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# Oncolytic Bacterial and Viral Therapies as Cancer Prevention and Treatment Options: A Comprehensive Review

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**Abstract**

Cancer has always been a severe threat to health and life. Since patients with advanced cancer often have a limited survival time and high treatment expenditures, routine therapies, such as surgery, radiation, and chemotherapy may help them live longer. However, the majority of these individuals cannot afford the excessive cost of care and have short life duration. With the introduction of oncolytic bacteria and viruses, a revolutionary therapeutic technique for the treatment and potential cure of malignant tumors has emerged. *Clostridium, Bifidobacteria, Salmonella typhimurium, Listeria monocytogenes,* and *Bacillus* are all oncolytic bacteria. *Adenoviruses, Vaccinia viruses, Reoviruses, Herpesviruses,* and *Coxsackieviruses* are all oncolytic viruses. This study aimed to review the current studies on the therapeutic potential of oncolytic bacteria and viruses as an alternate method for cancer prevention and therapy, including both experimental and clinical trials.

*Keywords:* Carcinogenesis, Human microbiota, Oncolytic bacteria and virus, Probiotics, Tumor therapy

## 1. Background

Uncontrolled cell development is the primary cause of cancer. When cancer spreads to other parts of the body, it is known as metastasis. Today's cancer treatment methods often include chemotherapy (1). Many of the most commonly used chemotherapeutic drugs are harmful to all cells in the body and do not target just cancer cells. Over time, the body may develop a resistance to some therapeutic medications (2). Cancer therapies that focus on the tumor's hypoxic zone may be more successful than conventional treatments (3). Anti-cancer bacteria and viruses are currently being studied by scientists as a consequence of these chemotherapy's adverse effects (4). In the context of oncolytic treatment, we refer to viruses that can target cancer cells. Viruses come in many shapes and sizes; however, not all of them are oncolytic. The characteristics of oncolytic viruses include non-pathogenicity, the capacity to target and kill cancer cells, and the potential to make tumor assassins by genetic engineering (5). Cancer patients in the 1950s and 1970s were given live viruses, which had a positive impact on their therapy or recovery. Several organizations have argued for the exclusive use of microbes to treat cancer for more than a century. The objective of this therapy is to induce an immune response that rejects the tumor and avoids recurrence. In the early 1900s, William Coley pioneered the use of microbes to cure cancer. Whenever the innate immune system identifies germs or tumors, this reaction takes place (6).

In this study, researchers looked into new findings about oncolytic bacteria and viruses and how they could be used in both experiments and clinical settings to prevent and treat cancer. The study protocol was approved by the Ethics Committee of Baqiyatallah University of Medical Sciences, Tehran, Iran (ethical code IR.BMSU.REC.1400.002).

### *1.1. Oncolytic bacteria*

Oncolytic bacteria, which are being developed as novel treatment agents for several forms of cancer, exert their effects in four distinct ways:

### *1.2. Using microorganisms as anti-cancer agents to strengthen the immune system*

The majority of bacteria include lipopolysaccharide, which is responsible for the production of cytokines. Temperature rise increases in the production of cytokines and cytotoxic T cells (CTL). Cancer cells are very sensitive to temperature changes. Infections that induce hemorrhagic necrosis might result in the collapse of tumor vasculature as a result of fever. Febrile illnesses may aid in cancer treatment by activating a cascade of proinflammatory chemicals capable of stimulating dendritic cells and ultimately activating T cells (7).

*1.3. Substances produced by anti-cancer bacteria*

Substances generated by some bacteria, such as enzymes, cause the tumors to cease growing. Several experimental investigations have shown bacteriocin's therapeutic potential against a variety of cancer cell lines. Bacteriocins are cationic peptides generated in the ribosome that are secreted by practically all bacterial species. Certain bacteriocins are more toxic to cancer cells than to normal cells (8).

*1.4. Production of biofilm by anti-cancer bacteria*

Certain bacteria induce infections in tumor tissue via the formation of biofilms. This infection initiates an immunological response that is marked by a rapid influx of neutrophils to the infection site (9).

*1.5. Bacteria as a transporter of anticancer agents*

One of the difficulties in cancer treatment is the limited penetration of anticancer medications (chemotherapy) and other biological treatments, such as monoclonal antibodies and cytokines, into tumor tissue. Bacteria are capable of penetrating malignant tissue and serving as carriers for therapeutic compounds and medications (10).

*1.6. Tumor cells are destroyed by oncolytic bacteria in two primary ways: direct and indirect.*

*2.6.1. Tumor cells are lysed directly*

Certain chemicals generated by bacteria, such as enzymes, have been shown to inhibit the growth of tumors. Numerous recent experimental investigations have shown the therapeutic potential of various chemicals generated by bacteria for the treatment of cancer cell lines, including Bacteriocins and Bacteria-mediated anti-angiogenesis therapy (10).

*1.6.2. Immune response induction*

The interaction between the host and microorganisms acting as a pathogen has been shown to boost the immune system. For example, some bacteria reduce tumor growth by stimulating the inflammatory cytokine interleukin-1ß in tumors (11). Experiments have shown that some bacteria induce the immune system and T-lymphocytes CD8, which can clear the tumor (12). Some facultative anaerobic bacteria increase the level of TNF-α and proinflammatory cytokines in the blood. This induces an influx of blood, bacteria, and neutrophilic granulocytes into the tumor tissue and destroys the tumor tissue (13).

*1.7. Important types of oncolytic bacteria*

*1.7.1 Clostridium*

*Clostridium spp. bacteria* are gram-positive, anaerobic, and capable of producing spores (14). Connell discovered *Clostridium histolytic* in 1935. It generated proteolytic enzymes that destroyed tumor cells without causing injury to healthy or normal tissues (15). Malmgren and Flanigan discovered in 1955 that clostridium might have a selective impact on necrotic or hypoxic malignant tissues (16). *Clostridium bacterium* was shown to be safe in early cancer treatment testing (17). For several reasons, concerns about the security of Clostridium-mediated cancer therapy can be dispelled. Clostridial spore injection was astonishingly well-tolerated even in the earliest tests (18). Janku et al. (2021) concluded from their clinical studies that the injection of *C. novyi-NT* caused a transient systemic cytokine response and improved systemic tumor-specific T-cell responses. Toxicities from *C. novyi-NT* intratumoral injection can be severe but are controllable. Additional research on *C. novyi-NT* in people is supported by the indications of anticancer efficacy and host immunological response (19). Systemic hydration may be used to successfully control the toxicity. While cobalt-related toxicity requires more research, the appropriate chemotherapeutic technique may aid in its management. Finally, one of the most significant advantages of employing live bacteria in cancer treatment is that the treatment may be discontinued at any moment if a patient has adverse consequences (20). In hypoxic settings, *C. novyi-NT* kills tumor cells. In clinical settings, the production of this kind of bacteria also results in a significant innate and acquired anti-tumor response. Before recombinant microorganisms may be employed therapeutically, antibiotic-resistant indications must be removed. Numerous scientific discoveries in recombinant microorganisms indicate that this issue will be resolved in near future.

*1.7.2. Bifidobacteria*

*Bifidobacterium* is a genus of gram-positive, obligate anaerobic bacteria. They are naturally occurring members of the main colonic microbiota, comprising up to 25% of cultivable fecal bacteria in adults and up to 80% in infants. No adverse consequences were seen when *Bifidobacterium bifidum* was administered intravenously into cancer mice. These bacteria alter tumor tissue by converting 5-fluorocytosin (5FC) to 5-fluorouracil (5FU), a routinely used anticancer drug that is highly specific for tumor tissues (20).

*1.7.3. Salmonella typhimurium*

*Salmonella typhimurium* is a gram-negative anaerobic pathogen that is mostly found in the intestinal lumen. It is capable of limiting tumor growth by delivering genes encoding cytokines and converting prodrugs into very effective anticancer drugs and toxins. For example, modified attenuated *Salmonella Typhimurium* may express cytosine deaminase capable of generating the anticancer agent

fluorocytosine 5, and uracil mimics the replication mechanism of tumor cells and suppresses tumor cell proliferation (21). Angiogenesis plays a vital role in the progression of cancer. Salmonella may potentially impair angiogenesis or cause damage to blood vessels in tumor tissue, thereby retarding tumor growth. Salmonella infection may inhibit one of the most important angiogenic factors, vascular endothelial growth factor (VEGF), both in vivo and in vitro (22-26). Despite the enormous benefits of Salmonella in cancer therapy, this bacterium is incapable of preventing metastasis or tumor formation on its own. In 1999, Salmonella was approved for Phase 1 clinical trials. It was administered intravenously to one patient with renal cell cancer and 24 patients with metastatic melanoma. According to the results, Salmonella might be used to treat cancer cells (27). Additional studies are required to determine the toxicity of the dosage and the proper placement of Salmonella. Three patients with refractory cancer were recruited in an exploratory experiment to assess the feasibility and effectiveness of intratumorally administering TAPET-CD, which seems to be without significant side effects (28). Numerous studies indicate that differences in the growth rates of human and animal cancers may influence the anticancer effects of modified *salmonella bacteria*, including tumor growth rate, bacterial entry into tumors, bacterial proliferation in tumors, and bacteria clearance from the environment and tumors. Nonetheless, there are some serious problems with the use of this modified anti-tumor bacterium. To begin, we may consider immunity, which is a key condition for treating *salmonella*-associated cancer. *Salmonella* may multiply in the bloodstream, create bacterial toxins, and possibly cause a severe septic shock if the germs are not sufficiently weak. However, genetic modification, such as gene deletions expressing LPS, amino acids, or purines, dramatically decreased the severity of *Salmonella* illness (29-31). Although the bulk of *Salmonella*'s anticancer activity has been shown in this study, the molecular mechanisms behind this action remain unclear. Numerous studies on various bacterial strains, animal strains, and tumor cell lines have been undertaken utilizing a number of different infection procedures and injection times, which adds to the complexity of *Salmonella* anticancer activities. Finally, the bulk of positive results from animal studies indicating the advantages of genetically-modified *Salmonella* cancer treatment have not been confirmed in human clinical trials. Preclinical research should be conducted on tumors in animals that have a high degree of similarity to human tumors.

*1.7.4. Listeria monocytogenes*

*Listeria monocytogenes (L. monocytogenes)* is a gram-positive rod-shaped bacterium that is facultatively anaerobic and incapable of generating spores (32). The human immune system often manages infections caused by *L. monocytogenes* through an innate and adaptive immune response. Due to these enticing properties, "the potential for repeated administration and utilization to enhance T cell response and the power of bacteria to induce innate and adaptive immunity," the majority of studies choose *L. monocytogenes* as an appropriate vector (33). Experiments using *Listeria* as a vaccine carrier for a number of ailments produced positive results. *Listeria* is capable of immunizing animals in high numbers against tumor proteins and overpowering their resistance; moreover, it is completely safe to use in people (34). Recent research indicates that vaccines derived from *Listeria* may selectively target CD105 expression in tumor vasculature. CD105 is a transmembrane glycoprotein with a wide transmembrane domain that is required for TGF- signaling and angiogenesis. Anti-CD vaccines derived from *Listeria* produced therapeutic responses in primary and metastatic tumors of the 4T1-Luc and NT-2 breast cancer animal models (35). Additionally, several studies have shown that attenuated *Listeria* is an ideal model for delivering anticancer agents, such as therapeutic radionuclides into metastases and the primary tumor microenvironment. Radionuclides release radioactive particles that harm and kill cancer cells, and these medicines are not susceptible to multidrug resistance mechanisms (36). *L. monocytogenes* is most often seen in immunocompromised individuals, such as babies, the elderly, and pregnant women. As a consequence, clinical vectors are much less effective than the wild strain. Because the carriers used do not include antibiotics, they are easily treated in the case of adverse responses after vaccination. *Listeria* flourishes in environments other than those containing animal products because, unlike viral vectors, the bacterium's DNA does not integrate into the host genome (37). Despite the many benefits shown in preclinical models of *Listeria* immunization, there is currently no FDA-approved vaccine available. Additional improvements in *Listeria* vaccine technology are expected to occur in the coming years as these trials progress. Indeed, after early clinical trials in which *L. monocytogenes* was employed to create a single tumor antigen, the technology for *L. monocytogenes* vaccines developed to include vaccines expressing several tumor antigens. By targeting a large number of antigens, it is possible to produce therapeutic responses that are less susceptible to antigen escape. On the other hand, vaccination against many tumor antigens may be inadequate to produce therapeutic responses. Tumors have a varied array of cell types, and it was previously believed that the tumor microenvironment acts as a protective barrier, containing antitumor responses

(34, 35, 38, 39).

*1.7.5. Bacillus*

*Bacillus spp.* are rod-shaped gram-positive bacteria that are either obligately aerobic or facultatively anaerobic and capable of producing spores. A study of *Bacillus thuringiensis'* insecticidal activity discovered a new protein capable of recognizing and destroying cancer cells. *Bacillus subtilis* and *Bacillus licheniformis* are also capable of generating compounds that are toxic to cancer cells. *Bacillus spp.* have considerable biological activity in vitro as a consequence of their ability to synthesize carcinogenic compounds. Experiments have shown that fengycin, an antimicrobial peptide generated by Bacillus subtilis, may prevent colon cancer development. Duarte et al. demonstrated that fengycin inhibits the growth of breast cancer cells (40). While theoretical studies suggest that fengycin may be useful in the treatment of colon cancer, more study of its molecular mechanism is required.

*1.7.6. Probiotics*

Probiotics are a kind of live microorganism that has a variety of health benefits. Probiotics are increasingly being utilized to prevent and treat gastrointestinal disorders, such as irritable bowel syndrome, inflammatory bowel disease, bacterial or viral infections, and antibiotic-associated diarrhea. Probiotics have been demonstrated in laboratory experiments to have anti-cancer properties. Certain lactobacilli possess antitoxin action against a range of carcinogens, including 1,2-dimethylhydrazine (DMH) and N'-nitro-N-nitrosoguanidine (41). These bacteria suppress the formation of azoxymethane-induced ectopic crypt foci, which are the most common kind of neoplasm. The mechanism through which probiotics fight colon cancer is unknown (42).

*1.7.7. A quick overview of oncolytic bacteria therapy*

Table 1 shows some selected clinical trials, cell culture, and animal model studies that have used oncolytic bacteria in the treatment of cancers.

**Table 1**. Oncolytic bacteria in the treatment of cancers

| Type of study | Author's name | Country | Bacteria type | Sample | Cancer type | Immunological mechanism | Result |
|---|---|---|---|---|---|---|---|
| Cell culture | Hoffman, Robert M et al 2016(43). | USA | Salmonella typhimurium A1-R | Stomach adenocarcinoma cell line, MKN45 | Stomach adenocarcinoma | *S. typhimurium*A1-R trigger cell-cycle transit of quiescent cancer cells | Corruption of cancer cells by S. typhimurium A1-R |
| | Olino, Kelly, et al.2012(44) | USA | Listeria monocytogenes | Colon cancer cell line, CT26 | Colon cancer | Decreased expression of molecules PD1 within the tumor environment | Enhance antitumor efficacy against CT26 metastases |
| | Cheng, W., et al.2016(45) | China | Bacillus subtilis | Colon cancer, HT29 | Colon cancer | 1) Apoptotic cells in control cells were increased in cells treated with fengycin. 2) Fengycin could induce cell cycle at G0/G1 stage | Fengycin cause Bax, Caspase-3, and Caspase-6 expressions were increased; however, Bcl-2, and CDK4/cyclin D1 expressions were decreased |
| | Parisa, Asadollahi, et al.2020(46) | Iran | Bifidobacteria (BC) | Colon carcinoma cell line, LS174T | Colorectal cancer (CRC) | 1) BC induced ~21% apoptosis among LS174T 2) BC decreased the expression of EGFR by 4.4 folds, HER-2 by 6.7 folds, and PTGS-2 by 20 folds among the LS174T cells | It prevented the tumor from developing to higher stages and worsening, improving intestinal length |
| | Yao et al. 2010(47) | Australia | Clostridium perfringens | (MCF7), (SW480), (A431), (A549), (Hela), (HUVEC), (DU145, PC3) | Human breast cancer , Colon cancer cell lines , Skin epidermoid cancer cell line , Lung cancer cell line , Cervical cancer cell line , Human umbilical vein endothelial , Human prostate cancer cell | C-CPE-ETA0 was fully effective towards CLDN-4 positive cancer cells, contrary to cells missing CLDN-4 expression. | The mixture, merging protein specifically bound was fast and fully internalised into CLDN-4 overexpressing cancer cells by endocytosis, and subsequentially it occurred in cell apoptosis, as argued to cell lines lacking CLDN-4 expression |

**Table 1.** Continued

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| **Animal model** | Ganai et al. 2009(48) | USA | Salmonella typhimurium | Mice | Breast cancer | Way has attenuated the initiation of NF-kB, heading to a definite death signal through p53-independent tools | Pre-clinical use of S. typhimurium as a sequence expression vector that completely decreases tumor mass and increases host continuance |
| | Loeffler et al. 2008(49) | USA | Salmonella typhimurium | Mice | Colon cancer breast cancer melanoma | Amount of interferon-γ (INFγ), CXCL9, and CXCL10 was significantly raised in tumors of mice | Engineering S. typhimurium to show the chemokine CCL21. These windings reach our recent interpretation of bacteria provided to express the cytokine LIGHT, a TNF-family member |
| | Gunn et al. 2001(50) | Belgium | Listeria monocytogenes | Mice | Tumor | Turned our consideration to important tumor special Ags, the HPV-16 proteins E6 and E7 that are constitutively displayed in HPV-16-associated tumors. E6 and E7 expression is adequate to praise murine or human cells. | This treatment caused regression in 75% of tumors revealing E7 antigen. This answer depends on TCD4+ and TCD8+ lymphocytes, as well as the INFg secretion |
| | Lemmon et al.1997(51) | USA | Clostridium. beijerinckii | Mice | EMT6 tumors | 22-fold increase in cell killing by cb 1954 when colesteridia-produce nitroreductase | Nitroreductase action raised in vitro antitumor activity of CB in 1954, by a factor of 22. |
| | Kim et al. 2008(52) | USA | Listeria monocytogenes | Mice | Breast cancer | Agents that repress the generation of IL-6 could enhance the potency of vaccination or chemotherapy for breast cancer | The most effective vaccine for breast tumors, reducing amount of metastasis by 96%, relating to a powerful CD8+ lymphocytic answer in the spleen after re-stimulation with antigen treatment |
| **Clinical trials** | Nemunaitis et al. 2003(21) | USA | Salmonella | Cases with excellent and/or metastatic cysts | Solid tumors | Change of 5-FC to 5-FU | A positive response was seen in 2 patients at their injection site. Bacterial CD dependent change of 5-FCto 5-FU. Conferred conflicting effects not related to therapy |
| | Toso et al. 2002(27) | USA | Salmonella typhimurium | 25 patients | Metastatic melanoma, Metastatic renal cell carcinoma | VNP20009 was also discovered to hinder tumor growth in mice, and therefore, was seen as a possible antitumor agent in cancer cases | From the 25 cases managed with VNP20009, none tried an measurable tumor regression. Dose-limiting toxicity was correlated To TNF-α and IL1-β excretion, despite the majority of conflicting effects recorded reversibility |
| | Roberts et al. 2014(53) | USA | Clostridium novyi-NT | - | High-grade gliomas | Intratumoral inoculation of C. novyi-NT spores could also obtain a dominant localized inflammatory answer, as well as an adaptive immune response upon tumor cells | Displayed lack of viable tumor cells. By day 55, it was shown with a pathologic break. Treatment increased the patient's quality of life |
| | Maciag et al. 2009(54) | USA, Serbia | Listeria monocytogenes | 15 patients | Advanced carcinoma of the cervix | Novel intracellular life cycle provides antigens secreted by Lm to be prepared and displayed in the setting of both MHC class I and II molecules, producing ineffective cytotoxic CD8+ and Th1 CD4+ T-cell-mediated immune responses | At the end of the study, 2 cases died, 5 developed disease progression, 7 showed stable disease, and incomplete tumor response was observed in one case |
| | Le et al. 2015(55) | USA | Listeria monocytogenes | 90 patients | Metastatic pancreatic cancer | Live-attenuated *Listeria* monocytogenes–expressing mesothelin, influences innate and adaptive immunity | Survival time was increased in treated patients |

*1.8. Oncolytic viruses*

Oncolytic viruses (OVs) which are used as new *therapeutic* strategies to treat different types of cancer exert their efficacy in three main different ways:

*1.8.1. Targeting cancer cells via infecting them results in a term called oncolysis which leads to the elimination of cancerous cells.*

Some OVs exert their influence through mechanisms, such as virolysis and the introduction of suicide genes, which are engineered accordingly.

*1.8.1. Enhancing the potency of the immune system to develop a long-lasting anti-tumoral and anti-viral immunity.*

The interaction of oncolytic viruses with the immune system involves restrictive and stimulant measures. The immune system counteracts the development of antiviral pathways (such as interferon type 1) and the imposition of inhibitory strategies (neutralizing antibodies). OVs also have the ability to provoke antitumor immunity by activating NK cells, T cells, and DC cells (56).

*1.8.2. Showing anti-angiogenic effects on tumor cells by either down-regulation of angiogenic or expressing anti-angiogenic factors.*

Oncolytic viruses (such as vaccinia and vesicular stomatitis virus) can inherently target and disrupt tumor vessels. These manipulated viruses are able to down-regulate angiogenic factors in tumor tissues or molecules or express anti-angiogenic factors (57).

*1.8.3. Infection mechanism of OVs*

Reduced IFN activity coupled with increased EGFR and downstream signaling pathways, such as Ras, PI3K, and MAPK activation, might let Ovs bypass the immune system and infect cancer cells while multiplying and spreading to produce progeny that could lead to the demise of tumor cells (58-60). Because cancer cells are able to evade the body's immune system in order to thrive, they provide a perfect environment for a wide range of viruses. Tumor cells have become an ideal host for a wide variety of viruses by preventing interferon signaling and avoiding apoptosis. Furthermore, cancer cells that overexpress a variety of genes, including CAR, CD155, and laminin, are more susceptible to viral infection. It is also important to note that many of the tumor microenvironment components that confer resistance on cancer stem cells (CSCs) to traditional therapies are ineffective against oncolytic viruses (61) (Figure1).

*1.8.4 OVs destroy tumor cells in two main ways: Direct and Indirect*

*2.8.4.1. Direct lysis of tumor cells*

Replication of viruses and producing viral progenies in cancer cells followed by the recognition of these cells by NK cells of the innate immune system and CD8+ cytotoxic T lymphocytes lead to tumor cell destruction (62). OVs infect and replicate cancer cells, inducing tumor cell lysis and releasing infectious viral progeny that spreads to surrounding tumor cells (amplification of oncolysis). Oncolytic vaccinia virus and vesicular stomatitis virus can target tumor vasculature affecting tumor blood supply, and therefore, tumor progression.

Oncolysis also releases tumor-associated antigens (TAAs), cellular damage-associated molecular patterns (DAMPs), and pathogen-associated molecular patterns (PAMPs) in a highly inflammatory process, termed "immunogenic cell death". Cellular detection of viral infection and the products of oncolysis trigger the rapid activation of a host antitumor immune response. The direct recognition and killing of tumor cells are primarily mediated by natural killer cells of the innate immune system and tumor antigen-specific CD8+ cytotoxic T lymphocytes (blue cells) of the adaptive immune system. Other roles that can be mentioned for NK cells include interacting with DCs and direct effects on T cells; affecting adaptive immunity, NK cell-derived IFN-γ, and TNF-α; better regulating excitatory and migratory markers; and production of IL-12 and IFN-γ, which DCs mediate, thereby incrementing their potency to prime cytotoxic T cells. (62).

*1.8.4.2. Indirect lysis of tumor cells*

OVs have the circumstantial ability to generate inflammatory reactions by PAMPs and release tumor Ags, thereby acting as in situ cancer vaccines. Genetic manipulation of immunomodulatory genes, such as cytokines (e.g., GM-CSF for T-Vec), or immunosuppressive inhibitors, enhances the immunity produced by OV against tumors (62) (Figure 2).

*1.9. Important types of oncolytic viruses*

*1.9.1. Adenovirus*

Many studies are being conducted on adenoviruses, which are double-stranded DNA viruses without an envelope. In order to create oncolytic adenoviruses, scientists had to make a number of alterations to the original adenoviruses' genomes due to their incapacity to selectively target tumor cells (63). Replication of the virus would be confined to cancer cells because of a minor loss of viral genes. The virus can only replicate in cells with a low level of P53 due to the deletion of E1B55K from its viral genome (64). It is necessary to eliminate E1B19K to increase viral progeny release in cancer cells by inhibiting FAS-mediated apoptosis and deleting eight amino acids from the Rb-binding region of the E1A proteins in cancer cells. Finally, cancer cells must be forced into entering the S phase of the cell cycle to allow virus replication in tumor cells while disrupting Rb function (65). In order to turn normal adenoviruses into oncolytic adenoviruses, tumor-

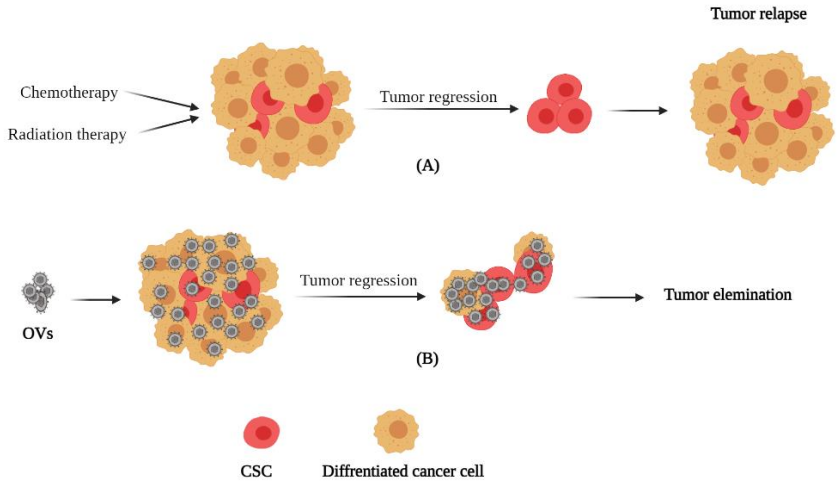


**Figure 1.** (A) The use of traditional therapies which are ineffective due to the presence of cancer stem cells and fail. These treatments can kill the differentiated cells that make the mass of the tumor, but they have no effect on the cancer stem cells, and as a result, the disease return is noticed after the initial treatment. (B) OVs can kill differentiated cells and CSCs, and therefore, may eradicate the disease

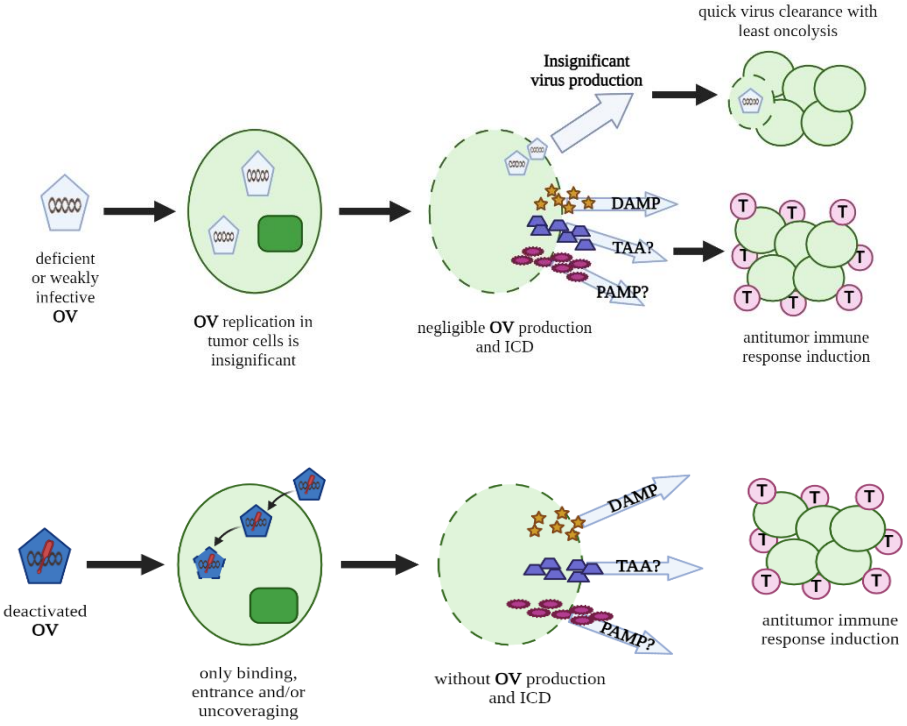


**Figure 2**. Mechanisms by which OVs may be associated with replication by efficacy. Defective or weak OVs after the infection of tumor cells, although producing small amounts of viral particles, do not cause the spread of infection due to the host antiviral response. (Quick virus clearance with least oncolysis) However, defective oncolytic viruses also have the ability to kill tumor cells in an immunogenic way. Binding of the virus alone or insignificant proliferation of the virus in the host, entrance and/or lack of coverage in tumor cells to form immunogenic cells results in the release of damage-related molecular patterns that ultimately activate or enhance the host's immune response to tumor cells (Purple cells: cytotoxic T lymphocytes). There are doubts about the activity of tumor-associated antigens or the degree of involvement of pathogen-related molecular patterns because they are unknown

specific promoters like the prostate-specific antigen promoter may be inserted into the adenovirus genome. For instance, the E1A genes are produced via the insertion of the E2F-1 promoter in adenovirus serotype CG0070 (66). TERT promoter usage to increase the expression of E1A-dependent genes in specific cancer cell types, such as glioblastoma, is another example of such insertions (67). For viral therapy, *adenoviruses* are a good candidate because of three key characteristics. Virus offspring may be

multiplied in huge numbers. It is possible to effectively transmit adenoviral genes to cells that are actively reproducing, as well as those that are not. The *adenovirus* structure can be modified genetically (68). Since adenoviruses have such broad tissue tropism, they may infect almost every kind of cell. The physicochemical stability of viral particles is also unusual (69). Tumor-specific promoters like Survivin, which are overexpressed in cancer cells, govern viral gene replication like E1 in adenovirus oncolysis. On the other hand, adenoviral gene expression may be regulated by malfunctioning biological transcriptional machinery if pRb and p53 activity is disturbed in most cancer cells. It is unlikely that *adenoviruses* would cause disease in humans since their DNA does not integrate into the cells of the host (70). People's pre-existing immunity to adenoviral therapy is compromised by their lifetime exposure to several serotypes of this virus, which is a significant impediment to their usage as oncolytic medicines (71, 72). The tropism of Adenovirus vectors also means that systemic administration might result in hepatotoxicity (72-75). Because of their non-specific trapping in organs like the spleen and liver and subsequent clearance by the immune system, *Adenovirus* administration is usually locoregional (76). More than 40 clinical trials have used a variety of oncolytic Adenoviruses. Oncologists have studied a variety of cancers, including glioma, prostate cancer, and hepatocellular carcinoma, throughout the years at different phases of clinical trials. There have been several studies that have employed oncolytic adenoviruses, such as H101 and DNX-2440, in combination with various injection techniques, including intravenous, intratumoral, and intraperitoneal (77). A 6-month complete response and a long-term response have been described in bladder cancer and glioblastoma patients receiving adenoviral therapy, respectively, as the best results (78, 79).

*1.9.2 Herpes virus*

*Herpes simplex virus (HSV)* types 1 and 2, which include double-stranded DNA and an envelope, have attracted substantial attention as modified oncolytic viruses. In order to gain oncolytic capabilities, T-VEC is a well-known oncolytic HSV-1. For cancer cells with a deficient PKR pathway, ICP34.5 gene deletion is employed to inhibit viral replication. Antigen recognition is improved when human GM-CSF is included in a virus' genome. Additionally, the ICP47 gene has been partially deleted to boost the virus's safety since it prevents normal cells from being evaded by the immune system. The US11 gene is quickly expressed after this deletion to compensate for the negative effect of the ICP34.5 deletions (80). One of the oncolytic HSVs is produced from *HSV* type 2 and possesses anticancer characteristics in metastatic malignancies, such as ovarian cancer, as well. When a specific domain (protein kinase) is removed from the ICP10 viral gene, the virus may thrive in tumor cells with an active RAS pathway. To treat ovarian cancers, this kind of virus has been shown to be more efficient than *HSV-1* (81). As a cancer-killer, *Herpes Virus* has several advantages over other viruses, and it is capable of infecting and quickly multiplying in a wide range of tumor cells. Genome-wide alteration and transgene inserts are possible because of its size. It is possible to utilize antiviral medicine to prevent this illness; however, the quantity supplied presents a danger to the patient's health. Its glycoprotein may be easily modified to improve cancer cell targeting (82). Although genetic alteration of *HSV* genes has made it safer and more appropriate to target tumor cells, overall efficiency has decreased. Another limitation is related to how the software is implemented. Since components, such as the extracellular matrix hinder the virus's propagation, direct injection is favored in cancer therapy as an example. The virus's reproduction and propagation may be hindered by acquired anti-virus immunity (83, 84). One of the several oncolytic *HSV* strains, T-VEC, has been approved by the FDA after thorough clinical testing. Studies like the HF-10 oncolytic *HSV* study of metastatic melanoma using this drug have already been concluded. M03 for malignant glioma is at the "recruiting" stage, whereas HF with gemcitabine and nab-paclitaxel for pancreatic cancer is now in the "active" stage of clinical trials. Each study has a specific goal that includes testing the virus's efficacy, safety, and tolerance in addition to testing the virus's exact dose (85).

*1.9.3. Vaccinia virus*

*Vaccinia virus* (VV), the genome of which is made up of double-stranded DNA, has a number of properties that make it a likely weapon in the fight against cancer. Due to the viruses' brief existence in the cytoplasm, it is able to attack cancer cells without being recognized by the body's immune system. This virus is an excellent candidate for eliminating cancer cells because of its high sensitivity to type 1 INF, its ability to connect to a variety of receptors, and its vast genome size (86). Wyeth, Lister, Western Reserve, and Copenhagen are all VV strains that have oncolytic activity, with Western Reserve being the most effective.

Oncolysis by VV happens in three unique ways:

• Direct lysis of tumor cells followed by necrosis and apoptosis as a consequence of the virus reproducing and producing progenies.

• Cell death is mediated by the immune system as a consequence of cellular signals, such as TAAs, DAMPs, and PAMPs.

• Apoptosis induction in uninfected cancer cells is characterized by vascular collapse and an insufficient blood supply to the tumor's core (87).

Numerous genetic alterations are being made to the VV in order to make it a more efficient tool for cancer cell eradication (88). JX-594, for instance, is a genetically engineered VV that lacks the thymidine

kinase gene but expresses the GM-CSF and Lac-Z transgenes. This mutation resulted in a more tumor-specific form of cancer cell destruction (89). The WR-4 virus was recently generated by deleting four viral genes: A48R, B18R, C11R, and J2R. This virus has been demonstrated to be effective in the treatment of tumors in melanoma animal models (90). VV is a prospective oncolytic agent for cancer therapy owing to a number of benefits, including its capacity to infect a broad range of cancer cells, a good safety profile in persons with a history of smallpox vaccination, and the ability to contain transgenes in their genome (91). Additional advantages of VV include rapid replication, a high level of gene expression, the capacity to pass cell to cell, and the fact that the virus's activity is unaffected by hypoxia or therapeutic irradiation (92). Furthermore, this virus does not infect normally healthy persons. The use of VV as an oncolytic agent has a number of disadvantages. For example, there is a weak viral infection and the tumor has a low selectivity. On the other hand, this virus may provide antiviral immunity (93). Additionally, there are a number of difficulties linked with the virus's systemic spread (94). This virus's genome has around 200 viral genes, half of which are unknown in function, making this virus very unpredictable. Another downside of employing VV to eliminate cancer cells is that the virus is incapable of infecting widely dispersed tumor cells across extended distances. Numerous preclinical studies on VV have been conducted, and few have moved to clinical trials. These preclinical investigations on melanoma (95), bladder cancer (96), and HCC (97) using the VG9-GMCSF virus were undertaken to assess the virus's antitumor activity. *Pexa-Vec* is an oncolytic vaccinia virus that was used in clinical trials for liver cancer to identify the viral dose required to induce anti-tumor immunity. A longer patient life was seen in correlation with the virus dosage supplied (98). GL-ONC1 is another oncolytic VV that is now being explored in head and neck cancer clinical trials (99).

*1.9.4. Reovirus*

*Reovirus* is a double-stranded RNA virus that has been utilized in the treatment of cancer as a biological agent. The virus enters cells through contact between its 1 protein and junctional adhesion molecule A, which results in the virus's outer capsid being eliminated in the endosomes of the cell. The next stage is the production of infectious subvirion particles. Following transcription and translation of the freed viral core in the cytoplasm, new viral progenies may elicit apoptosis, autophagy, or necroptosis in cells (100). The tumor selectivity of this virus is highly dependent on the RAS signaling pathway. *Reovirus* transcription may activate PKR in normal cells, thereby limiting viral replication. However, the RAS pathway is active in the majority of cancer cells, and PKR operates improperly. Therefore, the replication of the virus seems to be confined to cancer cells (101). Reolysin or Pelareorep (oncolytic *reovirus*) is a potent inducer of apoptosis and inhibitor of angiogenesis. By boosting CXCL10/IP-10, decreasing HIF activity, and limiting VEGF release, reolysin has been found to suppress angiogenesis in soft tissue sarcomas (102). *Reovirus* has a variety of advantages as an oncolytic virus. This virus has not been associated with any severe human illness. It has the ability to locate and kill tumor cells. Additionally, since the cytoplasm does not undergo DNA synthesis during its existence, DNA insertion mutations in the host genome are avoidable. Clinical studies have shown a minimal incidence of pathogenesis after systemic infection with this virus. This virus is very cytotoxic to cancer cells and quickly multiplies inside them (103). Additionally, oncolytic reovirus T3D offers a number of advantages, including an affinity for RAS mutant cancer cells, minimal toxicity, selectivity, and a dosage that is tolerable (104). On the other hand, T3D presents a number of difficulties, including the virus's inability to be neutralized by antibodies (105), off-target effects (106), and trouble with the systemic distribution. Numerous clinical trials are being conducted to determine the efficacy of *reovirus*. To assess the dosage and safety of *reovirus* therapy for brain tumors, the wild-type *reovirus* was used. Reolysin was used in combination with paclitaxel to evaluate the virus's efficacy in ovarian cancer when combined with paclitaxel. In clinical studies for myeloma, *reovirus* was used in conjunction with other agents, such as carfilzomib, dexamethasone, and nivolumab, to determine the best dosage (107).

*1.9.5. Coxsackievirus*

*Coxsackievirus* is a single-stranded RNA virus that comes in two strains: *CVA21 and CVB3*. A decay-accelerating factor called "ICAM-1" helps this virus penetrate cells and lyse them (108). In immunocompromised mice with melanoma, *CVA21* has been shown to reduce tumor cell volume in xenografts (109, 110). Additionally, *CVB3* has been shown to be an excellent cancer-fighting agent. Anti-apoptotic and mitogen-activated protein (MAP/ERK) signaling pathways were stimulated in vitro by *CVB3* injection. In addition, a significant amount of tumor regression was seen in the animal model. One of the many advantages of using *Coxsackievirus* as an oncolytic agent is that it has no oncogenes or carcinogens and can easily be genetically changed, making it a viable option for treating cancer (111). A study indicated that *CVB3* has several advantages, including the capacity to effectively administer the drug systemically and eliminate metastatic cancer cells (112). Clinical experiments have made use of the oncolytic coxsackie viruses *CVB3* and *CVA21*. It was tested in a mouse model of lung cancer for its antitumor effectiveness, and in a clinical study for its safety and anticancer potential, both of which were

found to be positive (113). Another oncolytic coxsackie virus, *CVA21*, has been used in preclinical and clinical studies on a wide range of cancers and has shown significant anticancer effectiveness and tolerance (114). Clinical trials with oncolytic viruses to treat cancer are summarized in Table 2.

**Table 2.** Oncolytic virus in the treatment of cancers

| Type of study | Author's name | Country | Virus type | Sample | Cancer type | Immunological mechanism | Result |
|---|---|---|---|---|---|---|---|
| Cell culture | Wang, Beibei , et al. 2018(115). | Japan | Coxsackievirus A11 (CVA11) | The CRC cell lines WiDr and Caco-2 | Colorectal cancer (CRC) | Several cytokines have been reported to enhance the expression of both CD55 and ICAM1, including tumor necrosis factor-α, transforming growth factor-β1, and interferon-γ | The level of expression of CD55 and ICAM1 mRNA was significantly upper in oxaliplatin-treated cells |
| | Thirukkumaran, Chandini, et al.2017 (116). | Canada | Reovirus | Breast Cancer cell lines HTB133, HTB 132, MCF7 and HTB30 | Breast Cancer (BrCa) | There are proofs that reovirus applies its oncolytic relic via apoptosis: 1) phosphatidyl serine expression 2) DNA fragmentation 3) mitochondrial dysfunction | Reovirus upregulates NF-kB and PUMA activity in BrCa |
| | Ding, Yuedi, et al.2020 (117) | China | Vaccinia virus-VG9-Luc | The mammary carcinoma cell lines MDA-MB-231 (human) and 4T1 (murine) | Mammary carcinoma tumor | vaccinia VG9-Luc activates the host cell immune response | Antitumor antibodies, such as IFN-γ produced by a viral infection, increase tumor-specific immunity |
| | Gholami, Sepideh, et al.2014 (118) | USA | Herpes simplex virus-1 (NV1066) | Breast Cancer cell lines, MDA-MB-231, HCC1806, HCC38, HCC1937, and HCC1143 | Breast cancer (BrCa) | NV1066 considerably down-regulates MEK/MAPK pathway in TNBC that infected | Significantly destroy TNBC through cytolysis induction |
| | Zhou, Yong-an, et al.2010 (119) | China | Adenovirus-PTEN | Esophageal adenocarcinoma cell lines Eca-109 and TE-1 | Esophageal cancer | Recombinant vector Ad-PTEN can impress the G2M cell-cycle detention affected by the overexpression of Bcl-2 | Overexpression of PTEN significantly suppressed the growth of and induced apoptosis |
| Animal model | Liu, Huitao, et al.2020 (112) | Canada | Coxsackievirus B3 (CVB3) | Mice | Lung Cancer | Insert several copies of the target sequences of the miR-145/miR-143 into the viral genome | Destroying both *KRAS* mutant lung adenocarcinoma and *TP53*/*RB1*-mutant SCLC |
| | Seyed-Khorrami, Seyed-Mahmood, et al.2021 (104) | Iran | Reovirus | Mice | Most cancer | Enhancement of the rate of stimulation and secretion of IFN-γ cytokine level | Increase in IFN-γ secretion |
| | Ding, Yuedi, et al.2020 (117) | China | Vaccinia virus-VG9-Luc | Mice | Mammary carcinoma tumor | Vaccinia VG9-Luc activates the host cell immune response | Antitumor antibodies, such as IFN-γ produced by a viral infection, increase tumor-specific immunity |
| | Benencia et al. 2008 (120) | USA | Herpes simplex virus-1(HSV-1) | Mice | Ovarian carcinoma | Increased DC maturation and tumor infiltration of INF-γ+ CTL | The antitumor immune responses are facilitated by oncolytic therapy with HSV-1716 |
| | Hassan, Faizule, et al.2018 (121) | USA | Adenovirus serotype 5 | Mice | Pancreatic and liver cancer | Increase in serum TNFα and IL-6 | Reduced cancer cell growth and increased chemotherapy susceptibility |

**Table 2.** Continued

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| **Clinical trials** | Müller et al.2019 (122) | United Kingdom | Coxsackievirus-A21 | Blood | Acute myeloid leukemia (AML) | 1) Intrinsic anti-tumor immunity, through by cytokine-induced spectator destroying and NK cell activation<br>2) TAA detected and adaptive antitumor immunity developed against it | Enhancement of the expression of interferon-excited genes by CVA21 |
| | Parakrama et al 2021 (123) | USA | Reovirus | Serum | Colorectal Cancer (CRC) | 1) Immune provocative (increasing the effect of immunochemo therapeutic drugs)<br>2) Reovirus, so obligation bimodally as an oncolytic agent causes the killing of tumor cells | Reductions in the exosomal expression of miR-29a-3p with reovirus |
| | Park et al.2015(124) | USA | Vaccinia virus | Plasma | Colorectal Cancer (CRC) | Stimulating EGFR commonly expressed in tumor cells | Vaccinia virus preferentially targets and replicates in tumors |
| | Streby et al.2107 (125) | USA | Herpes simplex virus-1(HSV-1716) | Serum | Various pediatric cancers | HSV1716 targets cancer cells for viral replication and cancer cell lysis | The antitumor immune responses are facilitated by oncolytic therapy with HSV-1716 |
| | Gatti-Mays, Margaret E., et al.2020 (126) | USA | Adenovirus 5 (Ad5) | Plasma | Colorectal cancer cholangiocarcinoma | The vaccinated by TAV, produced CD4+ and/or CD8+ T-cell reactions at minimum one TAA encoded with the vaccine | Antigen-specific T cells to MUC1, CEA, brachyury were generated by Ad5 |

*2.9.6. A quick overview of oncolytic virus therapy.*

Table 2 shows some selected clinical trials, cell culture, and animal model studies that have used oncolytic viruses in the treatment of cancers.

## 3. Conclusion

As a result, a variety of bacterial species have the ability to thrive in cancerous tissues; the gene is used to treat malignancies in both wild and engineered strains of these organisms. Some important obstacles remain to the practical implementation of oncolytic bacteria in hospitals despite recent developments. There are several variables, such as tumor size and location, which vary from patient to patient. Even though bacterial toxicity to cancer cells has been shown in animal models, the effectiveness of oncolytic bacteria in humans is still unknown owing to the compromised immune systems of people with cancer. Human trials have also been conducted. It is also possible that genetic instability will lead to an oncolytic phenotype that is ineffectual or dangerous. Anti-cancer medicines, such as oncolytic bacteria and viruses, must be used in the right mix to eliminate tumor cells. Another issue is how to improve the efficacy of chemotherapy and immunotherapy by employing oncolytic viruses. It is now safe and effective to utilize oncolytic viruses in combination or monotherapy clinical trials. In other words, if the issues surrounding oncolytic viruses can be resolved, they might one day be a painless treatment option for cancer patients everywhere.

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## Footnotes

**Conflicts of Interest:** The authors have no conflict of interest to declare.

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