

Oncolytic Viruses: Cancer Treatment Going Viral

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ABSTRACT

Certain viruses have a preference for targeting and eliminating cancer cells throughout the course of their natural lytic infection cycles. These preferential properties can be natural, adapted, or engineered to further enhance the safety and efficacy of the oncolytic properties of the virus. Research into the therapeutic applications of oncolytic viruses (OVs) have shown promise as a cancer treatment, and a select few have been manufactured, approved, and marketed as drugs. However, the last comprehensive academic review of the field was published in 2017. With the quickly changing world of cancer research and the significant advancements in oncolytic virus research, it was appropriate to provide an updated review of the state of therapeutic research. Significantly more viruses have been studied and utilized as oncolytic agents, and the rise of combination therapies has increased the viability of OVs as a safe and efficacious treatment for various cancers. Additionally, new methods of viral delivery, beyond the traditional intravenous or local administration, have been developed for the purpose of avoiding detection of the oncolytic virus by the body's immune system. Taken together, the current research demonstrates that the study of oncolytic virotherapy goes far past simply injecting an oncolytic virus (genetically modified or not) and hoping for good results, as was the basis of previous research.

Introduction/Background

Cancer and its Prevalence

Despite decades of research, cancer remains the second leading cause of death in the United States after heart disease, the number one cause of death in certain states, and the second leading cause of death in children aged 1-14. It was estimated that in 2019 there would be more than 1,760,000 new cancer cases diagnosed, and of these cases 606,880 deaths were projected [1]. In addition to its prevalence in the population, cancer is a disease with a heavy financial burden not only in the case of the individual patient but also to the United States as a whole. The estimated overall annual costs for cancer are about \$107 billion. This is broken down into \$37 billion for direct medical costs, \$11 billion for morbidity costs, and \$59 billion for mortality [2]. Thus, the burden of disease for cancer is extremely high and set to spiral as the population ages and grows.

The challenge that cancer forces doctors and researchers to face is that “cancer” is a broadly applied term that encompasses more than 100 different diseases that develop over time and originate from the uncontrolled division of cells in the body. While all cancer types have a common basis in rapid, uncontrollable cell division, depending on where it develops and what type of cancer it is the features can be completely unique [2]. However, despite major differences in presentation and attributes, there are six hallmarks of cancer that are shared among all cancer types. Cancer, regardless of classification, must be able to sustain proliferative signaling, evade growth suppressors, activate invasion and metastasis, enable replicative immortality, induce angiogenesis, and resist cell death. In addition to these six, there are two characteristics referred to as “emerging hallmarks” which have recently come to more attention as indicators of pathogenesis as the body of cancer research has expanded. These are the dysregulation of cellular energetics and avoiding immune destruction [3]. Taken together these eight characteristics of cancer make it incredibly difficult to treat. However, in recent years there has been more research into using these universal hallmarks as targets for therapies that could be broadly applied to different cancers.

Oncolytic Viruses (OVs)

While surgery, radiation, and chemotherapy still dominate the area of applied cancer treatments, there continues to be a need for less toxic and/or more effective ways to attack cancer. Immunotherapy has emerged as a potential treatment for cancer by stimulating and utilizing the body's natural defenses. Targeted therapies including small molecule drugs and monoclonal antibodies have also been FDA approved for clinical usage [4]. Many of these treatments are used in combination with other therapeutic options, however, the need for novel therapies is still very much present due to complications such as side effects and drug resistance that limit the scope of current therapies.

Oncolytic viruses are genetically engineered, adapted, or naturally selective viruses that preferentially infect cancer cells and leave normal cells unaffected [5]. Viruses have been used in gene therapy cancer treatments as transgene carriers, but oncolytic virotherapy is different in that the virus itself is causing the death of the cancer cell through lytic action. Although in depth study into the field is a relatively new area of research, the idea that viruses could inhibit tumor growth and treat cancer has been around since the late nineteenth and early twentieth centuries [6-8]. From 1896-1898, George Dock documented a case of leukemia in one of his patients that seemed to improve when she was infected with influenza. The patient's abnormally high white blood cell count decreased, as did the swelling in her spleen and liver during her viral infection [7]. Observations of naturally acquired, yet sometimes deadly, viral infections including rabies and varicella [6, 9] that improved the health of cancer patients would continue to be documented well into the twentieth century. However, the first instance of laboratory research into oncolytic virotherapy emerged earlier in 1922 when Constantin Levaditi noted that vaccinia virus reduced tumor size in mice and rats [6]. Despite these observations, most remission and improvement lasted only a month or two after clearance of the infection. Dedicated research and clinical trials would not begin until around the 1950s when, in 1949, 22 patients suffering from Hodgkin's lymphoma were infected with viral hepatitis B [17]. Clinical trials would continue into the 1980's, but not much headway was made in controlling virulence and creating a successful therapeutic [5, 8]. Following these years of study, research into the use of oncolytic virotherapy seemed to fade into the background until 1991 when Martuza et. al published results of a thymidine kinase (TK) negative HSV-1 mutant that selectively targeted cancer cells and showed

promise for treating brain tumors [10]. This breakthrough in viral genetic modification for the purpose of oncolytic virotherapy led to resurgence of research into the field and increasingly successful attempts to produce a clinical therapeutic. This renewed interest and research paid off, and the first oncolytic virus to be approved for treatment was H101, an E1B- adenovirus approved in China for head and neck cancer in 2005 [11, 12].

However, some viruses need no genetic modification to preferentially target cancer cells as they seem to have a natural propensity for targeting them. An example is Reovirus, whose specificity for cancer cell targeting theoretically depends on defects in the Ras signaling pathway of these cells [5]. Despite some of these natural propensities, genetic modification of various OV's can improve efficacy, safety, and selectivity as well as code for immunostimulatory cytokines to enhance the effect of immune activation as a result of viral infection and cell lysis. For example, GM-CSF, a known DC recruiter and activator, is the most commonly OV-encoded cytokine, and is encoded in T-Vec, an HSV-1 virus that was approved for use in the United States by the FDA for melanoma in 2005 [14].

Three main characteristics are usually used to identify and understand the general mechanistic action of OV's [13]. First, the virus must enter the tumor cell, this can be mediated by virus-specific receptors, or by exploiting consequences of tumor cell modulation such as defects in signaling pathways that control antiviral immunity [14]. While most entry is mediated by virus-specific receptors, selectivity could be improved by retargeting OV's to enter through tumor-specific receptors as well.

Once the virus has successfully infiltrated the tumor cell, rapid cell division and high metabolic activity make it an ideal environment for viral replication. The virus' natural lytic cycle of infection results in the death of the cancerous cell. This leads to subsequent infection of nearby tumor cells as well as targeting other cells in the tumor microenvironment such as tumor stromal cells, including CAFs (cancer associated fibroblasts), ECs (vascular endothelial cells) and pericytes. These effects on cells in the surrounding tumor environment can lead to a beneficial disturbance in the complex interactions and associations of cells that aid in tumor growth and survival [14]. Third, is that viral replication and subsequent killing of cancerous cells can induce antitumor innate and adaptive immunity. Immunogenic cell death is considered a vital factor that contributes to the effectiveness of oncolytic virotherapy. DAMPS (damage-associated

molecular patterns) are released as a consequence of the lysing of OV infected cells in the tumor microenvironment [14]. These DAMPS can then activate and attract dendritic cells in the tumor microenvironment [15]. Additionally, PAMPS (pathogen-associated molecular patterns) in the surrounding environment can be detected by PRRs (pathogen recognition receptors).

The types of oncolytic viruses can range from larger, DNA viruses all the way to tiny parvoviruses [13], but the most popular viruses under investigation and in use are dsDNA viruses such as Adenovirus, HSV-1, and Vaccinia due to their genetic manipulability [5]. However, there are other viruses that are being investigated for clinical use that have not been fully reviewed. The aim of this review is to provide a comprehensive, generalized overview of the current research in the field of oncolytic virotherapy that may have exceeded the scope of previous reviews. Newer, sometimes surprising viruses are being investigated for clinical usage, and there have also been advancements in the realm of dsDNA viruses as well since the last comprehensive overviews in 2017 [5, 13, 16]. The body of research into oncolytic virotherapy is constantly expanding as new oncolytic viruses are described and investigated for use. In addition, previously known oncolytic agents are being modified and further researched in order to increase their efficacy and safety.

Viruses: Information and Research Status

dsDNA Viruses

Adenovirus. Adenoviruses are double stranded DNA viruses that have been used in the majority of oncolytic research and clinical trials due to their genetic manipulability, well studied replication mechanisms, and relatively low pathogenicity [18]. The history, usage, and mechanisms of adenovirus as an oncolytic agent has been well reviewed [18-21]. Specifically, serotype Ad5 has been widely used and well-described both as a viral vector for gene therapy and as an oncolytic viral therapeutic due to its relative increased safety when compared to other serotypes [20]. H101, an approved treatment, and ONYX015, a virus under investigation and trials [19], were both isolated from the Ad5 pool. Both viruses utilize a deletion in the E1b gene to preferentially target and replicate in cancerous cells. The mechanistic actions behind the deletion of E1b potentially include “p53 inhibition, late viral mRNA export, and cell cycle disruption” [20]. H101 was the first approved oncolytic virotherapeutic and was approved to

treat head and neck cancers in China in 2005 [22]. However, new advances into the usage of other serotypes are being investigated alongside novel targeting mechanisms.

Some recent studies have sought to overcome the barriers of using Ad5 which include the presence of anti-AdHu5 immunity in most populations, extensive sequestration of AdHu5 by the liver, and the lack of CAR (coxsackie and adenovirus receptor) on cancer cells [23, 24]. Various methods have been investigated such as using simian adenovirus type 24 [23] as well as the creation of Ad657 from Ad6 and Ad57 [25]. Chimeric viruses, such as Ad5/3 have also been in development to attempt to subvert the necessity for CARs on cancer cells to facilitate infection [24]. Apart from using and/or creating new serotypes of adenovirus, other studies have focused on modifying the preexisting archetype of Ad5. DNX-2401 is a serotype 5 adenovirus in phase 1 clinical trials that utilizes gene modification of the E1a gene instead of E1b to selectively target cancer cells [26]. Other modified Ad5 viruses include telomerase specific targeting [27], $\alpha\beta6$ integrin targeting (Uusi), and targeting estrogen receptor positive breast cancer cells [28].

HSV-1 and other Herpesviruses. The first oncolytic virotherapy to be granted approval for use by the United States' FDA was a herpes simplex virus called Talimogene laherparepvec (TVEC) in 2015. TVEC, trade name Imlygic, was approved as a treatment for melanoma. It is engineered to express human granulocyte-macrophage colony stimulating factor (GM-CSF) to increase the innate antitumor immunity stimulated by the lytic action of the virus. TVEC's mechanism of action targets cancerous cells using surface nectins and exploitation of cancer-disrupted pathways, specifically protein kinase R (PKR) and the antiviral type I interferon pathway [29]. The exhaustive and precise details regarding the creation and mechanistic properties of TVEC have been well reviewed [29]. Real-world reports of treatment with TVEC continue to maintain that it is efficacious and safe for clinical use against melanoma [30]. However, just because it has been approved and is used for treatment does not mean that it is a perfect cancer treatment. Many times, it is more effective when combined with other treatments such as ipilimumab and kinase inhibitors, and it still falls victim to setbacks such as preexisting antibody prevalence in the patient [29].

Other modifications of HSV-1 have been recently investigated as oncolytic virotherapies following the FDA approval of TVEC. Seprehvir (HSV1716) is an HSV-1 virus currently in Phase 1 clinical trials for use in children and young adults to address the need for less-toxic

cancer treatments for patients in this age group. This virus was tested for intravenous use to target multiple types of metastasized cancers [31]. Ld0-GFP is another modified HSV-1 that has been specifically targeted to attempt to treat hepatocellular carcinoma by repeated cell passage in HCC cells [32]. Interestingly, one last modified HSV-1 is NG34scFvPD-1 which is genetically engineered to express a PD-1 binding antibody in the hopes of restoring anti-tumor T-cell activity alongside the lysis of glioblastoma cancer cells [33].

In addition to modified HSV-1, there have been a couple recent studies that involve other herpesviruses such as HSV-2 and HCMV. A chimeric HSV-1/HCMV called C134 was developed to investigate potential treatment of malignant gliomas. This intriguing virus contains IRS1 (a PKR-evasion gene from Human Cytomegalovirus) transferred from HCMV to “enhance its replication in tumor cells.” This treatment centered around the elicited immune response instead of lytic action and showed development of anti-tumor memory in mice. The authors concluded that this chimera C134 displayed “improved late gene expression, viral replication, and anti-tumor activity” [34]. Additionally, a comparison on the infectivity and spread of HSV-1 and HSV-2 oncolytic viruses has been conducted in order to evaluate potential treatment of HSV-1 resistant cancer cells with HSV-2 oncolytic viruses due to utilization of different nectins to facilitate viral entry and cell-to-cell spread. Between the two observed viruses, HSV-1 was able to produce a higher viral load, but, interestingly, an increased killing effect was observed with infection of the HSV-2 virus [35]. Overall, the study of other herpesviruses as oncolytic agents takes a backseat to the study of new HSV-1 modifications. This is most likely due to the fact that an HSV-1 has been FDA approved and generally shows more promise in novel cancer treatments.

Vaccinia and other Poxviruses. The second most common virus in oncolytic research, in terms of active clinical trials, is vaccinia. Due to vaccinia’s use as a smallpox vaccine there is an extensive understanding of the virus [36]. It also possesses high lytic activity, the ability to infect a range of tumors, and a highly described and manipulatable genome [37]. This has led to systemic studies of vaccinia both as a viral vector for gene therapies and independently as an oncolytic virotherapy. However, vaccinia initially showed low levels of clinical response as a monotherapy and safety concerns regarding case reports of vaccinia-associated encephalitis in populations of the immunosuppressed displayed the need for improved safety [36, 38].

Therefore, modifications to the virus have been explored to improve the safety profile and efficacy.

One of these engineered vaccinia viruses is known as Pexa-Vec, which comprises the majority of the current clinical trials involving vaccinia [39, 40]. Pexa-Vec, similarly to TVEC, has been modified to express GM-CSF to promote antitumor immune stimulation and disrupt the tumor vasculature [36, 41]. Many engineered vaccinia viruses focus on inhibiting vaccinia's thymidine kinase (VV-tk) production since it is a key virulence factor in infecting normal cells. Some of these strains include Guang9 and deVV5. Additionally, a recent study published their results regarding a vaccinia virus engineered to express HSV-tk for potential viral control with ganciclovir [37, 38, 42]. These investigations into thymidine kinase inhibition not only increase the safety of vaccinia but also its preferential replication in cancer cells. Other methods of improving vaccinia therapies include a deletion in the F4L gene encoding the cell-cycle-regulated small subunit of ribonucleotide reductase, which also plays a role in infecting healthy cells, as well as surface protein modifications to avoid viral destruction by immune responses [43, 44]. Despite these genetic modifications that have improved the efficacy and safety of vaccinia-derived OV, using vaccinia cancer vaccines in combination with other therapies still proves substantially more effective [36].

Even though vaccinia is the most extensively studied and commonly used poxvirus in the realm of oncolytic virotherapy there have been a few recent studies looking into the potential oncolytic properties of other members of the *Poxviridae* family. Three studies since 2017 have specifically focused on properties of Myxoma virus and its effects on varying cancers such as small-cell lung cancer and neurogliomas among others [45-47]. Myxoma virus naturally infects rabbits and the South American tapeti, but is harmless in humans, making it an attractive candidate for oncolytic research. Targeting seems to be based on interferon deficiencies in cancer cells and apoptosis of these cells is mediated by viral interactions with cell death regulators [48]. Other poxviruses have been significantly less studied, however, Ricordel et.al published a study in 2018 that evaluated the oncolytic properties of ten non-vaccinia poxviruses. Their findings pointed to raccoonpox as the prime candidate for future oncolytic studies and they subsequently developed a thymidine kinase deficient mutant expressing the suicide gene (apoptosis-inducing) FCU1, which is the standard genomic modification of widely tested vaccinia viruses [49]. The

continued research into genetic modifications of vaccinia and other poxviruses ideally increases their capacity as cancer therapeutics.

ssDNA Viruses

Parvovirus H1. Rat protoparvovirus (H-1PV) is a ssDNA virus and one of the smallest known viruses at 25nm. This is a pretty big shift from the previously discussed large, dsDNA viruses that encompass most of the past and present oncolytic viral research. However, there has been a shift into studying animal-specific viruses in order to avoid pathogenic responses and humans and any preexisting immunity that would limit the efficacy of the treatment. H-1PV is somewhat unique as it is the only ssDNA virus being studied for oncolytic potential, and similar to the previously discussed poxviruses, H-1PV as a monotherapy is also unable to eradicate tumors in vivo [50].

H-1PV is unique in that it is being studied for oncolytic virotherapy as a wild-type virus. It has a natural propensity for cancerous cells due to their increased proliferation, making them more advantageous for H-1PV DNA replication. The dysregulated signaling pathways in cancer cells also contribute to preferential infection as many overexpressed pathways control the viral life cycle and dissemination, and the down regulation of anti-viral type 1 interferon allows for more infection [51]. The subsequent cytotoxic effects are mediated by the non-structural protein (NS1), and H-1PV infection has been shown to result in both apoptotic and non-apoptotic death through NS1 mediated accumulation of ROS in tumor cells [51]. Particularly in glioma cells, the virus shows promise as it avoids the resistance that many glioma cells have against traditional cytotoxic methods like death ligands by using lysosome-dependent cell death [51, 52]. In addition to its mediation of cell death, H-1PV has also shown immunogenic properties and the induction of tumor-specific memory [53].

In 2011, a phase I/IIa trial was initiated with WT H-1PV named ParvOryx to treat glioblastoma, and, in 2015, the same company began another trial with the same virus to treat pancreatic carcinoma [52, 54]. Both of the trials determined that the use of H-1PV was safe and tolerable as a potential therapy. Despite the relative success of the clinical trials, there is a clear discrepancy in the effectiveness of the virus in vitro compared to in vivo, demonstrating a need for improved therapeutic function. One current idea is retargeting the virus to boost its preference for cancer cells by designing an insertion of arginine-glycine-aspartic acid (RGD)-4

cyclic peptide to the viral capsid to bind to two integrins commonly expressed on cancer cells, $\alpha V\beta 3$ and $\alpha V\beta 5$ [51]. While normal cells are not negatively affected by the virus, it still enters healthy cells leading to sequestering and decreased efficacy, highlighting the need for the proposed retargeting solution. Next generation H-1PVs could also be engineered to express immune stimulators as is common in many oncolytic virus modifications as well as arming with RNA interference triggers to silence gene expression of carcinogenesis. This is an attempt to overcome the heterogenous nature of some tumors that can resist viral therapeutics by “reverting the malignant phenotype” [51]. A genetically modified second generation of H-1PV could prove even more promising and efficacious as a potential virotherapy.

dsRNA Viruses

Reovirus. Respiratory Enteric Orphan virus or “Reovirus” is a ubiquitous, dsRNA virus that is weakly or completely non-pathogenic in humans. There are four main mammalian types of Reovirus with serotypes 1-3 being infectious in humans. So far, only the type 3 Dearing strain has entered clinical trials for oncolytic virotherapy (Reolysin™) and is the strain that has been given an orphan drug designation by the FDA for approved use in ovarian, gastric, pancreatic, glioma, and breast cancers. Of note, it is the only wild-type virus approved for clinical use as Reovirus is only modified through passaging selection [55, 56]. The detailed mechanisms of the viral cycle of Reovirus have been previously described [57], however, there is still some ambiguity surrounding the exact mechanisms of preferential targeting and replication within cancer cells. It is known that defective dsRNA activated PKR signaling can lead to infection of cancer cells, however, much research has instead centered around the potential role(s) of the Ras pathways regarding Reovirus infection [55]. While many instances show Ras pathway contributions, many others also display Ras signaling independence. Taken together, we can conclude that as of now the exact influence that the Ras pathway has on Reovirus remains unclear. As for cell death, Reovirus activates cell death pathways such as TRAIL, Fas ligand-associated, and p53 apoptotic avenues [58]. This displays both intrinsic and extrinsic apoptosis. Recently, it was also described that Reovirus can also induce cell death by way of the non-apoptotic mechanism of necroptosis [55]. Anti-angiogenic effects in the tumor microenvironment have also been observed in Reovirus infection of soft tissue sarcomas [59].

However, much like many other OV, the performance of Reovirus in vivo does not measure up to the effects demonstrated in vitro.

Despite the reduced efficacy in the clinic, Reovirus has still been shown to be able to prime and sensitize tumor cells to other immunotherapies such as checkpoint blockades, anti-PD-L1 therapies, and monoclonal antibodies [60-62]. Even when used in combination as a primer, the efficacy of Reovirus is negatively impacted by the presence of a serological response since most individuals have been exposed to the virus during childhood. Recent studies have started to investigate workarounds to avoid the body's natural immune response against a recognized virus by using carrier cells or an avian strain of Reovirus. Cytokine-induced killer cells and monocytes have both been explored as potential mechanisms of delivery to avoid neutralizing antibodies [63, 64], and an avian orthoreovirus demonstrated successful oncolysis and displayed marked differences in its homolog to the S1 protein of Reovirus that is primarily targeted by antibodies, thus avoiding neutralization [65]. While these advances serve to further the understanding of Reovirus and improve its efficacy, genetic engineering to further improve targeting, cytotoxicity, and other beneficial effects is still in very early stages. No doubt these genetic advances will only serve to increase the capacity of Reovirus both as a monotherapy and a combination drug.

ssRNA Viruses

Animal Viruses

Newcastle. NDV (Newcastle disease virus) is an avian paramyxovirus that primarily infects chickens. For years it has been well studied and commonly used as a challenge virus in the poultry industry for the development of vaccines. After the discovery of its oncolytic properties in the 1960s, research into its use as a cancer therapeutic truly began due to its weak pathogenicity in humans and avoidance to neutralizing antibodies. Since then, apoptotic effects have been documented in breast, brain, renal, colorectal, skin, bone, and cervical cancers as well as in leukemia [66]. Studies into the exact mechanisms of cell death and the immunogenic effects of infection with NDV have been extensively reviewed [66, 67]. Briefly, NDV is able to cause both intrinsic mitochondrial and extrinsic receptor-mediated cell death, and it elicits the Th1 antitumor response through the maturation of dendritic cells. Additionally, autophagy-induced cell death has also been recently documented in NDV tumor cells [68]. Kalyanasundram et.al. conducted an in-depth analysis of these immunostimulatory pathways. The tumor selectivity is

similar to many other OV's in that it is dependent on the compromised anti-viral IFN pathway and upregulation of anti-apoptotic factors which promote viral replication in cancer cells.

Oncolytic virus research with NDV continues to investigate the potential for both genetically modified and wild-type viruses as therapeutics. Much of the research has looked towards incorporation of immunostimulatory factors like IL-2, GM-CSF, TNF, and IL-12 among others [69]. However, wild-type adaptation to increase infectious titers through cell culture passaging is still being evaluated by research groups. Much of argument for WT over modified viruses is that genetic constructs need regulation of their biological properties and have mutagenic potential to develop human pathogenicity [70]. Regardless of wild-type or recombinant, NDV was dealt a setback in 2008 when the U.S. Department of Agriculture classified mesogenic (moderate) and velogenic (severe) strains of NDV as select agents because of its high pathogenicity in poultry. This has impeded clinical development since then [71]. Despite these classifications, strain AF2240 (velogenic) has entered clinical trials (phase I, II, and III) to treat colorectal cancer [67].

VSV. Vesicular stomatitis virus (VSV) is a rhabdovirus that infects livestock through insect vectors. This virus has multiple advantages for its use as an oncolytic virotherapy. In humans, the virus is generally asymptomatic and there is a lack of preexisting immunity to limit its efficacy. It also has the ability to rapidly display high titers in infected cells along with creating a strong immunogenic effect [72]. This is ideal for modification of the tumor microenvironment and also makes VSV a strong vaccine platform candidate [73]. Its tropism also lends itself to broad spectrum tumor treatment as it is a pantropic virus as opposed to adenovirus which requires the CAR to effectively target cancer cells. In addition to all of these advantages, VSV has a small, easy manipulatable genome. Specifically, this manipulability of the genome is important due to observed neurotoxic effects when using wild-type VSV as a potential treatment [73]. The viral M protein is mainly responsible for this toxicity due to the blocking of antiviral effects in normal cells. This has led to the creation of recombinants that have a modified or deleted residue at position 51 in the genome coding for M protein which prevents the blockage of these effects and prevent neurotoxicity [72].

VSV targets cancer cells with deficient type I interferon pathways and leaves normal cells unharmed due to its extreme sensitivity to type I IFN. Once inside, VSV mediates cell

killing through shutdown of host RNA and protein synthesis as well as inducing ER stress-mediated apoptosis [73, 74]. In addition to infection of tumor cells, VSV also tends to infect the surrounding tumor vasculature causing loss of blood flow to the tumor microenvironment and an influx of antiviral neutrophils [74]. Antiviral effects might seem counterproductive, but these virally induced activities of infected tumor cells and their surroundings can actually induce antitumor bystander effects [73]. Additionally, VSV also causes immune stimulation by inducing the maturation of dendritic cells.

These advantages also come with disadvantages, mainly ineffective delivery, and toxicity. Attempts to circumvent these problems have resulted in many recombinant strains of VSV to modulate toxicity and improve the overall safety. VSV-GP (the VSV glycoprotein G is substituted by the lymphocytic choriomeningitis virus (LCMV) glycoprotein GP) is a commonly tested recombinant and has shown some promise [75, 76], and there is even a chimeric virus of VSV and NDV [71]. Unfortunately, many VSV tests are solely preclinical and do not perform adequately in the clinic. However, this attenuation many times results in decreased efficacy of the viral treatment [72]. There are many ideas to help offset this lack of efficacy, and in a review published by Felt et.al, the different modifications to VSV made in the last 5 years are described at length, including the incorporation of cytokines into the genome [73]. Overall, VSV shows promise as a potential OV after continued research into genetic modification to improve both efficacy and safety without the loss of one or the other.

Maraba. Maraba virus is a rhabdovirus that is closely related to VSV. Much like VSV, it generally causes no symptoms in humans with only rare clinical manifestations of flu-like illness and fever. In addition to its low pathogenicity, there is only one documented case of seroconversion against viral antigens making Maraba an interesting and viable candidate for OV research [77]. It gains another point in its favor due to the fact that, just like VSV, it is pantropic and utilizes the pervasive LDLR cell receptor to mediate entry [77]. This potentially makes Maraba effective against many different types of cancer.

The most common Maraba strain researched for clinical use is MG1 which contains mutations in both the G and M proteins of the virus [72]. In general, this recombinant strain replicates faster, has a larger burst size, increased killing efficacy, is strongly attenuated in normal cells, and boosts NK cell activity in the tumor microenvironment [77]. MG1 has been

tested in multiple cancer types including, but not limited to, breast, prostate and sarcomas [78, 79], and shows good efficacy with in vitro and in vivo models. In addition to its increased killing, MG1 also promotes immune stimulation, and with more modification by the insertion of the IL-12 cytokine, further boosts NK cell recruitment for effective cancer eradication [80].

Maraba virus has also been studied as a potential cancer vaccine due to its relatively strong stimulation of anticancer immunity and memory. In a preclinical model, infusion of MG1 infected-infected γ -irradiated leukemia cells resulted in immune responses in 60% of animals. Moving even further, two recent first-in-human clinical trials (NCT02285816; NCT02879760) attempted to induce a T-cell response to tumor antigens by using a Maraba vaccine with previous priming by an adenovirus vaccine to boost the efficacy. Three out of the six patients displayed antitumor immunity, and over 1% of their circulating CD8 T-cells were reacting against the tumor antigen [77]. Taking these studies into consideration, Maraba could be a very capable cancer therapeutic as a vaccine as well as an OV in combination with other treatments.

Seneca Valley. Seneca Valley virus (SVV) is a picornavirus that has been observed to selectively infect and lyse neuroendocrine cancer cells, which mostly occur in the lungs, appendix, small intestine, rectum, and pancreas. The targets of SVV research have mainly focused on small cell lung cancers and pediatric neuroendocrine solid tumors [81]. It has shown no pathogenicity in any animals or humans and has a non-integrating RNA genome which alleviates many safety concerns [82]. The most recent research (2018) has focused on the identification of the attachment receptor, which was found to be ANTXR1 (anthrax receptor 1) also known as tumor endothelial marker 8 (TEM8). This surface receptor is upregulated in some tumor cells and correlates with cell susceptibility to SVV infection [81, 83]. However, TEM8 is not the only factor that mediates successful viral infection. As with the majority of OVs, a downregulation of type I interferon is also necessary [83]. Regardless, this identification of the attachment receptor has potential diagnostic implications when screening patients for potential success with SVV treatment.

Testing has shown xenograft tumor reductions in mice, but clinical trials were not as successful. Two phase I clinical trials, one in adults and one in pediatrics, determined that intravenous SVV was safe and tolerable, but was unfortunately associated with rapid recognition by even extremely immunocompromised individuals. A phase II trial was then initiated but

deemed futile and cancelled. So far, all early human trials have not demonstrated enough of a response from SVV, mainly because of neutralizing antibodies [82, 83]. In short, the future of SVV research lies in subverting the natural host immune response to effectively deliver and maintain viral replication within the tumor and surrounding environment.

Human Viruses

Alphavirus. Alphaviruses are one of the lesser studied viruses in the realm of OV research, but they are showing some promising results. There are many types of alphaviruses that have shown oncolytic properties including Semliki Forest virus [84], Sindbus virus, and M1 virus [85, 86]. M1 is the most commonly researched alphavirus in regard to potential oncolytic effects. The exact pathogenicity of M1 is unknown, but it was found to be the cause of a mild fever outbreak in the Hainan province of China and is closely related to another alphavirus that causes a mild, self-limiting illness (Getah virus) [85]. M1 has shown to have oncolytic effects in multiple cancer types including hepatocellular carcinoma, colorectal, bladder, and glioblastoma, and its observed method of cell death seems to be ER stress-mediated apoptosis. [85]. One of the most recent papers on M1 alphavirus also observed that deficiencies in the ZAP gene (which was present in about half of tested bladder cancer patients) was required for increased sensitivity to M1 infection [86]. Currently there are no registered alphavirus oncolytic virotherapy clinical trials registered with ClinicalTrials.Gov [39].

Coxsackievirus. Coxsackievirus is another member of the picornavirus family that has been studied for use as an oncolytic virotherapy. There are two types of coxsackievirus: A and B. One from each group, A21 and B3, have shown strong oncolytic properties and are currently under study. A21 is widely regarded as the safer of the two strains and uses DAF (decay accelerating factor) to bind to host cells and ICAM-1 (intracellular adhesion molecule 1) to internalize [87]. It has been manufactured by Viralytics Ltd. under the name CAVATAK® and has undergone clinical trials as a treatment for melanomas and other solid tumor cancers. In addition to its oncolytic effects, A21 has been observed to induce immune-mediated cell killing through activation of effector cells including NK cells and priming of tumor specific CTLs [87]. Currently, there are a number of clinical trials investigating the effects of A21 in combination with other immunotherapies [39].

Coxsackievirus B3 is lesser studied and has not made it into clinical trials due to safety concerns. This strain has been tested in various human cell lines and targets actively dividing cells using DAF and CAR to attach and internalize [88]. However, its somewhat broad tropism has led to myocarditis, pancreatitis, and aseptic meningitis in mouse models [89]. Most recent research has focused on reducing the toxicity of B3 while preserving its oncolytic function, and regulation by microRNA has shown promising results in improving safety while retaining efficacy [88, 89].

Measles. The measles virus is both an extremely well-known and well-studied virus. The oncolytic properties of measles have been noted as far back as a case report in 1949 regarding a Hodgkin's lymphoma patient with tumor regression after a measles infection [90]. Much of the past and present oncolytic measles research utilizes the Edmonton vaccine strain due mainly to its excellent safety profile. However, genetically engineered strains have also been created to improve efficacy and stimulate the immune system. Some of these modifications include the presence of neutrophil activating protein (NAP), suicide gene insertion [91], IL-12 [92], enhanced CTL activation designs [93], GM-CSF, coding of thyroidal iodine symporters (which induce iodine uptake into cancer cells), and many more [94]. Bhattacharjee and Robinson have both published reviews that contain an extensive lists and descriptions of genetically modified oncolytic measles viruses [94, 95].

Measles viruses preferentially targets cancer cells mainly through the upregulation of CD46, one of the three measles virus receptors [94]. Preclinical and clinical trials have shown very promising results from even a low viral dose of measles, but only when confined to intratumoral or intracavitary injection [90, 95]. This restriction to a specific cavity means that preexisting measles immunity in most of the population does not reduce the effect of the virus on the tumor and its microenvironment. Systemic application, however, requires study into mechanisms and potential genetic modifications to subvert the host immune response against the virus. One method has advanced to clinical trials (NCT02068794), and that is using cells as viral delivery vehicles to shield the virus from the immune system as it travels throughout the body [90, 96]. Other potential strategies are the creation of chimeric viruses with exchanged, non-cross-reactive glycoproteins and the use of protective “decoy” viruses [90, 97]. There are

currently seven active clinical trials involving monotherapeutic, genetically modified oncolytic measles viruses registered with the clinical trials database.

Zika. Zika virus is a flavivirus known to cause congenital defects including microcephaly and neurological disorders. After the Zika virus outbreak in Brazil in 2016, large amounts of resources were devoted to Zika virus research. From this research came the observation that many of the proteins implicated in Zika virus infection were also overexpressed in cancer cells including glioma multiforme [98]. Zhu et.al hypothesized that since Zika infected neural precursor cells, this natural tropism could be used to treat glioblastomas. Through their research it was observed that Zika possessed oncolytic characteristics and preferentially infected glioblastoma stem cells (GSCs). This occurrence was also not a natural property of neurotropic flaviviruses since testing with West Nile virus revealed no viral discrimination between healthy and cancerous cells [99]. Later experiments showed that a live attenuated vaccine strain of the virus, ZIKV-LAV showed the same GCS killing in mouse models [100]. There has also been reported Zika virus mediated killing of human embryonal cancer stem cells [101]. Research into the oncolytic activities of Zika virus is still early and, as such, the immune stimulation and potential effects on the tumor microenvironment have not yet been evaluated. However, it is not out of the realm of possibility that a genetically modified Zika virus expressing cytokines or other immune stimulatory factors could be developed in the future. The diagnosis of glioblastoma is a grim one, and the discovery of Zika's oncolytic properties could hold some potential for improved treatment for this fatal cancer.

Discussion/Conclusion

When looking at the changes to the field of oncolytic virotherapy research since the last comprehensive reviews in 2017, it is clear to see that research into this potential cancer treatment has not slowed down. Fukuhara et. al noted, at the time of writing, that there were around 40 ongoing clinical trials for oncolytic virotherapy [5]. As of April 2020, there are 40 clinical trials just for Adenovirus alone, and around 115 in total when combining trials from all of this article's reviewed viruses. Much of this rise has to do with the continued genetic improvements to safety and efficacy, especially with well-studied viruses such as Adenovirus. However, a few of these current trials come from newer viruses not reviewed in the previous literature. Maraba virus has 4 ongoing trials and Seneca Valley virus has one as well [39]. Alphavirus and Zika virus are also

new to the scene, and while there are not yet any clinical trials involving these viruses, they are progressing through cell-cultured and animal studies.

The main concern for future research from previous reviews was the recognition that immune evasion methods were needed to be able to apply these OV's effectively as treatments. In the past three years, more research into immune evasion has been one of the biggest additions to the field. From carrier cells to genetic modification of surface proteins, effective delivery of OV's has been in the spotlight of research [63, 64, 96, 102]. The immune system has proved to be a dual edged sword in OV research, and the other side of it is immunotherapy. While the immune system can reduce the efficacy of the treatment, it is also vital for its success. The immunostimulatory actions of OV's are crucial for effective killing of tumor cells. Today, many OV's are not used as monotherapies (as they were reviewed in 2017). Combination therapies with immunomodulatory drugs as well as some chemotherapies have proven to increase the effectiveness of treatment when combined with OV's [56]. Some of the most promising strategies have been the use of OV's in combination with checkpoint inhibitors such as CTLA-4 (ipilimumab), PD-1 (avelumab, atezolizumab), and PDL-1 inhibitors (lambrolizumab, pembrolizumab, nivolumab) [102]. There is even a Phase II clinical trial involving T-Vec and ipilimumab which has shown positive results from the combination and has most importantly indicated that as the virus changed the tumor microenvironment, it became more conducive to the checkpoint inhibitor. [14]. There has even been a study to use OV's to prime the tumor microenvironment for subsequent treatment with checkpoint inhibitors since OV's have been shown to upregulate PD-1/PDL-1 in tumors [61].

Recent years have shown that the study of oncolytic virotherapy goes far past simply injecting an oncolytic virus and hoping for good results. Realistically, there can be no one hit wonder for cancer treatment, and although OV's can be theoretically applied to all cancer types, in most cases they alone are not enough to be an effective treatment. Thus, the research into combination therapies in order to boost the efficacy of both treatments by using them together. Considering the ever-increasing number of viruses with oncolytic properties, the future challenge will be to identify which genetically modified viruses and subsequent combinational therapies will prove the most effective for the treatment of various cancers. Optimistically, the field

continues to grow, and it seems that OV's will supplant themselves as one of the new focuses in therapeutic cancer research.

ROLE IN PROJECT

Since my project was an academic review, my role in the project was to find, read, and compile the chosen research papers from 2017-2020 about all currently studied oncolytic viruses. Additionally, I had to synthesize these papers and draw novel conclusions on what has been added to the field since the last review papers, what new directions oncolytic viral research is heading towards, and its potentially impactful role in the future of cancer treatment.

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