COLUMBIA UNDERGRADUATE SCIENCE JOURNAL

Recent Progress in Engineered Oncolytic Viruses: A Thematic Review

Nihal Manjila[1]

[1]Department of Biology, Case Western Reserve University, Cleveland, OH, USA

KEYWORDS: Cancer Therapy, Oncolytic Viruses, Medicine, Oncology

ABSTRACT: This review examines recent research in engineered oncolytic viruses and applies a thematic analysis to identify trends in the field. The major themes identified are tumor microenvironment remodeling, enhancing virus efficiency, treating slow-growing tumors, tumor targeting specificity, and combination with chimeric antigen receptor-T cell therapy. The tumor microenvironment plays a significant role in cancer progression through factors like immunosuppression and hypoxia. Engineered oncolytic viruses may be used to remodel aspects of the tumor microenvironment to favor and facilitate an immune response. Another major consideration in the development of oncolytic viruses is their efficiency in inducing antitumor effects. Viral vectors may be engineered with pro-apoptotic or pro-inflammatory signaling molecules to cause cancer cell death or mediate immune cell infiltration, respectively. Slow-growing tumors present a challenge to oncolytic virotherapy since many viruses infiltrate tumors through infected daughter cells arising from cancer cell division. This challenge may be overcome by engineering viruses to maintain a high viral load in infected cancer cells and drawing a sustained immune response. A concern in the development of oncolytic viruses is the issue of viral tropism and infection specificity. Although some viruses have limited tropism, oncolytic viruses may be engineered to specifically target tumors using cancer-specific receptor-ligand mechanisms. Oncolytic viruses may also be used in conjunction with chimeric antigen receptor-T cell therapy to reduce immunosuppression and enhance chimeric antigen receptor-T cell infiltration of tumors. This poses a promising approach in oncology research and treatment.

INTRODUCTION

Cancer is a class of diseases characterized by the uncontrolled proliferation of cells in the body. Affecting approximately 20 million people globally, cancer has one of the highest mortality rates with 10 million deaths per year, posing as a significant issue in the medical field. Cancer incidence is exacerbated by the lack of non-invasive and side effect-free treatment options. While treatments like chemotherapy may be effective at combating cancer, the side effects take a toll on patients.[1] There are various types of cancer, each with its own genetic causes and clinical manifestations, which makes developing treatments more difficult, as an approach that is effective against one type of cancer may not be effective against another type. For this reason, more therapeutics for cancer are vital.

Engineered oncolytic viruses (OVs) are viral vectors designed to damage and destroy cancer cells. OVs can be developed using a number of different theoretical approaches and experimental modifications. Once OVs have been engineered and undergo basic testing, they can advance to animal models and clinical trials. In the past five years, there has been an increasing interest in OVs due to the treatment method's novelty and comparative safety, which has led researchers to perform studies taking different approaches to advancing the knowledge and repertoire of effective OVs for clinical application.[2] It is useful to review recent advances in the development of engineered OVs to determine contemporary research trends. This analysis can be used

to make predictions about possible directions that OV research may take in the future.

In this review, a total of 42 primary research articles were analyzed. The majority of analyzed articles involve the engineering and testing of OVs, but clinical trials and theory papers are also included. Among the virus engineering articles, herpes simplex virus (HSV) was the most represented (Table 1). This is due to the advantages that HSV possesses as a vector: it does not integrate into the host genome since it is a double-stranded DNA virus and possesses a large genome that can serve as a stable base for genetic modifications. Adenovirus and vaccinia virus were also well represented, with approximately half the number of articles as herpes simplex virus. Several other viruses were also utilized in the analyzed studies, including poliovirus and bacteriophage T7.

Viral Vector	Number of Studies
Herpes simplex virus	15
Adenovirus	9
Vaccinia virus	7
Vesicular stomatitis virus	3
Newcastle disease virus	2
Reovirus	1
Measles virus	1
Zika virus	1
Maraba virus	1
Poliovirus	1
Myxovirus	1
Bacteriophage T7	1

*A number of the viruses counted were one of multiple viruses used in a single study.

Table 1. Viral vectors represented in analyzed studies

Five major themes or approaches were identified in the included studies. First, remodeling of the tumor microenvironment led to improvement in immune cell infiltration. This approach allowed the immune system to target and destroy cancer cells with its activated response. Second, additional modification of engineered OVs further improved their antitumor effect. This strategy utilized the variety of OVs already designed by researchers and advanced their work. Third, specialized OVs showed antitumor effects in the treatment of slow-growing tumors. Viral replication or antitumor effects that relied on cancer cell division was ineffective in slow-growing tumors. Fourth, targeting of cancer cells by viruses or immune cells were improved using signaling molecules. Increasing infection specificity improved the effectiveness of OVs, as well as reducing the severity of potential side effects. Fifth, oncolytic virotherapy in conjunction with chimeric antigen receptor-T (CAR-T) cell therapy shows improved antitumor effectiveness. The theme of combinatorial therapy can be applied to other treatments as well, with the hope that the anti-tumor effect will be greater than either treatment alone.

Tumor Microenvironment Remodeling:

When cancer cells accumulate and form tumors, they create a local environment that decreases the effectiveness of the immune system in infiltrating the tumor and destroying the cancer cells.[3] One of the primary effects of this tumor microenvironment is the prevention of natural killer (NK) cells from infiltrating the tumor mass. NK cells are one of the immune system's major cell types, and they can target and destroy cancer cells. In a healthy individual, NK cells will identify and eliminate any abnormally dividing cells. In an individual with cancer, NK cells are unable to overcome the cancer cells. Other components of the immune activity may also become inhibited, allowing cancer cells to proliferate and cause metastasis. Therefore, remodeling and modifying the tumor microenvironment to facilitate targeting of tumor cells by NK cells could be a design goal for engineered OVs. Currently, there is a well-known engineered OV on the market known as talimogene laherparepvec (T-VEC), a clinical treatment that utilizes an engineered herpes simplex virus to combat cancer. It was thought that T-VEC

functioned through direct oncolysis, or the virus infecting cancer cells and causing them to die. Ramelyte and colleagues showed that T-VEC does not directly cause oncolysis after conducting a phase I clinical trial of cutaneous B cell lymphoma patients. Instead, research found that the treatment alters the tumor microenvironment and allows the patient's immune cells to infiltrate and attack the tumor. The researchers examined tumor appearance, sequenced cell genetics, and identified immune cells present in biopsy samples. They confirmed that the treatment reduced tumor scope, both in the tumor at the injection site and in distant tumors. They also found that multiple types of immune cells began to infiltrate the primary tumor as the treatment progressed. Lastly, they found that the viral genetic script was present in both cancer cells and healthy cells, indicating a nonspecific infection mechanism.[4]

These findings provide key insights into the significance of the tumor microenvironment and viral design in engineering effective OVs. Ramelyte and colleagues demonstrated that it was not necessary for an OV to directly destroy cancer cells. Instead, the OV could modify the tumor microenvironment and allow the immune response to take effect. This provides a mechanism for engineering OVs that would not infect healthy cells to a significant degree. One of the major strengths of OVs and modification of the tumor microenvironment is the specificity and efficacy that can be achieved relative to more traditional treatment approaches. Additionally, the tumor microenvironment contains many cell types and active pathways that can be investigated and targeted by future studies, potentially showing anti-tumor effectiveness that further improves on preexisting OVs or targets other cancer types.

The microenvironment remodeling approach has been utilized by a number of research groups addressing various pathways and cell types present in the tumor microenvironment. Cervera-Carrascon and colleagues developed an oncolytic adenovirus that causes infected tumor cells to release two key cytokines into the tumor microenvironment.[5] The cytokines signal local cytotoxic T cells to approach and target the tumor. The antitumor effectiveness of the adenovirus is enhanced by the addition of immune checkpoint inhibitor treatment that activates the immune response. Lin and colleagues engineered an oncolytic herpes virus that releases damage-associated signals to facilitate antigen presentation by dendritic cells and incite an immune response.[6] Rather than counteracting immunosuppressive signals, this approach utilized antigen presentation to incite infiltration of the tumor by immune cells. Gentile and colleagues created an oncolytic herpes virus that causes infected tumor cells to express adenosine deaminase, an enzyme that breaks down extracellular adenosine—an immunosuppressive signaling molecule.[7] The breakdown of adenosine in the tumor microenvironment allows local immune cells to become activated and attack the tumor cells.

Keshavarz and colleagues developed an oncolytic Newcastle disease virus transported by mesenchymal stem cells to deliver apoptosis signaling molecules to tumors.[8] Upon binding to the appropriate receptors in the tumor cells, the apoptosis signaling molecules will cause the cells to undergo cell death. Each of these studies used biochemical pathways as a mechanism of action for OVs to modify the signaling molecule repertoire present in the tumor microenvironment. Other researchers have built on the mechanism behind T-VEC's function, as in the case of the oncolytic herpes virus engineered by Haines and colleagues to not infect healthy cells but still trigger immune recognition of the tumor.[9] This is a significant advance in OV engineering as it improves on a virus in clinical use by increasing infection specificity.

The tumor microenvironment also includes several cell types that are normally found in a healthy body but may be present within a tumor and closely associated with cancer cells. Kurisu and colleagues used an oncolytic reovirus to target and destroy

tumor-associated fibroblast cells, which contribute to the immunosuppressive environment created by tumors.[10] The researchers also found that the OV did not harm healthy fibroblast cells, indicating a level of infection specificity. Infection specificity is important in this case since the OV directly causes fibroblasts, a cell type that also naturally exists in the body, to lyse and die. However, it poses potential risks and side effects since an OV with imprecise action could destroy healthy cell populations.

Another interesting method of approaching tumor microenvironment remodeling is to utilize a viral vector that would not normally infect human cells. Bacteriophages are viruses that infect bacterial cells instead of animal or plant cells. Like other classes of viruses, bacteriophages can also be genetically and structurally engineered. The challenge of using a bacteriophage as the vector for OV engineering is that it does not naturally infect cancer cells. Instead, they must be engineered to target and infect cancer cells through signaling or receptor interactions. Hwang and Myung engineered bacteriophage T7 to express a peptide that contributes to tumor growth inhibition and immune cell infiltration of tumors.[11] This result indicates that bacteriophages are viable bases for OV engineering and that additional studies could explore this class of viruses.

Remodeling of the tumor microenvironment holds massive potential for engineered OV research. T-VEC has already been implemented as a clinical treatment and has shown success in the clinical setting. Microenvironment remodeling also allows immune cells to infiltrate tumors and destroy cancer cells. This approach is generally safe since the direct cause of cancer cell death is the immune response. Additionally, there are a few signaling pathways that are upregulated in cancer cells but not in healthy cells. Upregulating or downregulating these pathways appropriately could disrupt the tumor microenvironment and reduce immune exclusion.

Enhancing Virus Efficiency

Engineered OVs are designed using one or more genetic and structural modifications to cause an antitumor effect. OVs with one or more such improvements can be further improved by making additional modifications that enhance efficacy without compromising safety. These modifications can take the form of increased levels of surface proteins or signaling molecules, as well as directly causing the death of cancer cells.[12] Enhancing modifications could also address different barriers to antitumor effectiveness, such as signaling from tumor cells or the immune system containing and destroying OV particles before they can take effect and destroy cancer cells. Some cancer types, such as acute myeloid leukemia, show limited susceptibility to OVs and require more effective viral vectors and active agents.

One molecule utilized by engineered OVs to cause an antitumor effect is tumor necrosis factor-related apoptosis-inducing ligand (TRAIL). This immune factor can be expressed on the surface of OVs and binds to TRAIL receptors on the surface of cancer cells to initiate a signaling pathway that causes cell death. Wang and colleagues aimed to enhance an engineered OV with additional TRAIL to cause a greater antitumor effect.[13] The researchers had previously engineered an OV to express TRAIL on its protein shell that showed antitumor activity. They recognized that additional TRAIL could be bound to their OV through a structural attachment and experimentally achieved this through a zipper domain. This addition of TRAIL improved the antitumor effectiveness of the OV against acute myeloid leukemia when compared to the first iteration of their TRAIL-enhanced OV.

Certain types of cancer, including acute myeloid leukemia, may be resistant to the effects of TRAIL signaling. The mechanism behind this resistance is a reduction of TRAIL receptors in the cancer cells, leading to fewer opportunities for TRAIL on the OV to bind and initiate the signaling pathway. Due to the nature of this resistance

mechanism, increasing the availability of TRAIL is not sufficient to induce cell death in cancer cells. Ginsenosides, compounds derived from ginseng, were previously shown to increase the availability of TRAIL receptors in cancer cells.[13] Wang and colleagues investigated the role of the ginsenoside Rh (steroid glycosides) in amplifying the antitumor effect of their TRAIL-enhanced OV. They found that Rh activity led to greater expression of TRAIL receptors on the surface of cancer cells. In conjunction, the inclusion of Rh in the treatment course increased cancer cell death as TRAIL had more receptors to bind to. This demonstrates that OV enhancement can be further improved using synergistic treatment with other compounds, including natural compounds as in the case of ginsenosides.

Other research groups have taken the approach of enhancing viral efficiency by focusing efforts on different structural and genetic targets. Menotti and colleagues modified an engineered herpes simplex virus to target cancer cell-specific receptors by inserting different single chain antibodies into the viral protein structure.[14] This modification increased the number of cancer cells that can be targeted by the OV since different antibodies could be inserted, making that virus more useful across more cancer types. Kim and colleagues increased viral expression of interleukin-12 and granulocyte-macrophage colony-stimulating factor to amplify immune response.[15] More cancer cells will be destroyed if more immune cells are activated and localize to the tumor site. Rivadeneira and colleagues engineered an oncolytic vaccinia virus to express the adipokine leptin in infected tumor cells.[16] Furthermore, Leptin activates a signaling pathway that contributes to an increased T cell response and prolonged immune memory. These studies demonstrate the variety of methods that are available for enhancing OVs.

A major barrier to achieving greater viral efficiency is the containment and destruction of OVs by the human immune system. If the immune response is activated by the presence of viral particles in the body, the OV will not have any significant antitumor effect since the virus will not reach the tumor in sufficient quantity. Atasheva and colleagues addressed this issue through the development of an oncolytic adenovirus that avoids immune response and containment by mutating the region of the viral protein that host antibodies bind to.[17] The resulting OV was able to cause a systemic antitumor effect without being hindered by the immune response since antibodies could no longer bind to the virus. This study highlights the need for OVs engineered to avoid triggering an immune response from the host organism, particularly as OVs become increasingly effective at destroying tumors. In a similar manner, Bah and colleagues engineered an oncolytic measles virus to better avoid targeting by the immune system by replacing key surface glycoproteins with those from another virus.[18] This is another method by which OVs can be engineered to demonstrate stealth against the immune system.

While further laboratory studies are being performed, a few OVs have also moved into the clinical phase. Zhang and colleagues performed phase I and II clinical trials on the antitumor effectiveness of an oncolytic herpes simplex virus designed to express granulocyte-macrophage colony-stimulating factor, which enhances immune response.[19] The researchers found that the OV treatment was effective in metastatic, treatment-resistant esophageal and rectal cancer. The addition of an antibody for a tumor cell receptor that suppresses immune activity enhanced the oncolytic effectiveness of the virus. Side effects observed in the patients were relatively mild, and improved immune infiltration of the tumor was demonstrated. These results are significant because they show that OVs that are not effective against certain types of cancer may be enhanced to become effective. Fares and colleagues found that an oncolytic adenovirus showed antitumor effect in a phase I clinical trial of glioblastoma patients.[20] Glioblastoma is a very aggressive cancer type, and results indicating antitumor activity could be indicative of the potential for clinical

application after additional research is performed.

Enhancing preexisting OVs provides more effective therapeutics. Due to the recent interest in oncolytic virotherapy, many different OVs have been engineered with a few entering clinical trials.[12] These OVs that have already shown antitumor effectiveness could be made more effective through different engineering approaches and antitumor mechanisms. Therefore, future research is not limited to the development of entirely novel OVs. It may also potentially be more efficient to push enhanced OVs into clinical trials if the parent strain was already in clinical trials.

Treating Slow-Growing Tumors

Different cancer types have different pathophysiology and rates of metastasis. Tumors grow at different rates depending on the type of cancer, anti-cancer immune response, and other host-related factors. The growth of tumors are directly the result of cancer cell proliferation and accumulation. Engineered OVs are able to target tumors that grow quickly by spreading through tumors via daughter cells produced by cancer cell division.[21] Slower-growing tumors undergo cell division at a slower rate, and therefore OVs that primarily proliferate in a tumor through cell division are not as effective at combating the cancer cells. Additionally, given that slow-growing tumors usually take an extended period of time before becoming significantly dangerous to patients, there is more time to administer treatments, and treatments that could be very effective in the long-term may have more utility than those of the short term. Slow-growing tumors may also exhibit treatment resistance, which necessitates OV engineering that overcomes resistance mechanisms.

OVs require additional engineering and investigation to move towards the development of novel OVs that utilize mechanisms of action that are effective against slow-growing cancers. Fukuhara and colleagues sought to develop an OV that could be an effective anti-cancer agent in slow-growing tumors. They engineered an oncolytic herpes simplex virus known as T-hTERT which retains the infection specificity of its parent strain while preventing protein synthesis shutdown in infected cancer cells, leading to virus proliferation that does not damage healthy cells. The researchers found that the OV was effective against slow-growing tumors by maintaining a high viral load despite lower cell division rates.[22] Additionally, they demonstrated that the virus was safe for application in brain tissue, which is quite susceptible to impairment due to tissue damage. These findings indicate that OVs utilizing similar mechanisms may be suitable for clinical use in the treatment of slow-growing tumors. OV effectiveness is shown to not be limited to fast-growing tumors and can be effective against slow-growing tumors given the necessary modifications. The researchers also showed that the OV is safe for healthy tissue and may not pose a significant risk to the host organism.

Other researchers have investigated the possibility of OVs that function effectively in slow-growing tumors or have the potential to be useful towards the development of such OVs. Cao and colleagues engineered an oncolytic vaccinia virus that draws macrophages towards infected tumor cells.[23] The involvement of another immune cell type increases the number of possible OV mechanisms that could be utilized by researchers. Liu and colleagues engineered an oncolytic herpes simplex virus to express cytosine deaminase for the treatment of uveal melanoma.[24] This OV was designed to treat a rare and chemotherapy-resistant cancer type, demonstrating the need for OVs for uncommon cancers. Khalique and colleagues created an oncolytic herpes virus that targets immunosuppressive tumors while simultaneously drawing an immune response to cancer cells.[25] Multi-function OVs could be effective against slow-growing tumors, particularly if the tumors grow at higher or lower rates over a period of time. Leoni and colleagues developed a non-cancer cell line for the development of OVs.[26] This cell line could be a useful tool in the engineering of more OVs, particularly OVs designed for slow-growing cancers.

Some research groups have worked towards the development of anti-cancer vaccines which enhance long-term, antitumor immune activity through the activity of viral vectors. Roy and colleagues furthered the approach of engineering OVs that do not rely on division to be effective. The researchers developed an anti-cancer vaccine utilizing a number of viral vectors with antigenic peptides. The separation of these peptide components allowed for anti-cancer immunity enhancement without requiring cell division.[27] Tian and colleagues also developed an anti-cancer vaccine using a herpes simplex virus to carry a monoclonal antibody.[28] This anti-cancer vaccine also downregulates cell adhesion, reducing the ability of a tumor to exclude immune cells. Priming the immune system to combat cancer cells has significant potential in reducing cancer incidence at an earlier stage of disease development. Additionally, increasing the natural immune response to cancer in individuals with a predisposition to cancer development could improve patient outcomes.

Improving immune memory through increasing memory cell counts or extending memory cell survival also contributes to viral effectiveness against slow-growing tumors. As a tumor grows, the immune memory of that cancer type could trigger a response and draw immune cells to the tumor. Thomas and colleagues developed an oncolytic herpes simplex virus that improves immune memory after responding to tumor cells.[29] The researchers achieved this by identifying a virus that demonstrated antitumor activity and expressed a truncated envelope glycoprotein in the virus. They found that the antitumor activity of this OV leads to the activation of signaling molecules that cause immune memory to form via memory cells.

Slow-growing tumors have gained interest as their low division rate causes OVs that rely on cell division to not be as effective. While this is a significant engineering challenge, recent studies have shown that there are still methods of overcoming or circumventing the low division rate. OVs that create high viral loads without quickly lysing cancer cells can gain the most benefit from each cell division in a slow-growing tumor. Progress has also been made on the development of research tools and early-stage mechanisms for treating slow-growing tumors. Anti-cancer vaccines have the potential to develop into clinical treatments. Enhancing immune memory via OV action also holds potential for treating slow-growing tumors through a natural, long-term immune amplification.

Tumor Targeting Specificity

As demonstrated by T-VEC, there is a need to improve the targeting specificity of OVs to infect tumor cells primarily or almost exclusively. This could effectively reduce any possible systemic effect of OV therapy and improve patient safety. One approach that has shown significant potential in facilitating improvements in infection specificity is the utilization of biochemical signaling pathway components as markers for OVs. Biochemical signaling pathways play a significant role in the regulation of tissue maintenance and cell division. Any imbalance or dysregulation in these signaling pathways can lead to the development of cancer. Ligand-receptor binding interactions also provide an opportunity for improving tumor targeting specificity by taking advantage of a signaling pathway that is dysregulated to facilitate localization by OVs.[30] There are many pathways that have the potential to be utilized as the basis of a tumor-targeting system.

One such ligand-receptor interaction that has been studied extensively is that between C-C chemokine ligand 5 (CCL5) and C-C chemokine receptor type 5 (CCR5). In many types of cancer, the CCL5/CCR5 ligand-receptor interaction functions improperly and contributes to tumor growth and metastasis.[31] Li and colleagues developed a pair of OVs that upregulate CCL5 in infected tumor cells and upregulate CCR5 expression in NK cells, respectively. Each of these components individually increased antitumor effectiveness, and the combined treatment system was more effective than either

component alone. The result of this bilateral oncolytic virotherapy was the establishment of a tumor-targeting system which led to an improved antitumor effect. NK cells were also more effectively able to target and destroy the corresponding cancer cells.[32] Additionally, the OV engineered to cause CCL5 expression did not infect NK cells in significant numbers. This is an important finding since NK cells with CCR5 expression would be drawn to NK cells with CCL5 expression, rather than the tumor cells.

Researchers have explored additional avenues to improve target specificity. Vijayakumar and colleagues engineered an oncolytic Newcastle disease virus to deliver immunotherapeutic molecules to tumors, eliciting a targeted antitumor response.[33] Zuo and colleagues developed an oncolytic vaccinia virus that encodes for a single-chain variable fragment that targets immune cells.[34] Wang and colleagues created an oncolytic vaccinia virus that inhibits PD-1, an immunosuppressive signaling molecule, and elicits a cancer-specific immune response.[35] Wu and colleagues engineered an oncolytic vesicular stomatitis virus that also inhibits PD-1, causing an antitumor effect.[36] Walton and colleagues developed an oncolytic poliovirus that proliferates rapidly, evades immune response, and is cytotoxic to infected cancer cells to treat glioblastoma, an aggressive brain cancer.[37] These studies utilize different structural interactions and signaling pathways to effectively target tumors and combat cancer cells.

Triple-negative breast cancer is very aggressive and difficult to treat. OVs engineered to take advantage of signaling pathways specific to triple-negative breast cancer can demonstrate infection specificity while also being more effective. Rodriguez Stewart and colleagues developed an oncolytic reovirus that activated the caspase-mediated apoptosis signaling pathway in a novel manner.[38] The caspase pathway involves a series of caspase signals that end with caspase-3, triggering apoptosis of the cell. The researchers found that the caspase pathway was activated but caspase-3 was not, indicating that some other signaling mechanism was responsible for apoptosis of tumor cells.[38] This difference in signaling pathway activity may explain why the tumor cells were affected with specificity while also causing apoptosis. Additionally, this study indicates that variations of previously understood signaling pathways may potentially facilitate the discovery or design of other OVs with specific tumor targeting and antitumor effectiveness.

Other types of cancer, such as pancreatic ductal adenocarcinoma (PDAC), have also demonstrated resistance to treatment. Seegers and colleagues engineered an oncolytic vesicular stomatitis virus that effectively infects treatment-resistant PDAC cells due to enhanced viral attachment. The researchers used directed viral evolution to create mutations in the viral genes and identified two mutations that appeared in more than one evolved virus. OVs with these mutations were advanced to testing and demonstrated increased replication in treatment-resistant PDAC cells.[39] This finding indicates that directed evolution could be used to identify other beneficial mutations in OVs. Additionally, other treatment-resistant cancers could potentially be treated more effectively by OVs which are designed to utilize targeting mechanisms that are not involved with the cancer's resistance to treatment. An OV that relies on a different targeting mechanism could identify and infect cancer cells more effectively than an OV that relies on a mechanism which is made ineffective by a mutation in the cancer cells.

Significant improvements have been made in tumor targeting specificity by OVs and immune cells. This approach includes a number of different specific mechanisms that have been utilized to improve oncolytic virotherapy. OVs can infect cancer cells and cause the expression of different signaling molecules and other proteins on their surfaces that aid immune cells in identifying them. OVs can also be engineered to improve their infection specificity and more selectively infect cancer cells. Engineering of OVs can also be specialized to different cancer types in order to take advantage of

certain receptor-ligand interactions that may be upregulated in different cancers. This could lead to the development of an OV repertoire that demonstrates a significant degree of precision in its mechanism and cytotoxicity.

Oncolytic Viruses and CAR-T Cell Therapy

Certain types of cancer, particularly blood cancers, can be treated using chimeric antigen receptor T (CAR-T) cell therapy. This treatment utilizes immune cells taken from the patient and engineered to carry receptors that better allow them to target cancer cells. This method is beneficial because it relies on the patient's own cells and reduces the likelihood of severe side effects. The immunosuppressive nature of the tumor microenvironment decreases the efficiency of immune cell-based therapies in the same manner as it decreases the efficiency of natural immune responses. This means that CAR-T cell therapy could potentially be ineffective against tumor growths that express high levels of immunosuppression.[40] Combining CAR-T cell therapy with another form of treatment could potentially overcome the challenge of immunosuppression and improve the antitumor effectiveness of the therapeutic approach.

Oncolytic virotherapy can be utilized to counteract immunosuppression in the tumor microenvironment. After OVs have been implemented to cause the tumor region to become immunologically active, CAR-T cells can more easily infiltrate the tumor and cause cancer cell death. McKenna and colleagues developed a multi-component oncolytic therapeutic delivered by mesenchymal stromal cells combined with CAR-T cell therapy to achieve a greater antitumor effect. The first stage of this therapeutic strategy is the delivery of oncolytic viruses by the mesenchymal stromal cells. After the viruses have been delivered, they infect the tumor and reduce immunosuppression in the microenvironment. The final stage involves infiltration of the tumor by CAR-T cells and the death of cancer cells. This combined treatment was more effective than the engineered OVs alone.[41] The researchers also found that T cells were identified in larger numbers within the tumor mass. This result indicates that the immune response is amplified in addition to the CAR-T cell activity.

The concept of utilizing chimeric receptors and combination therapy for anti-cancer activity has been investigated by researchers developing OVs as well. Froechlich and colleagues engineered an OV targeted to mesothelin, a molecule expressed by cancer cells.[42] They achieved this by creating a chimeric receptor that included an immune component and the binding site for the cancer ligand target. Huang and colleagues developed an oncolytic adenovirus that contains a gene circuit for the control of viral replication after infecting cancer cells.[43] Simulation of the population dynamics of the OV revealed that controlling immune signaling at the tumor site would be more effective than combination therapy using an OV with immune signaling. This finding is interesting but was not experimentally determined to a sufficient degree.

Combination therapy using engineered OVs and CAR-T cell therapy can also be utilized to overcome the challenge of homogenous expression of CAR targets. Tumor cells and healthy cells both express certain CAR targets, which causes CAR-T cell activity to be nonspecific to tumors. Park and colleagues developed an OV that causes infected tumor cells to express truncated CD19 on their surface before lysing due to viral activity. CAR-T cells engineered with CD19 were drawn to infected cancer cells due to the binding mechanism of CD19. Native T cells were also signaled and drawn towards the tumor. These findings indicate that CAR-T cell activity can be made specific through the use of OVs to express chimeric antigen receptor targets in tumors.[44] The researchers concluded that they had developed a method of improving the tumor targeting of CAR-T cells using OVs and that this interaction had potential therapeutic applications in the future.

Combination therapy utilizing oncolytic

virotherapy and other cancer therapeutic strategies, such as CAR-T cell therapy, holds significant potential for improving patient outcomes. Multiple therapeutic strategies that can function effectively could have additive antitumor effects and prolong life or destroy tumors. Additionally, therapeutics that do not cause severe side effects could be implemented in the clinical setting without causing great discomfort or additional risks for patients. Currently, oncolytic viruses are entering clinical use and CAR-T cell therapy has been in use for treating patients suffering from blood cancers. It is reasonable that two clinically approved treatment strategies could be utilized as a combined treatment in the future.

CONCLUSION

Significant advances have been made in research on engineered oncolytic viruses in the last five years. Several studies have led to the development of novel OVs which have demonstrated antitumor effectiveness, including in vivo. Researchers have also taken many different approaches to developing engineered oncolytic viruses. Tumor microenvironment remodeling has been investigated as a method of improving immune cell infiltration of tumors. Genetic and structural modifications have been utilized to enhance the antitumor effectiveness of OVs. Specialized OVs have entered development for the treatment of slow-growing tumors and rare cancers. The targeting and infection of tumor cells has been improved through signaling and receptor upregulation. Oncolytic virotherapy is effective in combined treatment with CAR-T cell therapy. These approaches address a variety of issues and challenges in the development and refinement of OVs for potential clinical treatment.

Outside the direct development of OVs with genetic and structural modifications, researchers have approached the question of engineered OV implementation in a number of ways. Engineering experts have applied their expertise to the delivery of OVs, as in the application of control theory for the regulation of OV dosage.[45] Theoretical biologists have also approached the question of implementation through mathematical models, such as a Voronoi cell-based model for viral spread in tumors.[46] These types of studies contribute additional information towards the larger effort to optimize and implement OVs in the clinical setting. Some researchers have also published the specific methods they used to engineer OVs and quantify them, allowing others to pursue their own virus engineering projects.[47,48,49] Clinical trials in veterinary medicine are also being conducted, with the same types of cancers being treated in animals as in humans.[50] This variety of research approaches is additional evidence for the interest engineered OVs are gaining.

In conclusion, OVs demonstrate significant potential for treating cancers with efficacy and safety. OVs can be engineered to deliver signaling molecules to cancer cells, facilitate immune cell infiltration of tumors, and upregulate immune response to tumor growth. There are many viruses and viral modifications that are suitable candidates for engineering into OVs. Cancer treatment strategies such as chemotherapy often cause systemic symptoms and side effects that are very unpleasant for patients. These symptoms may be mitigated by OVs since the viruses can be engineered to selectively target tumors and utilize safe molecular components. As more OVs are engineered and move into clinical trials, there will likely be a greater number of OV therapeutics available for medical use in the next decade. The future of oncolytic virus research appears very promising as it provides hope for the development of meaningful cancer therapeutics.

AUTHOR INFORMATION

Corresponding Author

Nihal Manjila - nihal_manjila@hms.harvard.edu

ACKNOWLEDGEMENTS

The author expresses his gratitude to Professor Phoebe Stewart (Department of Pharmacology, Case Western Reserve University School of Medicine) for guidance and feedback in the preparation of the article.

ABBREVIATIONS

OV: Oncolytic virotherapy
HSV: Herpes simplex virus
CAR-T: Chimeric antigen receptor T
NK: Natural killer
T-VEC: Talimogene laherparepvec
TRAIL: Tumor necrosis factor-related apoptosis-inducing ligand
hTERT: Human telomerase reverse transcriptase
CCL5: Chemokine ligand 5
CCR5: Chemokine receptor type 5
PD1: Programmed cell death protein 1
PDAC: Pancreatic ductal adenocarcinoma
CD19: B-lymphocyte antigen CD19

REFERENCES

[1] O. Hemminki, J. M. Dos Santos, A. Hemminki, Oncolytic viruses for cancer immunotherapy. *J Hematol Oncol* 13, 84 (2020).

[2] P. K. Bommareddy, M. Shettigar, H. L. Kaufman, Integrating oncolytic viruses in combination cancer immunotherapy. *Nat Rev Immunol* 18, 498-513 (2018).

[3] E. Ylosmaki, V. Cerullo, Design and application of oncolytic viruses for cancer immunotherapy. *Curr Opin Biotechnol* 65, 25-36 (2020).

[4] E. Ramelyte *et al.*, Oncolytic virotherapy-mediated anti-tumor response: a single-cell perspective. *Cancer Cell* 39, 394-406 e394 (2021).

[5] V. Cervera-Carrascon *et al.*, Tumor microenvironment remodeling by an engineered oncolytic adenovirus results in improved outcome from PD-L1 inhibition. *Oncoimmunology* 9, 1761229 (2020).

[6] C. Lin *et al.*, Intratumoral Delivery of a PD-1-Blocking scFv Encoded in Oncolytic HSV-1 Promotes Antitumor Immunity and Synergizes with TIGIT Blockade. *Cancer Immunol Res* 8, 632-647 (2020).

[7] C. Gentile *et al.*, Generation of a Retargeted Oncolytic Herpes Virus Encoding Adenosine Deaminase for Tumor Adenosine Clearance. *Int J Mol Sci* 22, (2021).

[8] M. Keshavarz *et al.*, Oncolytic Newcastle disease virus delivered by Mesenchymal stem cells-engineered system enhances the therapeutic effects altering tumor microenvironment. *Virol J* 17, 64 (2020).

[9] B. B. Haines *et al.*, ONCR-177, an Oncolytic HSV-1 Designed to Potently Activate Systemic Antitumor Immunity. *Cancer Immunol Res* 9, 291-308 (2021).

[10] N. Kurisu *et al.*, Oncolytic reovirus-mediated killing of mouse cancer-associated fibroblasts. *Int J Pharm* 610, 121269 (2021).

[11] Y. J. Hwang, H. Myung, Engineered Bacteriophage T7 as a Potent Anticancer Agent in vivo. *Front Microbiol* 11, 491001 (2020).

[12] S. Chaurasiya, Y. Fong, S. G. Warner, Oncolytic Virotherapy for Cancer: Clinical Experience. *Biomedicines* 9, (2021).

[13] Z. Wang *et al.*, Enhancing the antitumor activity of an engineered TRAIL-coated oncolytic adenovirus for treating acute myeloid leukemia. *Signal Transduct Target Ther* 5, 40 (2020).

[14] L. Menotti *et al.*, HSV as A Platform for the Generation of Retargeted, Armed, and Reporter-Expressing Oncolytic Viruses. *Viruses* 10, (2018).

[15] K. J. Kim *et al.*, Antitumor effects of IL-12 and GM-CSF co-expressed in an engineered oncolytic HSV-1. *Gene Ther* 28, 186-198 (2021).

[16] D. B. Rivadeneira *et al.*, Oncolytic Viruses Engineered to Enforce Leptin Expression Reprogram Tumor-Infiltrating T Cell Metabolism and Promote Tumor Clearance. *Immunity* 51, 548-560 e544 (2019).

[17] S. Atasheva *et al.*, Systemic cancer therapy with engineered adenovirus that evades innate immunity. *Sci Transl Med* 12, (2020).

[18] E. S. Bah, R. A. Nace, K. W. Peng, M. A. Munoz-Alia, S. J. Russell, Retargeted and Stealth-Modified Oncolytic Measles Viruses for Systemic Cancer Therapy in Measles Immune Patients. *Mol Cancer Ther* 19, 2057-2067 (2020).

[19] B. Zhang *et al.*, Intratumoral OH2, an oncolytic herpes simplex virus 2, in patients with advanced solid tumors: a multicenter, phase I/II clinical trial. *J Immunother Cancer* 9, (2021).

[20] J. Fares *et al.*, Neural stem cell delivery of an oncolytic adenovirus in newly diagnosed malignant glioma: a first-in-human, phase 1, dose-escalation trial. *Lancet Oncol* 22, 1103-1114 (2021).

[21] M. Mondal, J. Guo, P. He, D. Zhou, Recent advances of oncolytic virus in cancer therapy. *Hum Vaccin Immunother* 16, 2389-2402 (2020).

[22] H. Fukuhara, Y. Takeshima, T. Todo, Triple-mutated oncolytic herpes virus for treating both fast- and slow-growing tumors. *Cancer Sci* 112, 3293-3301 (2021).

[23] F. Cao *et al.*, Engineering Oncolytic Vaccinia Virus to redirect Macrophages to Tumor Cells. *Adv Cell Gene Ther* 4, (2021).

[24] S. Liu *et al.*, Antitumor efficacy of oncolytic HSV-1 expressing cytosine deaminase is synergistically enhanced by DPD down-regulation and EMT inhibition in uveal melanoma xenograft. *Cancer Lett* 495, 123-134 (2020).

[25] H. Khalique *et al.*, Oncolytic herpesvirus expressing PD-L1 BiTE for cancer therapy: exploiting tumor immune suppression as an opportunity for targeted immunotherapy. *J Immunother Cancer* 9, (2021).

[26] V. Leoni *et al.*, A Strategy for Cultivation of Retargeted Oncolytic Herpes Simplex Viruses in Non-cancer Cells. *J Virol* 91, (2017).

[27] D. G. Roy *et al.*, Adjuvant oncolytic virotherapy for personalized anti-cancer vaccination. *Nat Commun* 12, 2626 (2021).

[28] C. Tian *et al.*, Enhanced anti-tumor response elicited by a novel oncolytic HSV-1 engineered with an anti-PD-1 antibody. *Cancer Lett* 518, 49-58 (2021).

[29] S. Thomas *et al.*, Development of a new fusion-enhanced oncolytic immunotherapy platform based on herpes simplex virus type 1. *J Immunother Cancer* 7, 214 (2019).

[30] M. M. Rahman, G. McFadden, Oncolytic Viruses: Newest Frontier for Cancer Immunotherapy. *Cancers (Basel)* 13, (2021).

[31] D. Aldinucci, C. Borghese, N. Casagrande, The CCL5/CCR5 Axis in Cancer Progression. *Cancers (Basel)* 12, (2020).

[32] F. Li *et al.*, CCL5-armed oncolytic virus augments CCR5-engineered NK cell infiltration and antitumor efficiency. *J Immunother Cancer* 8, (2020).

[33] G. Vijayakumar, S. McCroskery, P. Palese, Engineering Newcastle Disease Virus as an Oncolytic Vector for Intratumoral Delivery of Immune Checkpoint Inhibitors and Immunocytokines. *J Virol* 94, (2020).

[34] S. Zuo *et al.*, An engineered oncolytic vaccinia virus encoding a single-chain variable fragment against TIGIT induces effective antitumor immunity and synergizes with PD-1 or LAG-3 blockade. *J Immunother Cancer* 9, (2021).

[35] G. Wang *et al.*, An engineered oncolytic virus expressing PD-L1 inhibitors activates tumor neoantigen-specific T cell responses. *Nat Commun* 11, 1395 (2020).

[36] C. Wu, M. Wu, M. Liang, S. Xiong, C. Dong, A novel oncolytic virus engineered with PD-L1 scFv effectively inhibits

tumor growth in a mouse model. *Cell Mol Immunol* 16, 780-782 (2019).

[37] R. W. Walton, M. C. Brown, M. T. Sacco, M. Gromeier, Engineered Oncolytic Poliovirus PVSRIPO Subverts MDA5-Dependent Innate Immune Responses in Cancer Cells. *J Virol* 92, (2018).

[38] R. M. Rodriguez Stewart, V. Raghuram, J. T. L. Berry, G. N. Joshi, B. A. Mainou, Noncanonical Cell Death Induction by Reassortant Reovirus. *J Virol* 94, (2020).

[39] S. L. Seegers, C. Frasier, S. Greene, I. V. Nesmelova, V. Z. Grdzelishvili, Experimental Evolution Generates Novel Oncolytic Vesicular Stomatitis Viruses with Improved Replication in Virus-Resistant Pancreatic Cancer Cells. *J Virol* 94, (2020).

[40] R. C. Sterner, R. M. Sterner, CAR-T cell therapy: current limitations and potential strategies. *Blood Cancer J* 11, 69 (2021).

[41] M. K. McKenna *et al.*, Mesenchymal stromal cell delivery of oncolytic immunotherapy improves CAR-T cell antitumor activity. *Mol Ther* 29, 1808-1820 (2021).

[42] G. Froechlich *et al.*, Generation of a Novel Mesothelin-Targeted Oncolytic Herpes Virus and Implemented Strategies for Manufacturing. *Int J Mol Sci* 22, (2021).

[43] H. Huang *et al.*, Oncolytic adenovirus programmed by synthetic gene circuit for cancer immunotherapy. *Nat Commun* 10, 4801 (2019).

[44] A. K. Park *et al.*, Effective combination immunotherapy using oncolytic viruses to deliver CAR targets to solid tumors. *Sci Transl Med* 12, (2020).

[45] A. J. N. Anelone, M. F. Villa-Tamayo, P. S. Rivadeneira, Oncolytic virus therapy benefits from control theory. *R Soc Open Sci* 7, 200473 (2020).

[46] A. L. Jenner, F. Frascoli, A. C. F. Coster, P. S. Kim, Enhancing oncolytic virotherapy: Observations from a Voronoi Cell-Based model. *J Theor Biol* 485, 110052 (2020).

[47] F. Hamdan *et al.*, GAMER-Ad: a novel and rapid method for generating recombinant adenoviruses. *Mol Ther Methods Clin Dev* 20, 625-634 (2021).

[48] L. E. Torres-Dominguez, A. L. de Matos, M. M. Rahman, G. McFadden, Methods for the Construction of Recombinant Oncolytic Myxoma Viruses. *Methods Mol Biol* 2225, 63-75 (2021).

[49] R. E. Means, S. G. Roy, S. G. Katz, The Propagation and Quantification of Two Emerging Oncolytic Viruses: Vesicular Stomatitis (VSV) and Zika (ZIKV). *Methods Mol Biol* 2097, 253-263 (2020).

[50] N. B. Omar *et al.*, Safety and interim survival data after intracranial administration of M032, a genetically engineered oncolytic HSV-1 expressing IL-12, in pet dogs with sporadic gliomas. *Neurosurg Focus* 50, E5 (2021).