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Review: CRISPR-Augmented Oncolytic Virotherapy as an Effective Treatment of Metastatic Cancer

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Abstract: ***Oncolytic viruses (OVs) preferentially infect and induce lysis in cancer cells. They have been well tested as a monotherapy and in combination with other immunotherapies to treat isolated tumors. In comparison to other immunotherapies and current cancer treatments, oncolytic virotherapy offers an excellent safety profile, dual-action treatment, and potential for more targeted treatment with genetic modification. However, T-VEC, the only FDA-approved oncolytic virus drug, is limited in its application to other types of cancer by adverse effects and contraindications. In addition, the application of oncolytic virotherapy to treat metastatic cancer is under-discussed, despite its potential to improve the current poor prognosis. In this literature review, we will examine the role of CRISPR-Cas9 gene editing in creating more targeted, efficient oncolytic viruses and their clinical application to metastatic cancer.***

Keywords: cancer immunotherapy, oncolytic viruses, metastatic cancer

Research Question: How can CRISPR-Cas9 gene editing be utilized to augment the efficacy of oncolytic virotherapy in the treatment of metastatic malignant carcinomas?

1. Introduction

Despite advances in treatments, cancer remains one of the leading causes of death worldwide, accounting for one in every six mortalities [1]. Moreover, current treatments for cancer, such as radiotherapy, chemotherapy, and surgery, have severe side effects including weakened immunity, hair loss, extreme fatigue, and a generally lower quality of health. Even then, these treatments are limited in their effectiveness by the size, shape, and stage of the tumors [2]. For this reason, research into alternative therapies that provide a more holistic treatment option for advanced stages of cancer, while remaining economical, is a pertinent subject of interest in the world of biochemistry and oncology.

One such alternative therapy is immunotherapy. Cancer cells have adapted to disguise themselves from T-cells, suppress anti-tumor immune responses, and inhibit the body's innate immune system to improve their survival. These immunosuppressive features include T-cell suppressive cytokines released from the tumor microenvironment (TME), tumor-associated macrophages, and immune checkpoints expressed on the membrane surface of cancer cells. Immunotherapy uses drugs and treatments designed to circumnavigate these inhibitory mutations to enhance the immune system's ability to recognize and destroy cancer cells, optimizing a pre-existing, personalized defense system against cancer.

Immunotherapy's application to oncology can be traced back to 1893, when Dr. William Coley first propagated the idea of using "toxins" to stimulate the immune system, enhancing its innate ability to fight cancer. However, due to inconsistencies in his method and poor replicability of his results by other medical professionals, his work was largely rejected by the medical community. It wasn't until the mid-20th century when breakthroughs like the invention of the first-ever cancer vaccine and the discovery of T-cells were made, that immunotherapy was reintroduced as a viable treatment in the field of oncology.

In recent years, cancer immunotherapy has seen immense development with breakthroughs, especially in the field of oncolytic virotherapy with the FDA's approval of the first oncolytic virus drug, Imylgic [3]. Since then, research into expanding and improving the efficacy of immunotherapy and oncolytic virotherapy has only grown, with the application of CRISPR-Cas9-guided genetic modifications to tailor viruses that could redefine our current oncological approaches to the treatment and survival of patients with advanced, rare, or complicated forms of cancer. In this review, we will explore the development of oncolytic virotherapy and CRISPR-Cas9's role in enhancing its efficacy to treat metastatic cancers.

2. Current Cancer Immunotherapies for Metastatic Cancer

Cancer immunotherapy, however novel, has already seen considerable success in the treatment of a wide range of cancers. Among these immunotherapies are the most promising: immune checkpoint inhibitors, CAR T-cell therapy, macrophage-based therapy, and oncolytic virotherapy.

2.1 Immune Checkpoint Inhibitor Therapy

Immune checkpoints are normal features of non-cancerous to prevent a hyperactive immune response that harms healthy cells. However, the expression of immune checkpoints on cancer cells disables the T-cell's recognition of and action against cancer, weakening the body's anti-cancer immune response. Immune checkpoint inhibitor therapy uses molecules that block surface proteins like the programmed cell death protein 1 (PD-1), protein ligand 1 (PD-L1), or cytotoxic T lymphocyte-associated protein 4 (CTLA-4) to reverse T-cell anergy and allow a normal anti-tumor T-cell action to take place [4].

Immune checkpoint inhibitors have had immense success in the treatment of a wide range of cancers. A recent study conducted at the *Johns Hopkins Bloomberg-Kimmel Institute*

for Cancer Immunotherapy revealed that immune checkpoint inhibitors may be more effective than chemotherapy at treating an aggressive type of skin cancer, Merkel cell carcinoma [5]. 3 categories of inhibitors and 16 inhibitory drugs have been approved by the Food and Drug Administration (FDA), making immune checkpoint inhibitor therapy one of the most widely researched and accepted immunotherapies [6].

However, cases of hyper progressive disease, a condition that accelerates tumor growth and clinical deterioration [7], post-anti-PD-1 treatment [8], coupled with the novelty of the therapy, have introduced significant safety concerns, especially in the application of this treatment to pediatric oncology [9].

2.2 Macrophage-based Therapy

Macrophage-based immunotherapy follows the idea of manipulating the tumor microenvironment to overcome its immunosuppressive characteristics. Macrophages are a group of immune cells responsible for a multitude of homeostasis-related functions. In order to achieve these functions, macrophages undergo polarization—a process that is extremely sensitive to changes in the microenvironment. Typically, healthy macrophages are polarized to carry out vital roles in tissue development, wound healing, regulation of inflammatory responses, and elimination of pathogens [10].

However, when in the vicinity of the TME, a specific type of macrophage, "alternatively activated M2 macrophages", is polarized to form Tumor-Associated Macrophages (TAMs) that promote tumor progression by producing T-cell suppressing cytokines and upregulating angiogenesis within the TME. Macrophage-based therapy works by blocking a protein-ligand receptor on TAMs, thus disabling their immunosuppressive functions and allowing T-cells to detect and destroy tumor cells, as would naturally happen [11].

Macrophage-based therapy works effectively in combination with Immune-checkpoint inhibitors, but given the minimal research in this field and the risk of blocking non-tumor-associated macrophages and their functions vital to homeostasis, macrophage-based therapy remains less-favored immunotherapy to immune-checkpoint therapy or CAR T-cell therapy.

2.3 CAR T-cell Therapy

CAR T-cell therapy, or Chimeric Antigen Receptor Therapy, is a cell-based gene therapy where T-cells collected from a patient's blood are genetically modified to introduce antigen receptors (CARs) onto the T-cell surface membrane. The CAR T-cells are then multiplied, or "expanded", and are reintroduced into the patient. The expression of this Chimeric Antigen Receptor enhances the T-cell's recognition of cancer cell surface markers, thus aiding the immune system's identification and destruction of cancer cells [12].

CAR T-cell therapy has been most successful in the treatment of hematological malignancies, in cancers such as Leukemia, Lymphoma, and Myeloma. However, the replicability of this success in solid tumors has been poor, as the effector T-cells must penetrate the tumor, after which the acidic, immunosuppressive TME may prove to be unfavorable for the survival of the effector cell. In addition, solid tumor cells express tumor-associated antigens rather than unique cancer cell surface markers. Tumor-associated antigens are not specific to tumor cells, as they are also expressed in healthy, non-cancerous cells at lower concentrations. In two clinical trials with patients treated with the CAR T-cells Her2 and GD2 for Metastatic colorectal cancer & neuroblastoma [13], this non-specificity of the detectable surface marker caused on-target, off-tumor toxicity, a dangerous condition that caused a hyperactive immune response that threatened healthy cells as well [14].

CAR T-cell therapy, though it has seen success in blood and lymph cancers, is ineffective in treating advanced or tumor-localized cancers, making it a very specific immunotherapy with limited clinical application.

3. Oncolytic Virotherapy

Oncolytic virotherapy is a type of immunotherapy that is mediated by modified or wild-type viruses [15] that preferentially infect and kill cancer cells, through the process of oncolysis. The lysed cell will then release new virions, or infectious virus particles, that will infect other cells in the tumor, [16] making this process a self-sustaining treatment option. In addition, oncolytic viruses (OVs) are a subject of interest in their ability to trigger a T-cell-mediated immune response against the tumor, as some lysed cells will also release tumor-specific antigens [17] that allow the body's own immune system to identify the cancer cells and kill them. Thus, using a more holistic approach to cancer treatment, oncolytic virotherapy has a promising future in oncology.

3.1 Mechanisms of Oncolytic Virotherapy

Oncolytic viruses are a favored cancer treatment for their two-part action, allowing for widespread anti-cancer action.

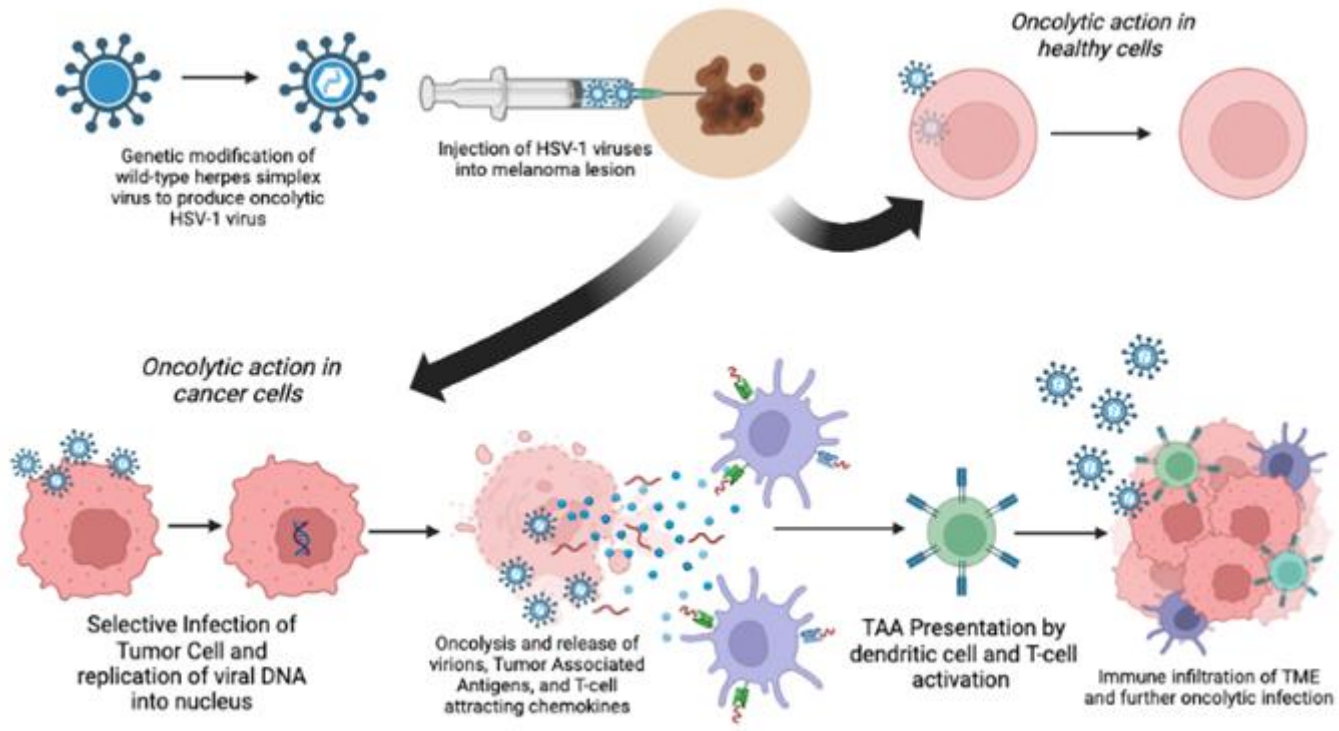

Figure 1: The mechanism of oncolytic virotherapy action of herpes simplex virus 1 in the treatment of inoperable melanomas. Oncolytic viruses replicate within healthy cells, causing cancer-cell lysis and the subsequent release of T-cell-attracting chemokines, tumor-associated antigens, and virions, which aids in T-cell recruitment to TME and a chain-reaction infection of the whole tumor. The dual action model of OVs allows for the treatment of uninfected tumors as well

The first part is a direct viral action inside the cancer cell. By replicating viral DNA into the cancer cell's DNA, the virus infects the cancer cell and begins its replication. The presence of OVs also stimulates some dysfunction of the endoplasmic reticulum and mitochondria, compromising the function of the cancer cell, and can stimulate oxidative stress that contributes to the stabilization of the TME [18], creating a more favorable environment for immune cells [19]. After millions of copies of the oncolytic virus are created within the cell, the cell undergoes oncolysis and bursts to release virions and tumor-associated antigens. These tumor-associated antigens are responsible for triggering the second part of the anti-tumor response.

The second part of oncolytic action is the stimulation of a secondary immune response that supports cell lysis. The viruses enhance the body's innate anti-cancer mechanisms through the release of key chemical markers during the oncolysis of tumor cells. Tumor-associated antigens are one such marker that helps the immune system recognize and target tumor cells, even uninfected ones. This secondary immune response makes oncolytic virotherapy an attractive treatment option for metastatic cancers, as it enables the simultaneous destruction of cells in metastasized cancers even outside of the treatment site.

4. CRISPR/Cas9 and Genetic Modification

CRISPR is a family of DNA sequences with repetitive bases, commonly found in bacteria and archaea. First applied in gene editing in 2017 by Japanese researchers, CRISPR has quickly become one of the most widely researched biotechnologies used in experimental treatments for diseases such as transthyretin amyloidosis, cystic fibrosis, and most relevant to this paper, cancer.

Genetic modification in the DNA exchange between organisms dates back to 1973 when biochemists Herbert Boyer and Stanley Cohen inserted the DNA of one bacteria into another. With this rudimentary version of gene editing came a possibility to create targeted changes to an organism's genome, altering and manipulating the function of that organism. Today, gene editing has evolved into numerous protein and RNA-guided processes, the most prominent being CRISPR.

CRISPR stands for "clusters of regularly interspaced short palindromic repeats", referring to the region of DNA commonly found in prokaryotic organisms of repeated nucleotide sequences with distinct, nonrepetitive "spacer" sequences in between. Researchers found that the spacers exactly match viral sequences, post-viral infection of the bacteria. It was then theorized that in a form of adaptive immunity, CRISPR-associated nucleases process and incorporate foreign viral and macrophage DNA as spacers into the CRISPR locus of host genomes [20]. In the event of future infections, spacers are used as transcriptional templates for producing RNA that guides endonucleases such as Cas9 in recognizing and cleaving segments of the viral DNA, thus disabling the virus and protecting the bacteria.

This method of selective DNA cleavage and translation proved to be of interest for modifying DNA sequences of

other organisms including multicellular eukaryotes such as rice and rats.

In its application to more complex gene editing, the CRISPR/Cas9 system works in the following manner: the Cas9 endonuclease first recognizes the protospacer adjacent motif (PAM) site, then, guided by the single guide RNA (sgRNA), creates a double-strand break in the target DNA sequence. Depending on the number of breaks produced, varied natural DNA repair processes take place, allowing for the insertion, deletion, and substitution of nucleotide sequences [21].

5. Engineered Oncolytic Viruses and a New Horizon of Treatments for Metastatic Cancers

The application of oncolytic viruses to small tumors and early-stage cancers has been widely discussed, but these cancers are already being treated by well-tested, highly-successful approaches like chemotherapy and even other forms of immunotherapy such as immune-checkpoint inhibitors.

Metastasic forms of cancer, on the other hand, are largely incurable by today's chemotherapies, immunotherapies, and other treatments. At this stage, the cancer has advanced into an aggressive, systemic form whose growth can only be slowed, resulting in low recovery rates and life expectancies for these patients.

The 5-year survival rate of metastatic malignant melanomas is a grim 5-19% [22]. For many patients with inoperable melanomas or cancers that have reached vital organs, the prognosis becomes about end-of-life care, with little hope for a cure. It's in this type of cancer that oncolytic virotherapy has the most potential—in patients where advanced cancers become too large or pervasive for effective chemotherapy and radiotherapy, oncolytic viruses have created hope for survival.

The first FDA-approved oncolytic virus, created from a weakened form of the Herpes Simplex Virus (HSV-1), has seen immense clinical success in the treatment of inoperable melanomas. This particular treatment, known as talimogene laherparepvec (T-VEC), is genetically modified to lose its natural neurovirulence [23]. Patients who were treated with the commercial form of T-VEC, Imylgic, saw a durable response in the first year and have seen a 95% reduction in the risk of death [24]. From the phase III clinical trials, T-VEC treatment saw a 67% [25] response rate and destroyed nearly half of all injected melanomas, including lesions that hadn't been treated, but was likely affected by a secondary immune response stimulated by Imylgic [26]. T-VEC's application in other forms of cancer is still being researched, including in locally recurrent breast cancer (NCT 02658812), liver metastases (NCT 02509507), and sarcomas (NCT 02453191).

However, with T-VEC, there is a risk of herpes infection in the patient and others in close contact with the treated melanoma lesion or fluids from the patient [27]. Symptoms of mouth sores, fevers, and chills are common side effects, associated with a mild herpetic infection in the patient.

In immunocompromised patients, the side effects of Imylgic treatment have not been sufficiently studied. Still, it is suggested that patients with weakened immune systems are at higher risk of disseminated herpetic infection, even after the completion of the treatment course. In a nude mice model, fatal disseminated viral infection was observed in 14% of mice treated with Imylgic [28].

Thus, congenital or acquired immunodeficiencies have been declared as contraindications for Imylgic treatment. This raises an important question: if T-VEC originates from a weakened, albeit pathogenic, virus and still poses a risk of infecting neighboring healthy cells, is oncolytic virotherapy as widely applicable as initially theorized? If oncolytic viruses still retain their virulence, they'd be ill-suited in the treatment of patients who have recently undergone chemotherapy, have cancers like leukemia or lymphoma, or are diagnosed with other conditions such as HIV/AIDS or common variable immunodeficiency [29], due to the threat of infection posed to the patient's immunocompromised body. This significantly limits oncolytic virotherapy's potential to treat metastasized cancers—that is if the replication potential of oncolytic viruses in healthy cells is not regulated.

5.1 Tropism-Modification to Genetically Engineer Selectively-replicative Viruses:

All oncolytic viruses are replication-competent, unlike replication-defective viruses that are commonly used in today's gene therapy. Especially in wild-type oncolytic viruses, this replication competence raises the concern of uncontrolled replication in both tumor and healthy cells, causing unintended damage to the surrounding tissue.

T-VEC's success in treating advanced melanomas has solidified oncolytic virotherapy as one of the only treatments with realistic potential in metastatic cancers. Given the high dosages required to treat metastatic cancers, it's important that the risk of uncontrolled replication in T-VEC is mitigated. Several studies have built on T-VEC to explore reducing the pathogenicity of HSV-1, by using CRISPR-Cas9 to target genes such as UL21, UL7, UL23, and most notably, UL39.

The HSV-1 UL39 gene encodes for ribonucleotide reductase (RR), a key enzyme in synthesizing deoxyribonucleotides, a building block for DNA replication. The enzyme is overexpressed in cancer cells to support their high replication rate. Hence, deleting the UL39 gene produces viruses with selective tumor-tropism in cancer cells that supplement the missing RR for the oncolytic virus to continue replication [30].

This principle of gene editing has seen success even when applied to other species of oncolytic viruses. In one study, native adenoviral promoters that initiated normal viral replication were replaced with a promoter region for genes overexpressed in cancer cells [31]. The resulting oncolytic

adenovirus displayed cytolytic activity that was confined to cancer cells, mitigating damage to healthy cells [32].
These promising results have revealed a more targeted approach to the creation and modification of oncolytic viruses. By designing oncolytic viruses that rely on the tumor overexpression of specific molecules to supplement deleted genes, the uncontrolled replication of cancer cells can be manipulated into its own oncolytic stimulant.

5.2 Clinical Application of Oncolytic Virotherapy to Metastatic Cancer

Patients with metastatic cancers are relatively under-researched in clinical contexts with oncolytic viruses due to their discouraging survival rates and relatively poor responses correlated with the aggressiveness of their cancer. The following are clinical trials researching metastatic cancers that have provided valuable insights on the application of OVs to more advanced forms of cancer.

In a 2010 clinical trial, 21 patients were treated with a single round of the oncolytic adenovirus ICOVIR-7 to treat advanced or metastatic solid tumors. 9 out of 17 patients saw objective anti-tumor activity. In radiological analyses, 5 out of 12 of the evaluable patients displayed stabilization or reduction of tumor growth. The treatment was relatively well tolerated, with only mild side effects of fever and hyponatremia [33].

Herpes Simplex Virus (HSV) is a commonly used oncolytic virus. In this pilot study, an HSV-1 was modified with the UL56 gene (a gene responsible for infection of the nervous system) deleted to improve its safety profile. This new HSV-1 mutant, HF10, saw between 30 and 100% cancer cell death in patients with recurrent metastatic breast cancer, with no adverse effects [34].

In another phase I trial of a clinical trial where the wild-type respiratory enteric orphan virus (reovirus), Pelareorep, was administered to 18 patients with advanced metastatic solid tumors, there was an overall response rate of 45%. All patients developed neutralizing antibody response, and a portion saw symptoms of viral shedding. In patients with viral shedding, the response rate was a more encouraging 67% [35]. There appeared to not be a maximum tolerated dose, meaning that the treatment was well tolerated with minimal side effects. This clinical study highlights the potential of wild-type viruses that don't require genetic modification to have promising anti-tumor activity.

This success has not been replicable in all clinical settings. A double-blind study using Seneca Valley wild-type oncolytic virus conducted by Schenck et al. had to be terminated for producing futile results [36]. The study used participants with extensive stage small cell lung carcinomas, where placebos displayed similar results of progression-free survival to those of treated patients. From this study, we can take away that the efficacy of oncolytic virotherapy in more extensive cancers is limited by the nature of the cancer, its proliferation, and the virus used. This particular study used a first generation Seneca Valley virus—one that had not been modified with exogenous genes to promote its efficacy. Perhaps using a genetically modified OV would result in more promising response rates. In addition, small cell lung carcinomas are an aggressive cancer type, so the weak Senecavirus was insufficient in fully killing cancer cells. The immunogenicity of the cancer defines its response to immunotherapy, and lung cancers are considered to possess a reasonable mutational burden, meaning that they would be ideal candidates if treated by a more potent oncolytic virus [37].

5.3 Considerations of Metastatic Cancer Treatment with OV:

In more localized forms of cancers, OVs can be administered intratumorally or as in the case with Imylgic, into the lesion itself. Given the extensivity of cancer in metastasis, the most effective method of administration would be systemically—directly into the patient's bloodstream or lymphatic system. However, with the systemic administration of OVs, possible obstacles arise.

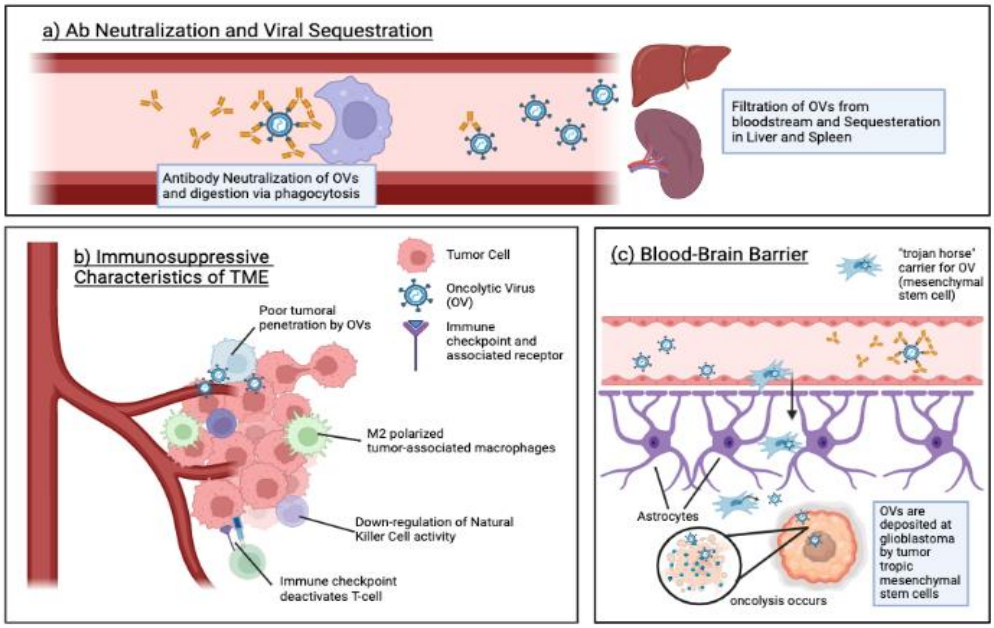

Figure 2: The obstacles of systemic oncolytic administration in the treatment of metastatic cancer. Antibody and complement neutralization causes an unwanted immune response against the treatment, sequestration of OVs in the liver and spleen reduces viral dose delivery to target tumors and could correspond to dose-limiting toxicity, and the blood-brain barrier poses a hurdle for treatment of brain metastases

5.3.1 Viral Sequestration in Liver and Spleen

Viral sequestration in the liver and spleen has been a common feature seen in the systemic administration of any virus, especially adenoviruses [38], a highly potent OV. The viral sequestration paradigm occurs in part due to the mononuclear phagocytic system in the liver and spleen [39]. In some cases, the systemically injected adenovirus took only minutes to clear the circulatory system and accumulate in the liver [40]. This can cause dose-limiting toxicities in the treatment of metastatic carcinomas, in the strain high concentrations of virus pose on the liver environment. A strategy to address viral sequestration would be the conjugation of the viral protein coat with polyethylene glycol (PEG), a biocompatible polymer that prolongs protein and liposome circulation. This method was shown to slow the clearance of adenovirus 5 from the bloodstream, improving its oncolytic activity post-IV administration [41].

5.3.2 Complement and Antibody Neutralization of Oncolytic Viruses

Oncolytic viruses are also susceptible to the body's natural immune response against pathogenic viruses such as complement or antibody-mediated neutralization. Neutralizing antibodies will bind to the capsid or membrane of the virus and prevent its entry into cells, including the target tumor cells for non-pathogenic oncolytic viruses. Neutralizing antibodies will also often signal white blood cell identification of pathogens, so protecting oncolytic viruses against neutralizing serum factors is necessary to avoid an immune response that would hinder the immunotherapy's progress. Researched methods to circumvent neutralization include alternative delivery of the oncolytic virus by immune effector cells such as macrophages or lymphocytes [42]. Another method of concealing the virus from neutralizing serum factors is through the use of "trojan horses" [43] or carrier stem cells that disguise the oncolytic virus from the immune system during systemic administration. Mesenchymal stem cells are ideal trojan horses for their tumor tropism [44]. This method is currently being investigated in two early-phase clinical trials for oncolytic adenovirus strains in the treatment of malignant gliomas and so far, has seen considerable response [45].

5.3.3 The Blood-Brain Barrier

Brain metastases are a delicate and dangerous form of metastatic cancer, with a median survival rate of 4.4 to 4.7 months post-diagnosis [46]. Despite medical advancements, the sensitivity and fragility of neural networks and brain tissue make prognosis poor for patients with brain metastases. Surgical procedures are difficult, radiotherapy has increased risk of impairing brain function, and chemotherapy is limited in its efficacy by the blood-brain barrier (BBB).

The blood-brain barrier (BBB) is a selectively-permeable network of blood vessels that vascularize the central nervous system. The BBB strictly regulates the movement of ions, molecules, and cells between the blood and the brain, ensuring that toxins and viruses remain outside the brain and spinal cord. As a result, the BBB poses a major hurdle in ensuring efficacious levels of drugs reach the brain metastases. This could potentially limit the viral load received by the brain tumor, especially in OVs such as T-VEC, where the HSV-1 is engineered to reduce its neurovirulence and subsequent risk of severe herpetic infection.

Viral penetrance into the central nervous system occurs most optimally in oncolytic viruses with natural tropism for neuronal tissue. Naturally neurovirulent oncolytic viruses such as the Semliki Forest virus [47], vaccinia virus [48], Mengovirus [49], and Seneca Valley virus-001 [50] have seen immense success in penetrating the BBB in both animal models and human trials. For viruses that struggle to pass the BBB, there exists the option of using mesenchymal stem cell trojan horses to carry the virus to target brain tumors, which is currently being explored by two clinical trials of malignant gliomas (NCT03896568) (NCT03072134).

5.3.4 Intra-tumoral Penetration of OVs:

As with CAR T-cell therapy, poor intra-tumoral penetration is a hindrance to the efficacy of oncolytic virotherapy. Due to the limited-replicative potential engineered into most oncolytic viruses, large tumors, especially ones metastasized into the brain or liver are most resistant to treatment by virotherapy due to their difficult vasculature and size. To overcome this, a study investigating genetic modifications that can be made to oncolytic viruses revealed that hyaluronidase expression—the expression of the enzyme responsible for the breakdown of hyaluronic acid—improved the efficacy of virotherapy and T-cell recruitment. In a xenograft rat melanoma model, engineering greater hyaluronidase expression into oncolytic adenoviruses improved the spread and speed of oncolytic degradation of the melanoma, when compared to the unmodified virus [51].

Immunotherapy also sees lower response rates in cold tumors such as pancreatic and ovarian tumors. Cold tumors are regarded as a therapeutic challenge for oncolytic virotherapy as they are nonimmunogenic—there is a paucity of T-cell infiltration into the TME and in the microenvironment surrounding cold tumors, there are regulatory T-cells and myeloid-derived suppressor cells, which further dampen any immune response [52]. In cold tumors, the most successful outcome of oncolytic virotherapy is when it is used in combination with other treatments, such as chemotherapy or radiotherapy.

6. Synergistic treatment of metastases with combination immunotherapy

6.1 Oncolytic Viruses and Conventional Cancer Treatments:

Resistance to conventional therapies is a significant dose-limiting factor. Cancer's high mutation rate often results in

new chemotherapy or radiotherapy-resistant cancer cells, whose treatment would require lethal doses of these treatments. Several pre-clinical trials have seen immense success in treating drug-resistant or radio-resistant cancer cells using a combination treatment approach with oncolytic virotherapy.

A combination treatment of chemotherapy and oncolytic virotherapy enhances induction of apoptosis in cancer cells. One study explored the use of an oncolytic adenovirus to enhance the therapeutic efficacy of gemcitabine, a popular chemotherapy drug used against pancreatic cancer, to treat pancreatic cancer that had mutated to become chemoresistant. In the pancreatic xenograft model, treatment with monotherapeutic gemcitabine exhibited low cytotoxicity towards cancer, whereas, when partnered with an oncolytic virus that was engineered to express the matrix-degrading protein relaxin, the chemotherapy-virotherapy combination saw potent anti-cancer effects in the induction of apoptosis [53].

Radiotherapy is used to break down inoperable tumors located in high-risk areas such as around a major blood vessel or in the brain/spinal cord. Yet, the therapy itself can often result in tumor recurrence due to radio-resistant cells and result in irreversible damage to stem cells, tissue damage, and acute radiation damage to highly-proliferative cells such as epithelial cells in the skin or digestive tract [54]. To avoid collateral damage to surrounding healthy tissue, one treatment takes advantage of the selective replication of oncolytic viruses in tumors to deliver radionuclides directly to cancer cells, making for more targeted radiotherapy [55]. One oncolytic vaccinia virus was genetically modified to express a membrane protein (the sodium iodide symporter) that drives cellular uptake of radionuclides. The trial resulted in increased cellular concentration of radioiodine and a more pronounced broad-spectrum antitumor response against prostate cancer [56] as compared to any isolated oncolytic virus or radioiodine treatment. Combination treatment opens up the scope for treating more cancers than just melanomas with oncolytic virotherapy.

6.2 Oncolytic Viruses and Other Immunotherapies:

Oncolytic virotherapy is also regarded as an attractive synergistic cancer immunotherapy for its successful use alongside other combination immunotherapies. OVs are advantageous in synergizing with immunotherapies as they help prime the TME for optimal function of other immunotherapies, for example by boosting T-cell recruitment to TME.

The therapeutic potential of OV and immune checkpoint inhibitor combination therapy has been verified by clinical data as well. Some types of cancer simply respond better to combination immunotherapies: Triple-Negative Breast Cancer appears more likely to respond to immunotherapy because of an increased mutational burden, and higher expression of PD-L1, that makes it an ideal candidate for immune checkpoint inhibitor-oncolytic virotherapy combination therapy [57]. In one phase II clinical trial where T-VEC and the anti-CTLA-4 blockade ipilimumab were used to treat previously unresectable stage IV melanomas, the combined therapy provided higher response rates (38% in combined v.s. 18% when just treated by ipilimumab) [58]. In addition, this combination therapy saw success in both localized tumors as well as metastatic cancers, likely due to the systemic reach of the immune response.

OVs induce immunological infiltration into the TME, making them an ideal pair for improving CAR-T cell therapy efficacy in solid tumors. For example, using a vaccinia virus vector to deliver the $CXCL_{11}$ chemokine directly into the tumor augmented CAR-T cell concentration in the solid tumor [59]. The immunosuppressive nature of TME could still be a roadblock to using CAR-T cell therapy to treat solid tumors, but even then, CAR-T and oncolytic virotherapy synergy can be used to address systemic metastases of the lymph or circulatory system using CAR-T cells as well as the original tumor with intratumoral administration of Ovs [60].

7. Comparative Evaluation of Oncolytic Virotherapy

Oncolytic virotherapy is regarded as a synergistic treatment for its strong success when used alongside other cancer treatments in the treatment of metastatic cancers. CRISPR-Cas9 can be used to improve the specificity of this immunotherapy in comparison to macrophage-based therapy, which often disables healthy macrophages too. Oncolytic viruses also have an excellent toxicity profile. When compared to other immunotherapy counterparts such as macrophage-based therapy and CAR-T cell therapy, oncolytic virotherapy is regarded as a relatively safer treatment, as collateral damage of non-cancer cells can be mitigated through genetic modification to create selectively replicating oncolytic viruses.

Immune checkpoint inhibitors work only enhancing by enhancing the immune system's natural function, so it is regarded as a more passive treatment of cancer. Conversely, oncolytic virotherapy utilizes a dual-action approach that involves the direct destruction of cancer cells as well as boosting the immune system's response—a more widespread approach than most other immunotherapies [61]. Oncolytic virotherapy also offers an alternative mechanism of cytotoxicity, helping address cancer cells resistant to current treatments.

Though the efficacy of oncolytic virotherapy is limited by the size of the tumor, due to poor tumoral penetration from the restricted replication potential of OVs, location of the tumor, such as in the brain, and natural immune responses against viruses, these limitations can be addressed through specific modifications or the use of carrier cells. The most evident limitation of this immunotherapy is its novelty. There is still scope for research in areas of pediatric use, use in immunocompromised patients, and even in metastatic cancers, that could reveal new facets of this therapy that improve our understanding of oncology.

8. Conclusion

Oncolytic viruses are a promising new field of cancer immunotherapy. Its success in brain metastases, breast cancer metastases, and advanced melanomas warrant further exploration of its application to other metastatic, inoperable, and advanced-stage cancers as well as in pediatric and other cases. Oncolytic virotherapy sees the greatest results when used in combination with other immunotherapies and current cancer treatments and can be used to enhance the efficacy of immunotherapies in nonimmunogenic cancers.

Gaps still exist in our understanding of barriers to the application of oncolytic viruses in immunocompromised patients and rare, complicated forms of metastatic cancer, but CRISPR has opened a whole new spectrum of prospective OVs. Wild-type OVs can possess modified tropisms, barriers to tumor penetration and limited viral dissemination can be overcome, and pathogenic viruses can be edited into new oncolytic viruses. With this cost-effective CRISPR-guided gene editing, the field of oncolytic virotherapy will become a more targeted, effective treatment option for cancer, with minimal side effects.

Oncolytic virotherapy, as with cancer immunotherapy, is still a very novel field in oncology. With success that is still barely comparable to that of well-tested alternatives such as chemotherapy, the justification for investing in the extensive research still required for immunotherapy lies in its application to metastatic cancer. In a field with little progress from current treatment options, oncolytic virotherapy stands to distinguish itself as the future of cancer treatment, creating hope for better survival rates and increased longevity in a previously grim disease.

9. Conflict of Interest

The researcher claims no conflict of interest as there were no affiliations with any organizations that may have had a stake in the outcome of this review article.

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