

Developments on Cancer Therapy through Oncolytic Virotherapy

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Abstract

Cancer is a complex disease and is difficult to treat. Until the early twentieth century, it was treated by surgical removal which later on was combined with chemotherapy, radiotherapy and immunotherapy. However, advances in molecular biology and genetics lead to the establishment of virotherapy. The genetic modification of oncolytic viruses has improved their tumor specificity, targeted delivery and increased efficacy, leading to the development of new weapons for the war against cancer especially in those cancer patients in which tumor is inoperable. In this review, we describe the basis of oncolytic virotherapy and how the genetically modified tumor-specific viruses are developed. Utility of oncolytic virotherapy to treat cancer, clinical trials and their success rate are also discussed. We conclude with current and future challenges in oncolytic virotherapy and the safety concerns raised by the trials conducted so far.

Keywords: Cancer; Oncolytic Viruses; Cancer Therapy

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Introduction

Incidence of cancer (excluding skin cancer) is 10,055.6 per 100,000 people and the mortality rate is 6,208.7 per 100,000 people [1]. Cancer is a complex disease and difficult to treat. The early 1900s produced significant advances in cancer therapy including surgery, radiotherapy, chemotherapy and immunotherapy but none of them was applicable to all tumors at all stages. Tumors could neither be removed surgically all the time nor be killed all the time with high-energy beams of radiation or with poisons delivered to them intravenously. Even the combination of these proved to be insufficient.

History and basis of virotherapy

The past decade has played a great role in our understanding of the genetic basis of human diseases. Among them the most profound impact has been in the area of cancer genetics, where the

explosion of genomic sequence and molecular profiling data has illustrated the complexity of human malignancies. This complexity often interferes with the therapeutic regimes. It is reasonable to suggest that sophisticated therapeutics that can attack cancers in multiple, but targeted ways, will be necessary in order to improve current success rates. The Oncolytic Viruses (OVs), which have the intrinsic ability to selectively replicate in and kill cancer cells, have found application in the treatment of primary and metastatic cancers with tremendous potential to revolutionize the management of what has become one of mankind's disaster. A number of viruses are being developed around the world for this purpose [2]. The use of viruses in the treatment of cancer was not by chance but rather was considered from the observation that, sometimes, cancer patients who had an infectious disease went into brief periods of clinical remission. In the case of leukemia, it was well recognized that influenza

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virus infection sometimes produced beneficial effects [3-4]. Although no cases were reported where an accompanying infectious disease led to complete cure of leukemia, it was anticipated that the treatment based on the causative agent may provide an alternative to the ordinary treatment of leukemia [3].

The first clinical trials involved transmittance of body fluids containing viral particles to other cancer patients and observing the result, without knowing their biological behavior. The viruses were destroyed by the immune system and were eliminated from hence failed to destroy the malignant cell growth. In the 1950's and 1960's, many attempts were made to develop viruses with greater tumor specificity [5]. The mouse sarcoma 180 could be completely destroyed when treated with oncolytic virus proved to be a landmark for virotherapy [6]. With a better understanding of virology, as well as experience using viruses in cancer gene therapy, has prompted a new wave of oncolytic virotherapy. The use of genetically engineered, tumor-specific viruses as oncolytic agents recently has emerged as a promising method for cancer therapies. ONYX-015 (an oncolytic adenovirus) was the first oncolytic virus which demonstrated both the safety and anti-tumor potential of this approach [5].

Mechanism of antitumoral efficacy of oncolytic viruses

There are different mechanisms utilized by the oncolytic viruses to destroy the cancer cells. The virus can destroy tumor cells by replicating. The initial infection of only a few tumor cells would initiate a chain reaction and will cause the subsequent destruction of the surrounding tumor cells. Once the virus reaches and infects the surrounding normal tissue, replication of a virus that is selective for tumor cells would be aborted, sparing normal tissue. Moreover, this feature of viral replication provides continuous amplification of the input dose which continues until stopped by the immune response or a lack of susceptible cells [6]. Secondly some oncolytic viruses synthesize certain proteins during their replication which are directly cytotoxic to tumor cells. Thirdly they can initiate specific and nonspecific anti-tumor immune responses. It is well known that the tumor cells are inherently weakly immunogenic because they express low levels of major histocompatibility complex (MHC) antigens and stimulatory signals such as cytokines which activate a local immune response. Adenoviruses express E1A protein during replication, which mediates killing of tumor cells by increasing their sensitivity to tumor necrosis factor (TNF) [7]. Viral peptides are presented on the cell surface with MHC class 1 proteins; this complex is recognized by cytotoxic T

lymphocytes (CTLs) and are attracted by the virallytransduced tumor cell which intern can acquire specificity for tumor-specific antigens and kill the cells by an unknown mechanism [6].

Fourth mechanisms by which oncolytic viruses can lyse the tumor cells are enhancing the sensitivity of tumor cells to chemotherapy and radiation therapy. The adenoviral E1A gene product is an example. It is a potent chemo sensitizer in cells with functional p53. It can induce high levels of p53 in these cells and make them susceptible to DNA damage from chemotherapy and radiation. Normal, non-transformed cells remain unaffected. This gene product can sensitize tumor cells to chemotherapeutic agents even in the absence of functional p53, though the mechanism is known yet [8].

A final mechanism by which oncolytic viruses mediate antineoplastic activity is by the expression of therapeutic transgenes which is inserted into the viral genome. These therapeutic viruses offer a specific advantage over the replication-incompetent viruses that have been employed in the vast majority of gene therapy applications up till now. As the virus replicates, there is a simultaneous amplification of the inserted gene expression, which intern produces an amplified antitumor effect. Some researchers have inserted prodrug-converting enzymes, such as viral thymidine kinase and bacterial cytosine deaminase (CD), into replication conditional adenoviruses to enhance tumor cell lysis [6,8]. Others have introduced various immunostimulatory genes such as interleukins-4 (IL-4) and -12 (IL-12) into oncolytic herpes viruses, hence attempting to augment the antitumor immune response of the tumor cell [6].

Development of oncolytic Viruses

Chemotherapy and radio-therapy are current mainstays to treat advanced cancers but have limitations such as, tumor cells develop resistance to these agents and a relatively have narrow therapeutic index. Moreover, increased dose or combination therapies designed to overcome this resistance or increase tumor cell lysis are limited by toxicity to normal tissues. Oncolytic viral therapy, on the other hand, is able to increase the therapeutic index between tumor cells and normal cells when viral replication proceeds preferentially in tumor cells. While using the oncolytic viral therapy to treat cancers the main challenge faced is the immune system of the host. The use of rapidly acting, genetically engineered, tumor-targeting viruses are a promising new area for novel cancer therapies [9]. Following are the techniques to modify the viruses and to enhance their clinical utility.

Selection criteria

The virus should be able to tolerate storage and production at high titers. A double-stranded DNA genome is advantageous because it has greater stability during storage hence reducing the chances of hazardous mutations. Viruses like adenoviruses and herpes simplex virus are the most suitable, and have been the most extensively used and studied.

Generating tumor selectivity

The oncolytic viruses can be made tumor specific and selective by transductional and nontransductional targeting.

Transductional targeting

It involves modification of the specificity of viral coat protein, thus increasing entry into target cells while reducing entry to non-target cells. This approach has mainly focused on adenoviruses, although it is entirely viable with other viruses too. The most commonly used group of adenoviruses is serotype 5 (Ad5), whose binding to host cells is initiated by interactions between the cellular coxsackievirus and adenovirus receptor (CAR), and the knob domain of the adenovirus coat protein. These receptors are necessary for adenovirus infection by showing that CAR-negative cells could be made adenovirus-sensitive by transfection with CAR cDNA (9). In addition, the viral internalization depends on an Arginine-Glycine-Asparagine (RGD) motif at the base of adenovirus coat protein that binds to integrins, causing endocytosis. It has been suggested that CAR has a role in cell adhesion, and possibly tumor suppression. Although expressed widely in epithelial cells, CAR expression in tumors is extremely variable, leading to resistance to Ad5 infection. Retargeting of Ad5 from CAR, to another receptor that is ubiquitously expressed on tumor cells [6,9], can be done in one of two ways explained below:

Adapter molecules

Specific adapter molecules can be administered along with the virus to redirect viral coat protein tropism which are fusion proteins and are made up of an antibody raised against the knob domain of the adenovirus coat protein, fused to a natural ligand for a cell-surface receptor. The use of adapter molecules has proved to increase viral transduction. However, adapter molecules add complexity to the system, and what effect is produced on the stability of the virus is uncertain [10].

Coat-protein modification

Genetic modification of the fiber knob domain of the viral coat protein is done to alter its specificity.

If short peptides are added to the C-terminal end of the coat protein, successfully alters viral tropism. The addition of larger peptides to the C-terminus is not viable because it reduces adenovirus integrity, possibly due to an effect on fiber trimerisation. The fiber protein also contains an HI-loop structure, which can tolerate peptide insertions of up to 100 residues without any negative effects on adenovirus integrity [10]. Insertion of RGD motif in the HI loop of the fiber knob protein, shifts specificity toward integrins, which Oncolytic virus 4are frequently overexpressed in Oesophageal Adenocarcinoma. When combined with a form of non-transductional targeting, these viruses proved to be effective and selective therapeutic agents for Oesophageal Adenocarcinoma [11].

Non-transductional targeting

It involves altering the genome of the virus so that it can only replicate in the tumor cells. This can be achieved either by transcription targeting, where genes essential for viral replication are placed under the control of a tumor-specific promoter, or by attenuation, which involves deletions of the viral genome that results in the elimination of such functions which are necessary for replication and cytolytic effects of that particular virus and are dispensable in cancer cells, hence making the oncolytic viruses, tumor specific [6].

Transcriptional targeting

By using this technique essential viral gene is put under the control of a tumor-specific promoter. It is well known that the gene is only expressed in cell types where all the transcription factors required for promoter function are active. A suitable promoter should be active in the tumor but inactive in the majority of normal tissue, particularly the liver, which is the organ that is most exposed to blood born viruses. Different promoters have been identified and studied for the treatment of a range of cancers. Cyclooxygenase-2 enzyme (Cox-2) expression is elevated in a range of cancers, and has low liver expression, making it a suitable tumor-specific promoter. Cox-2 is also a possible tumor-specific promoter candidate for other cancer types, including ovarian cancer. Another suitable tumor-specific promoter is prostate-specific antigen (PSA), whose expression is greatly elevated in prostate cancer. CN706 is a CRAd with a PSA tumor specific promoter driving expression of the adenoviral E1A gene, required for viral replication (See figure: A and B) [12].

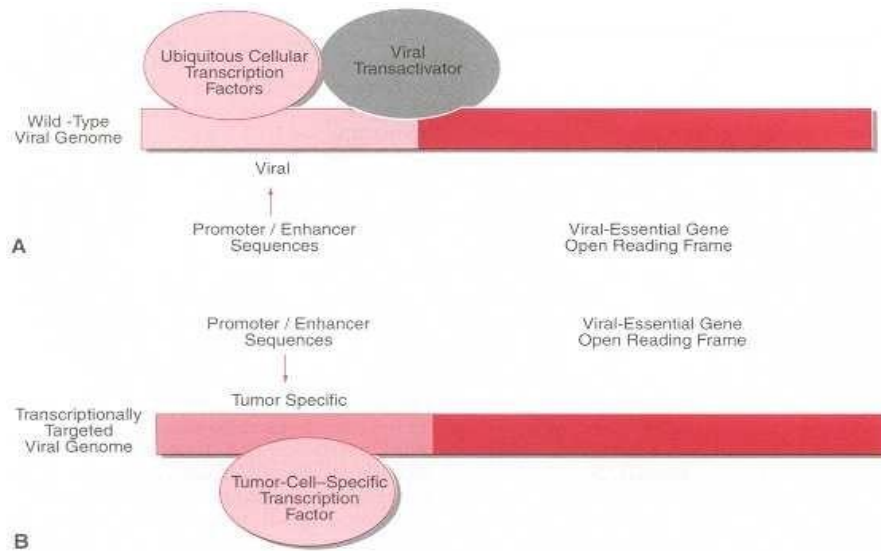


Figure 1. CN706 is a CRAd with a PSA tumor specific promoter driving expression of the adenoviral E1A gene, required for viral replication.

Attenuation

Cancer cells and virus-infected cells have similar alterations in their cell signaling pathways, particularly those that govern progression through the cell cycle. A viral gene whose function is to alter a pathway is dispensable in cells where the pathway is defective, but not in cells where the pathway is active. Attenuation involves deleting viral genes, or gene regions, to eliminate viral functions that are expendable in tumor cell, but not in normal cells. For adenovirus replication, the host cell must be induced into S-phase by viral proteins interfering with cell cycle proteins. The adenoviral E1A gene is responsible for inactivation of several proteins, including Retinoblastoma, allowing entry into S-phase. The adenovirus E1B55kDa gene cooperates with another adenoviral product, E4ORF6, to inactivate p53, thus preventing apoptosis. For example, in Ad5- Δ 24E3, there is 24 base pair deletion in the retinoblastoma-binding domain of the E1A protein, making it unable to silence retinoblastoma, and therefore unable to induce Sphase in host cells. This means Ad5- Δ 24E3 is only able to replicate in proliferating cells, such as tumor cells. Moreover, the herpes simplex virus genome contains the enzymes thymidine kinase and ribonucleotide reductase, whose cellular forms are responsible for the production of dNTP's required for DNA synthesis and are only expressed in Oncolytic virus 5 during the G1 and S phases of the cell cycle. These enzymes allow herpes simplex virus replication in quiescent cells, so if they are inactivated by mutation the herpes simplex virus will only be able to replicate in proliferating cells, such as cancer cells. A LacZ insertion in G207 herpes simplex virus mutant, inactivates ribonucleotide reductase [6, 9].

The HSV-1 viral mutant d120 is deleted of the viral essential gene α 4 and does not replicate upon infecting cells. The transcriptionally targeted HSV-1 G92A was created by placing the α 4 gene under the transcriptional control of the albumin enhancer/promoter elements and restricted viral replication to albumin expressing cells. Wild-type HSV-1 replicated efficiently in cells irrespective of albumin expression, while virus G92A replicated more efficiently in albuminexpressing cell lines compared to nonalbumin-expressing cell lines. In a similar vein, the calponin promoter has also been used to control expression of HSV-1 α 4. Calponin mRNA is overexpressed in soft-tissue/bone tumors, thereby providing a means to target such tumors with replication-conditional viruses.

The drawback of the above-mentioned transcription-targeted approaches is that individualized viruses have to be created for each tumor type. Also, tumors within a given tissue type also can vary in their expression of specific transcription factors, limiting the utility of such viruses for broad tissue types. To circumvent these problems, an adenovirus was constructed that targets tumor endothelial cells. The benefits of targeting the tumor vasculature are twofold: genetic stability and a common process among multiple solid tumors. The process of angiogenesis by endothelial cells involves the upregulation of multiple endothelial cell receptor complexes such as VEGF and tumor growth factor (TGF)- β . By placing the adenoviral E1A and E1B genes under the transcriptional control of the Flk-1 (VEGFR-2) and endoglin (CD105/TGF- β receptor component) promoter/enhancer sequences, the adenovirus preferentially replicates in dividing endothelial cells compared to tumor cells [12].

Therapeutic Gene Delivery with Replication-Competent Viruses

Therapeutic Genes have been employed with replication-selective tumor viruses as well. These strategies have included the delivery of immunomodulatory genes, prodrug-converting enzymes (suicide gene therapy), and cytotoxic genes. Replication-competent HSV-1 has been created to express the immunostimulatory IL-12 gene and the prodrug-converting enzyme cytosine deaminase gene. Replicating adenoviruses have also been created that express the cytotoxic TNF- α gene and the genes encoding the prodrug-converting enzymes cytosine deaminase and thymidine kinase. Delivery of a cytotoxic gene in combination with a replication-competent virus seems counterintuitive. However, these approaches appear to augment the therapeutic effect of antitumor therapy. The therapeutic gene delivered may enhance tumor cell kill by eliciting a bystander effect and results in the cell death of neighboring tumor cells that are not infected by the virus [9, 12].

Combination Chemotherapy/Radiotherapy with Replication-Competent Viruses

Finally, the researchers have also started to assess the utility of combining standard anticancer agents with replication-competent viruses. Ionizing radiation enhances the therapeutic potential of both replication-competent adenovirus and HSV-1 in part by increasing the replication potential of the viruses. A variety of chemotherapeutic agents have also been reported to increase the efficacy of replication-conditional adenovirus and HSV-1. Especially intriguing in this treatment paradigm are results of a Phase II clinical trial for recurrent head and neck cancer treated with E1B-deleted adenovirus (ONYX-015) in combination with 5-FU and cisplatin.

Preliminary results suggest that the chemotherapy augments the therapeutic effect of replicating conditional adenovirus [9].

This is how knowledge of cell and viral molecular biology has provided the basis for the construction of genetically engineered viruses that selectively replicate in tumor cells. Other replication-competent viruses under study include Newcastle disease virus and parvoviruses.

Multiple clinical trials are underway to determine the therapeutic efficacy of the current generation of replication-conditional viruses. At the same time, further basic science research in both tumor and viral biology is providing means to create more potent oncolytic viruses with increased safeguards to specifically target the tumor. Most of the current studies have focused on the use of replication-conditional viruses for regional therapy (i.e., direct tumor inoculation); however, studies are also

evolving that use of the virus as a systemic therapeutic agent could target metastases too. This would involve the virus surviving the systemic circulation and then homing in on malignant cells. Such viruses could bind selectively to tumor-cell-specific receptors to gain entry and have their gene expression driven by tumor-cell-specific transcription factors [6].

The Clinical Trials of the oncolytic viruses and their Success Rate

Oncolytic virotherapy has success to some extent, even at this initial stage. Herpes virus, adenovirus and many others are being evaluated in ongoing clinical trials for intractable cancers [13]. The very first viral therapy used for cancer treatment is adenoviral therapy and the virus used is ONYX-015. ONYX-015 is a manipulated adenovirus that lacks the viral E1B protein [16]. In the absence of this protein, the virus is not able to replicate in cells with a functioning p53 pathway as mentioned earlier. In most tumors, this pathway is defective or non-functional due to mutations, thus allowing ONYX-015 to replicate and lyse the cancer cells [13]. In squamous cell carcinoma of the head and neck, ONYX-015 has been used in phase I and II trials, resulting in tumor regression which is correlated to the p53 status of the cancer. Tumors with an inactive p53 pathway had a better response. ONYX-015, when used in combination with chemotherapy in phase II, showed better tumor response, leading to phase III trials [14].

In addition, ONYX-015 is now a day being evaluated as a preventive treatment for precancerous oral tissue, as in precancerous cells, p53 pathway-inactivating mutations will allow ONYX-015 to destroy and eliminate the precancerous cells before the tumor develops [15].

The virus CV706, in which the prostate-specific antigen (PSA) gene promoter-enhancer element is inserted upstream of E1A gene is another adenovirus. It replicates specifically in tissues with high PSA expression [15]. This viral vector is being evaluated in a phase I/II dose-escalation trial of intra-prostatic injection in patients with non-metastatic recurrent prostate carcinoma after definitive radiotherapy. Results have shown that this treatment has significant anti-tumor activity (Table 1).

Gene manipulations in the viruses ensure their tumor specificity. NV1020, has various mutations, including a deletion in the thymidine kinase region and a deletion across the long and short components of the genome, moreover an insertion of thymidine kinase gene under the control of the $\alpha 4$ promoter [6]. G207 is mutated in such a way that it has attenuated neurovirulence and is not capable of replicating in non-replicating cells [6]. These viruses have different cell targeting mechanisms.

The lytic portion of the cell cycle kills cells directly, and the thymidine kinase that is expressed from the viral genes sensitizes cells to ganciclovir. They have been tested in animal models and *in vitro* against a wide range of solid cancers successfully. G207 is being tested in treatment of malignant glioma in a

phase I clinical trial; NV1020 is in phase I and phase II clinical trials for the treatment of colorectal cancer metastases to the liver. This virus has also been evaluated for the treatment of glioblastoma [16].

Table 1. Partial list of oncolytic viruses in clinical trials, *Adapted from* Viral Oncolysis. John T, Mullen, Kenneth K, Tanabe, *The Oncologist* 2002; 7:106-119.

Name	Strain	Genetic alterations	Disease	Phase
ONYX-015	Adv 2/5 chimera	E1B-55 kD deletion	Head and neck cancer Ovarian cancer Primary and secondary liver tumors Pancreatic cancer	II-III I I-II I
CV706	Adv 5	Regulation of E1A under the PSA promoter; E3 deletion	Prostate cancer (organ-confined)	I-II
CV787	Adv 5	Regulation of E1A under the rat probasin promoter and E1B under the human PSA promoter; wild-type E3	Prostate cancer (organ-confined and metastatic)	I-II
G207	HSV-1	<i>lac Z</i> insertion into ICP6 gene; deletion of both copies of γ_1 34.5 gene	Malignant glioma	I-II
NV1020	HSV-1/HSV-2	700 bp tk deletion + 15 kb deletion across the joint region, which contains an exogenous copy of tk gene under control of HSV-1 $\alpha 4$ promoter and a 5.2 kb fragment of HSV-2 DNA	Colorectal carcinoma liver metastases	I
Vaccinia-GM-CSF	Vaccinia	Insertion of GM-CSF and <i>lac Z</i> genes into viral <i>TK</i> locus	Melanoma	I-II
PV701	NDV	Naturally attenuated	Advanced solid cancers	I

OncoVEXGM-CSF is a 2nd generation oncolytic *herpes simplex* type 1 virus, encoding human GM-CSF. OncoVEXGM-CSF represents an improvement over previous vaccine and virus-based therapies for the treatment of cancer, as has been genetically reprogrammed to attack cancer cells only. The insertion of the gene for human GM-CSF into the viral genome enhances the antitumor response both locally and at sites located distant. Expression of GM-CSF in the local tumor environment serves to achieve several biologic goals:

- (a) Induces local inflammation
- (b) Enhances dendritic cell activity
- (c) Produces antiangiogenic effect
- (d) Increases HLA class II expression

OncoVEXGM-CSF was easily added to a standard chemoradiation regimen along with each cycle of cisplatin, without significant additional toxicity being observed [16].

Reovirus (respiratory enteric orphan virus) is a double-stranded RNA virus and is associated with mild upper respiratory infections or enteritis. Reovirus infection results in activation of transcription factors (NF κ B), MAP kinase

pathways, and cell-cycle arrest, as well as apoptosis. As it is a double-stranded RNA virus, replication of the viral genome activates double-stranded RNA-activated protein kinase (PKR). Activated PKR phosphorylates the translation initiation factor, eIF-2 α , resulting in the cessation of protein synthesis. For the viral replication, the actions of PKR need to be stopped or inhibited. This can be achieved by activating mutations in the Ras signaling pathway by transfecting genes encoding proteins that activate the Ras pathway, i.e., EGFR, v-erbB oncogene, or SOS. Ras activation induces an inhibitor of PKR [16]. Thirty percent of human tumors have Ras-activating mutations. Tumors having such mutations would be predicted to permit reovirus replication. Initial results in tumor models appear to support such a hypothesis. Tumors with activated Ras (i.e. gliomas, colorectal, and ovarian cancers) are sensitive to reovirus infection. The interest in reovirus oncolytic therapy rests in its natural safety profile and lack of associated disease pathology upon wild-type reovirus infection [12, 17].

Vesicular stomatitis virus (VSV) a rhabdovirus, consists of 5 genes encoded by a negative sense, single-stranded RNA genome. In nature, it infects insects as well as livestock and causes a relatively localized and non-fatal illness. Since VSV undergoes

a rapid cytolitic replication cycle, infection leads to death of the malignant cell and roughly a 1000-fold amplification of virus within 24h. VSV is therefore highly suitable for therapeutic application, and several researchers have shown that systemically-administered VSV can be delivered to a tumor site, where it replicates and induces disease regression

leading to durable cures most of the time. Attenuation of the virus by engineering a deletion of Met-51 of the matrix protein ablates virtually all infection of normal tissues, while replication in tumor cells is unaffected. Recent research has shown that this virus has the potential to cure brain tumors [18- 20].

Table 2. Oncolytic viruses and their advantages and disadvantages when used for cancer therapy, *Adapted from Viral Oncolysis.* John T, Mullen, Kenneth K, Tanabe, *The Oncologist* 2002; 7:106-119.

Vector (size)	Advantages	Disadvantages	Insert size
Adenovirus (36 kb)	Infects dividing and nondividing cells Efficient gene transfer Nontoxic to host cells High viral titers possible Antigenicity generates immune response	Antigenicity generates immune response Small insert capacity	7.5 kb
HSV-1 (152 kb)	Infects dividing and nondividing cells Potential for prolonged gene expression Large transgene capacity High viral titers possible Sensitive to acyclovir/ganciclovir Antigenicity generates immune response	Possibility of herpes encephalitis Antigenicity generates immune response	40-50 kb
Vaccinia virus (187 kb)	Transient gene expression Efficient gene transfer and expression Large transgene capacity Antigenicity generates immune response Infects most mammalian cell types	Antigenicity generates immune response Safety concerns in immunosuppressed patients	25 kb
Reovirus (RNA)	Mild pathogenicity Unable to infect normal cells Antigenicity generates immune response	Infects only cells with an activated <i>ras</i> pathway	?
NDV (RNA)	Not pathogenic in humans Does not establish a permanent infection in host High potency	Mechanism of selective tumor cell lysis unclear Transgene insertion reduces viral replication	?

Conclusion

Viruses are able to target and kill cancer cells in human cancer patients. Cancer gene therapy is a field which is progressing and attaining maturity very rapidly. No doubt oncolytic viruses will be a part of future cancer therapies. Researchers are making efforts to overcome the challenges which are faced in the viral therapy. With the advances in genetic engineering and biotechnology, a number of viruses are being processed and modified to produce virus with improved safety and efficacy. Various clinical trials are being made in different types of cancers. Most of these clinical trials have had good results with high success rates using oncolytic virotherapy, and many more clinical trials are in progress with new viral vectors for the treatment of untreatable cancers.

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