human CD133 mRNA showed enhanced interleukin (IL)-12 production at 24, 48h following maturation. CD133 DC-mRNA vaccine strongly induced significant tumor strangling ability in vitro in murine models. The CTL assays in Figure 2 show that *in vivo* immunization led to positive responses from CD4 helper and CD8 cytotoxic T cells.

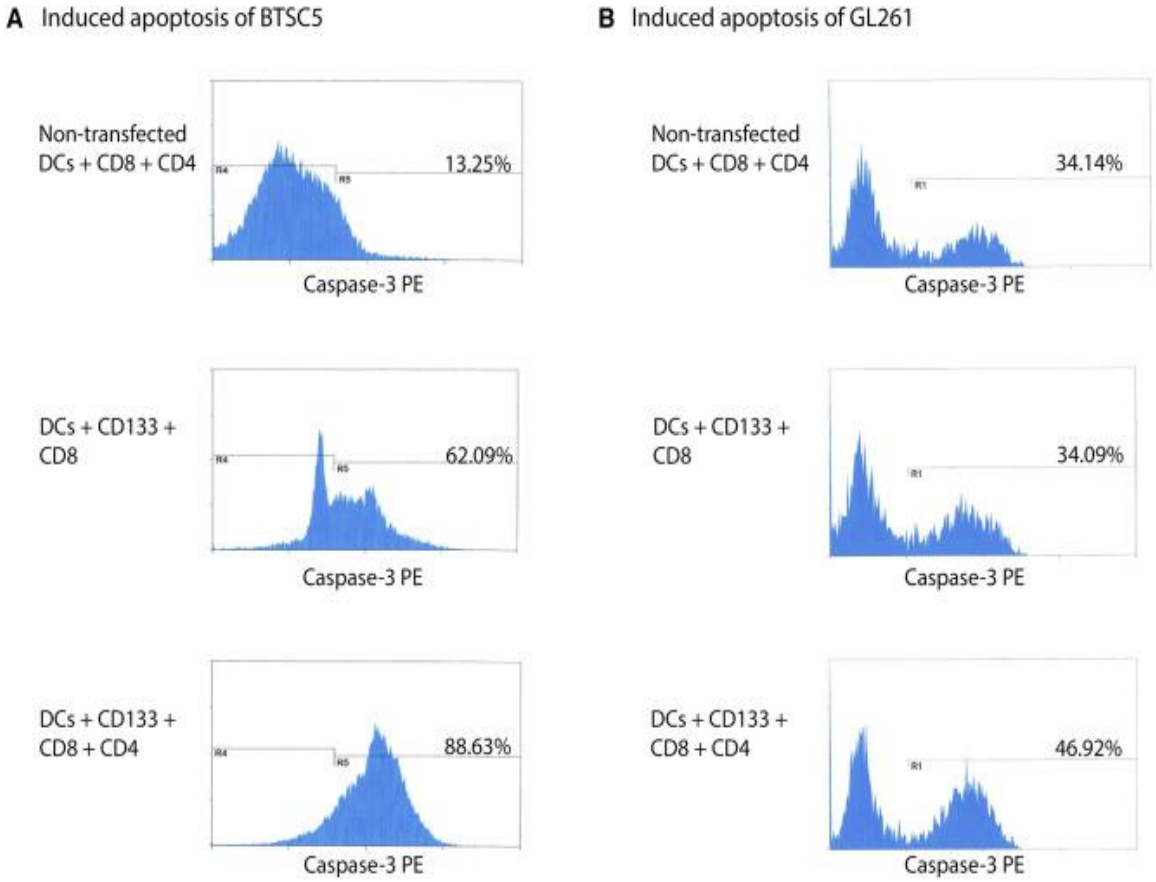

Figure 2. Tumor killing activity *in vitro*. After DCs transfected with cd133 mRNA

In the *in vivo* portion of this study, human peripheral blood mononuclear cells (PBMCs), which were used to treat NOG mice with immunosuppression along with busulfan injection, were used to isolate CD34-positive hematopoietic stem cells. The humanized mice maintained an insufficient CD8-positive T cell productivity, and naive T cells (CD4) under the secretion of IL-2 produce the desired T cell [20]. Once the immune T cells dropped down to the criteria, the transfected DCs with modified CD133 mRNA were then injected as it satisfies the requirement to increase IL-2 secretion by twofold, enhancing the expansion of in vivo CD8-positive T cells.

2.3. In vitro approach against

A phase I trial conducted by Vik-Mo et al. that followed the established European Organization for Research and Treatment of Cancer (EORTC) protocols produced encouraging results as well, with the treated group obtained a median survival of 759 days compared to 585 days during observation after receiving the treatment [21]. From 2009 to 2010, the Oslo University Hospital recruited patients and subjected with any immune or nero related disease like autoimmune or immunodeficiency diseases were excluded from this trial. Also, the use of corticosteroid, very efficiently reducing neurologic symptomatology regarding GMB, is considered as an impeder immune response. The criteria were the same for 77 patients in the Control group, receiving adjuvant temozolomide as placebo. For mRNA - DC transfection, 102 μg were used on average each time. It was a common practice to perform an MRI six months after surgery or whenever new symptoms developed.

Although a cell possessing a stem cell can be identified from both normal and cancer cells, even in vitro, it can still be judged by how well it forms spheres of cells. The "robust approach" for glioma stem cell (GSC) isolation and multiplication, as well as the initial step in the culture of GSCs, DCs generated from monocytes were transfected with amplified GSC-mRNA. Autologous DCs transfected with autologous GSC-mRNA were used to elicit an immune response against the patient's GSCs. Autologous dendritic cells transfected with autologous GSC-mRNA are employed to elicit an immunological response to patient-specific GSC. HTERT and surviving can be translated by GSC-mRNA to trigger an immune response. The production of privately customized vaccines can be divided into the following steps: Generation of GSC cultures-RNA isolation and amplification-DC generation-Immune monitoring (Figure 3). All patients in the treated group are requested to receive 12 doses of vaccination to complete the trial.

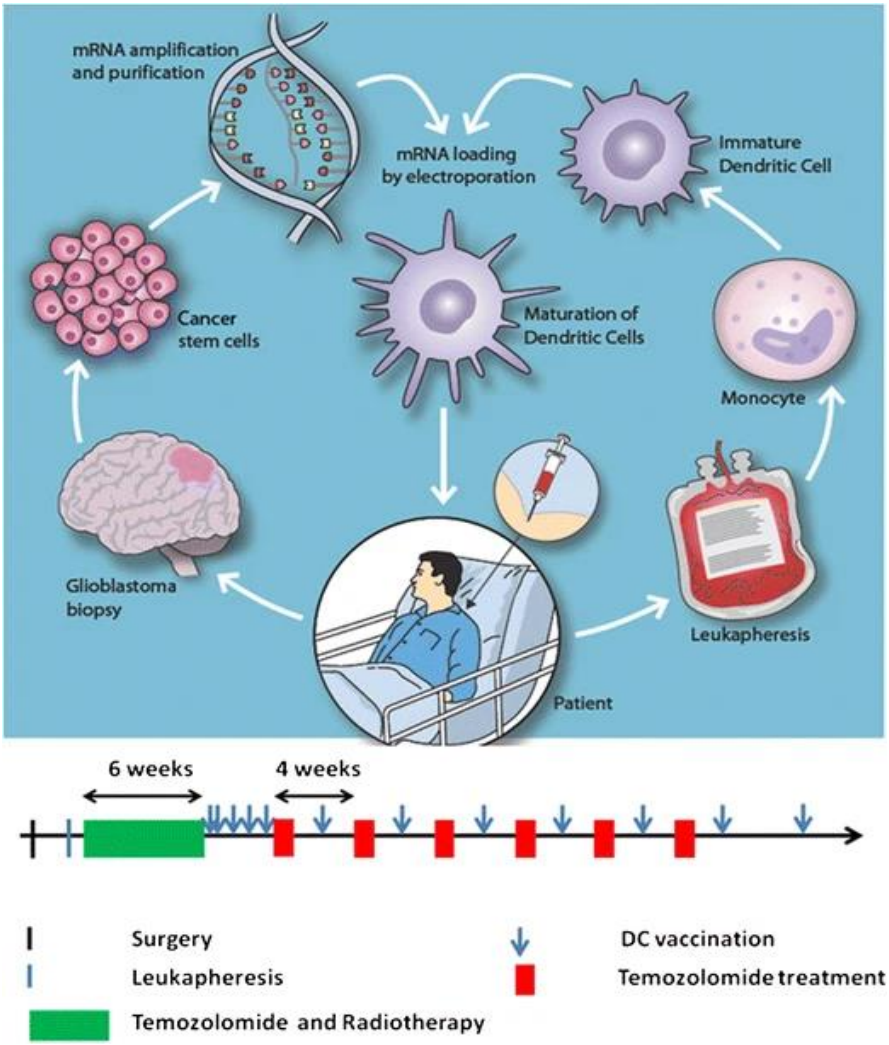

Figure 3. Process for subject receiving autologous GSC vaccination

Consequently, the experiment was successful in eliciting an immune response specific to GSCs without producing any negative effects, the majority of which were grade 1 fatigue and anorexia. The outcome supported the CSC hypothesis that was put forth before to the trial's execution and suggests that a therapeutic approach that targets the CSC population may be successful. Potential inflammation is indicated by the formation of inflammatory responses against such cells in the eye [22]. Another notable accomplishment, besides extending participants' overall survival times, is the diminution of tumor size. After the first round of vaccination, it reduced from a maximum mean volume of 805 mm^3 to a minimum of 209 mm^3.Unfortunately no assay can determine whether this reduction is because of the vaccination or surgery. Also, the biggest issue remained with such frequent vaccination. Equipment for autologous GSC vaccination is complex and complicated, and high frequency vaccination stifles

the ability of mRNA to be manufactured quickly and cheaply. Although clinical research did not exclude patients with severe or critical brain cancer, due to cancer cell's immunosuppressive will still limit this vaccine to a low born of disease (Figure 4) [23].

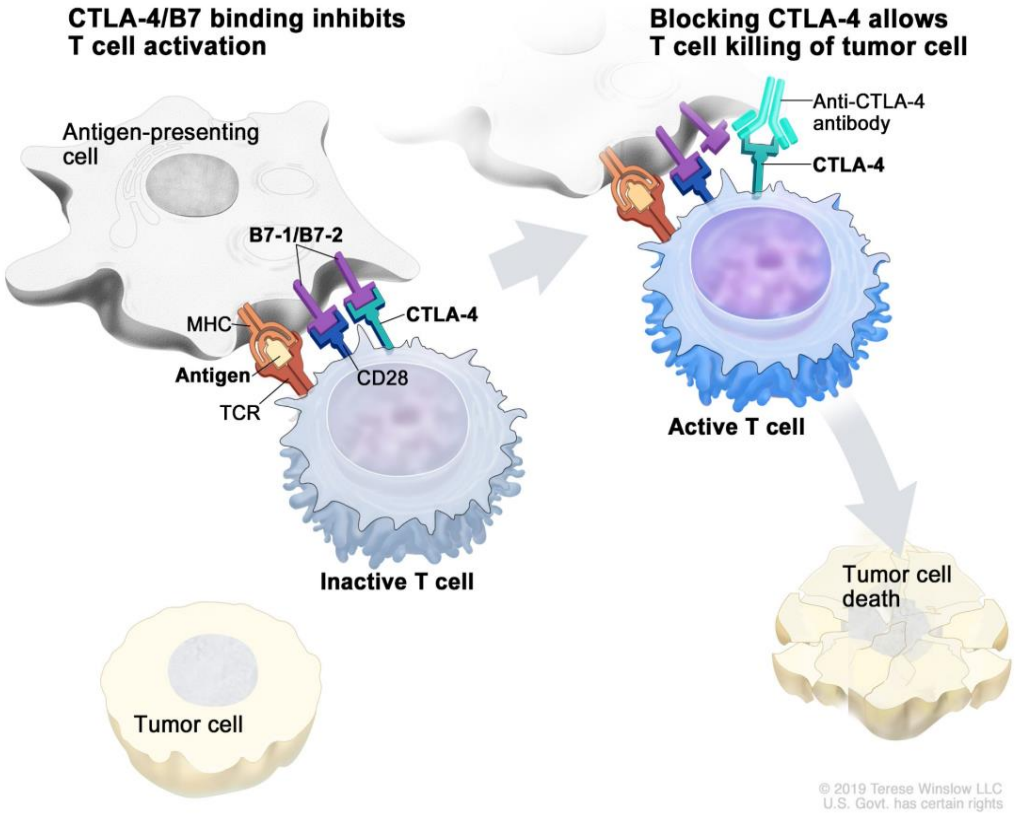

Figure 4. Mechanism of VLP vaccines

3. VLP vaccines

Virus-Like-Particles (VLPs) is a multiprotein structure mimicking virus with particles able to self-assemble without genome, therefore, they are not able to infect the host cells and then assemble new virus [24]. Hepatitis B virus and Human papillomavirus are widely used as virus-like particles to deliver drugs and genetic information [25]. Compared to polymeric micelles and liposomes, VLP has a more homogeneous structure and the process of producing it is relatively inexpensive. In addition, VLP illustrates less cytotoxic issues because of degradability as well as being able to interact with DCs, which are the most potent antigen-presenting cells responsible for MHC I and MHC II presentation. Due to the mobility of DC cells, they move to the lymph node and activate T cells there [26]. Unlike MHC-II, which can activate CD4+ T cells, MHC-I can only stimulate CD8+ T cells. By displaying the tumor antigen on MHC-I and MHC-II, dendritic cells identify and endocytose VLPs before presenting them to CD8+ T cells and CD4+ T cells for activation. After that, CD4+ T cells undergo differentiation into TH1 and TH2 cells, which control the inflammatory response and maintain the peak activity of CD8+ T cells, which have cytotoxic effects on cancer (Figure 5) [27,28].

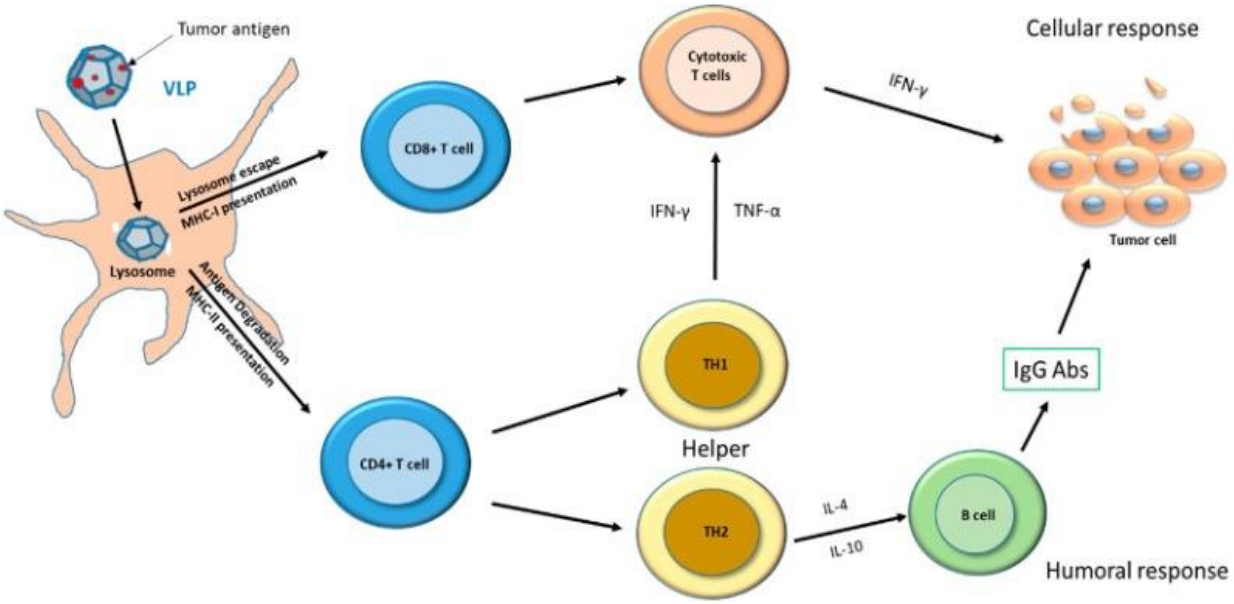

Figure 5. Mechanism of using VLP-based vaccines to target cancer cells

For the choices of VLPs, most researchers use spherical VLPs in morphology; some research has revealed that non-spherical VLPs may have favorable tumor uptake which may be used in GBM further studies [29]. There are some pre-clinical trials to evaluate the VLP with glioblastoma as nanocarriers. MS2 in a 27 nm sphere, TMV disc in 18 nm and a nanophage filamentous rod are considered. Surface holes of 2 nm on MS2 VLPs allow for inner capsid alteration and drug release [30]. For TMV VLPs which is a nanodisc consisting of tobacco mosaic virus with double arginine mutant, they can maintain structure properties in all biological conditions which can mimic the conditions in patients. Nanophage VLP was described as a possible nanocarrier in recent reports, but there are no current studies functionalized and evaluated as a drug delivery. Cysteins (red), reactive amines (green), and non-canonical p-aminophenylalanine moieties (purple) are examples of reactive handles for bioconjugation [29].

Typical protein modification involves 10 equiv maleimide, 40 equiv isothiocyanate, 10–20 equiv of PEG and 1–2 equiv of DOX-EMCH. All protein conjugates are purified through elution. For the cell culture experiment, U87-MG cells were trypsinized and diluted into 50,000 per mL. Various doses of doxorubicin are injected to the tumor, and a dramatic decrease in cell viability is observed. After the protein modification and survival studies in glioblastoma mice, TMV-DOX demonstrated enhanced survival rates when only a minor dose of DOX was administered to the mice. Additionally, it has been found that mice survival is dependent on tumor size, showing that the environment for GBM has much improved [29]. The doxorubicin concentration change is displayed in Figure 6.

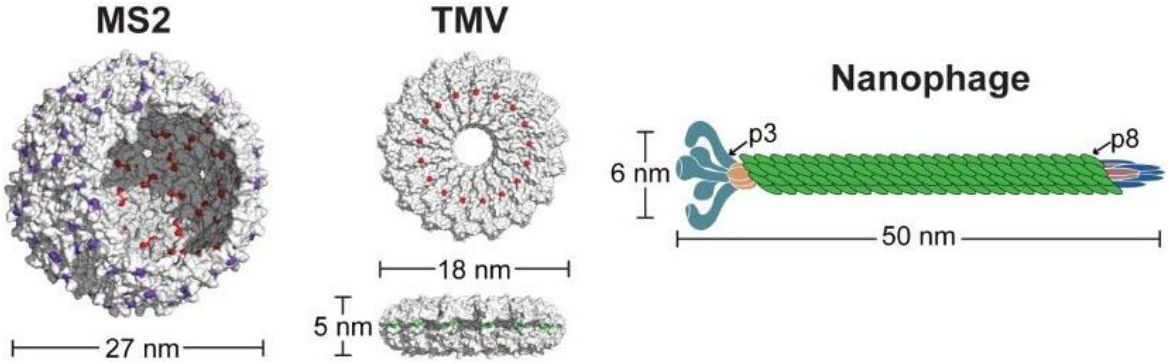

Figure 6. Three virus-like particles which are MS2 spheres, TMV disks and nano-phage filamentous rods are evaluated drug delivery efficacies

VLPs can also be combined with apoptosis enzymes to kill malignant cells. And they have better targeting effects than traditional chemotherapy as they target all rapidly dividing cells. Since VLPs

don't carry any infectious nucleic acids, viral genome integration can be prevented by utilizing enzymes with them (Figure7) [31].

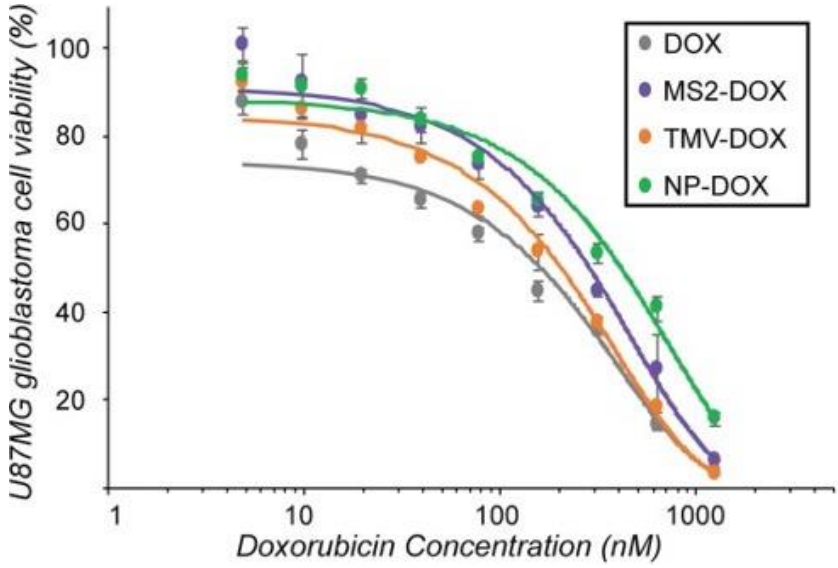

Figure 7. Delivery of doxorubicin to U87-MG cells through VLP delivery

There are 2 approaches to use enzymes for targeting cancer cells.

First, by altering a natural intracellular target like ricin, some enzymes can cause apoptosis. This can catalyze ribosylation of elongation factor 2 and cytolytic fusion protein for apoptosis [18,32]. Many induced apoptosis enzymes have been tested but only carboxypeptidase was tested in clinical trials (ADEPT).

Also, we can deliver DNA to construct apoptosis enzymes *in vivo*. Gene-directed enzyme prodrug treatment (GDEPT) and virus-directed enzyme prodrug therapy can be used to achieve this (VDEPT). Among two approaches, the most commonly used sequence is Herpes Simplex Virus 1 thymidine kinase (HSV1-TK) gene. If 41 kDa HSV-TK are exposed to cancerous cells, it can change prodrug ganciclovir into monophosphate derivative and then triphosphate by endogenous kinases. Neighboring cells can be killed by the diffusion effect of phosphorylated drugs through gap junctions.

According to Del Valle and Pia-Oviedo, JCPyV virus is an opportunistic infection agent of Progressive Multifocal Leukoencephalopathy (PML) and is present in severe immunosuppressive diseases like AIDS [33]. VP1, VP2 and VP3 are the main protein components in JC virus and VP1 is caused by receptor binding and the virus's outermost coating. In terms of structure, hemagglutination activity, and the capacity to infect cells and splice viral genetic material into nucleus DNA, JCPyV VP1 are equivalent. Additionally, research has demonstrated that JCPyV can cause a variety of brain tumors, including oligoastrocytomas, medulloblastomas, and glioblastomas. In glioblastoma malignancy, MCPyV early DNA sequence was discovered, and the viral early protein T-antigen was also discovered in the nucleus. The thymidine kinase suicide gene is produced when the Herpes Simplex virus (HSVtk) gene and the prodrug Ganciclovir are coupled (GCV). These demonstrate that using JCPyV as a VLP, it is possible to deliver the thymidine kinase suicide gene for the therapy of glioblastoma.

JCPyV VLPs carrying the gene for the green fluorescent protein (GFP) were transduced into U87-MG cells. After 72 hours, observe the results under microscopes. Control group is used with VLPs without tk and GFP genes. U87-MG cells can either be transduced by gfp-VLPs or control VLPs. The GFP was visualized through a fluorescence microscope after 72 hours (Figure 8) [34].

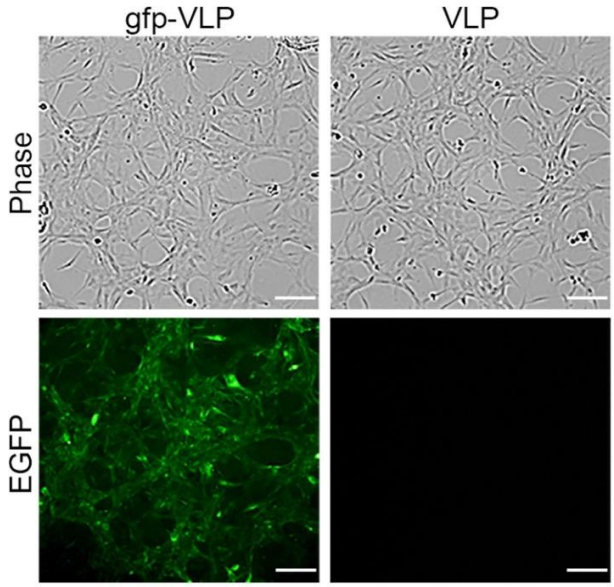

Figure 8. Transduction of the EGFP gene into U87 cells through JCPyV VLPs

After the in-vitro test, in vivo pre-mixed tests, which can increase the interaction between U87 cells and tk-VLPs, in nude mice are used to examine the drug delivery efficacy of VLP in an orthotopic animal model. iRFP, a sensitive marker for tumor development expressed by U87-L-iRFP cells, is utilized to employ fluorescence to examine the progress of a tumor. Either U87 cells combined with tk-VLPs and GCV are given to the mice, or they are given U87 cells with empty VLPs as the control group. The nude mice are implanted with U87-L-iRFP cells, and one week later, tk-VLPs are administered. The experimental group receives 2 doses of tk-VLPs with GCV on days 7 and 18, whereas the control group receives PBS/GCV or tk-VLPs/PBS. After day 26, there is less tumor visible in the iRFP fluorescence test when comparing the experimental group to the control group. In the brain slice, tumors are still visible, and ex vivo FMT scans and a confocal microscope both identified iRFP fluorescence (Figure 8).

In order to examine the efficacy of using JCPyV VLPs in mammals' circulatory system, whether VLPs are able to protect tk genes and finally reach U87-MG cells and express them, tail vein injection in U87-MG expressed mice was done. In the histopathological results, only subcutaneous U87-MG tumors were able to express GCV. In addition, in tk-VLP/GCV groups, the progression of subcutaneous U87 tumors was decreased. The mean weight of 3 groups didn't show any difference (Figure 9). It is rational to suggest that JCPyV VLPs are able to protect the tk genes in the circulatory system and produce therapeutic effects while non-TK-expressing VLPs have no effect.

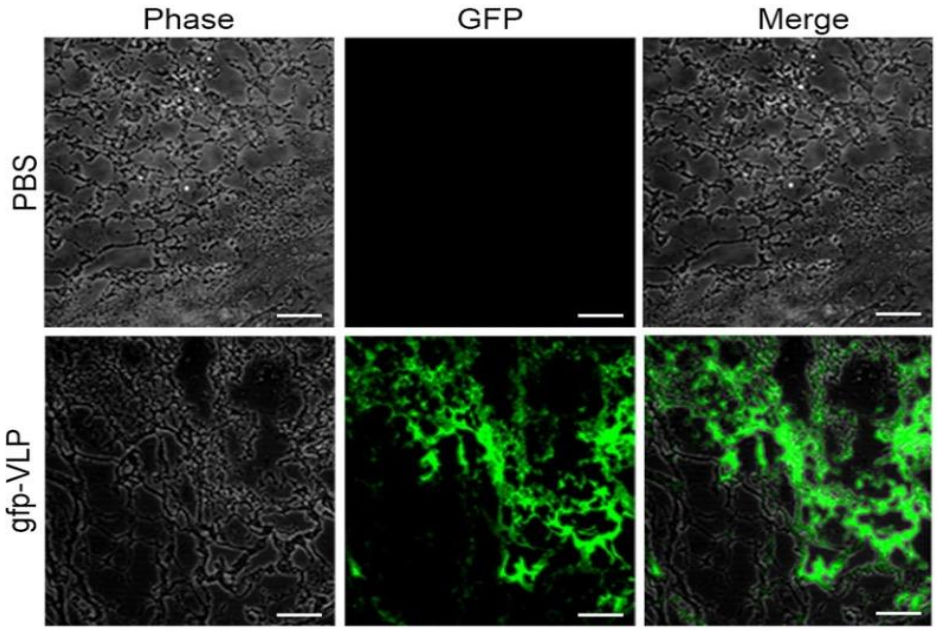

Figure 9. GFP-VLP and PBS were injected to nude mice with subcutaneous U87-MG cells

4. Conclusions

The mRNA vaccination offers a novel and unconventional method for treating gMB. Such vaccines can accomplish precise, safe, and well-regulated treatments that are not possible with other treatments by choosing the right vehicles to carry mRNA against pathogens, such as particular receivers or special cells. In general, LNP-based mRNA vaccines are a great way to combine medicine and material science, improve communication between different fields, and incorporate various cutting-edge methods. The experiments that were mentioned, whether they involved human or animal subjects, all produced encouraging results with hardly any negative or unforeseen outcomes. GMB is a very deadly but uncommon cancer, so the investment in this is unfortunate as more human experiments on the use of mRNA vaccine in this type of cancer are still lacking.

The experiments that were mentioned, whether they involved human or animal subjects, all produced encouraging results with hardly any negative or unforeseen outcomes. Unfortunately, the application of mRNA vaccine in this type of cancer still lacks more human experiments, not only because GMB is a very deadly but rare cancer, so the investment here is insufficient. Only one phase I experiment for the personized mRNA vaccine was carried out *in vitro* in 2010; the other two were not transferred from animals to humans for additional clinical use.

For the types of VLPs in glioblastoma application, TMV discs show the highest efficacy with low doses. More VLP based applications targeting cancerous cells could consider using TMV as a carrier. The pre-clinical research using JC virus with thymidine kinase suicide genes from Herpes simplex virus 1, shows the potential of using VLPs in combination with other medications to destroy cancerous cells. The control group receives PBS/GCV or VLP/PBS and the experimental group receives VLP/GCV for comparison. VLP based vaccines can be used in conjunction with other therapies including chemotherapy and checkpoint inhibitors to increase patients' median survival spans.

Therapeutic vaccines offer a novel immunotherapy for recurrence GBM patients. In this review, we only evaluate the pre-clinical trials on U87-MG cells and nude mice, more clinical trials can be done and more data can be conducted to fully illustrate the efficacy of TRIAL mRNA vaccines, DC vaccines and VLP vaccines. Hopefully through more available immunotherapy, the mortality rate of GBM could gradually decrease.

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