УДК 616-006-085.277.3:578.7:615.03
https://doi.org/10.20538/1682-0363-2024-4-158-168

Clinical trials on oncolytic viruses

Golovinov I.V.[1], Goncharova A.S.[1], Shulga A.A.[1], Vlasov S.N.[2], Dimitriadi S.N.[1]

[1] *National Medical Research Center for Oncology*
63, 14 Liniya Str., Rostov-on-Don, 344037, Russian Federation

[2] *Rostov State Medical University*
29, Nakhichevanskiy Av., Rostov-on-Don, 344022, Russian Federation

ABSTRACT

Oncolytic viruses (OVs) are a new class of targeted anticancer drugs with unique mechanisms of action. Oncolytic virotherapy has evolved from the use of in vitro-passaged strains (first generation) to genetically engineered viruses with increased selectivity (second generation) and, ultimately, to recombinant OVs expressing a transgene (third generation).

The aim of the review was to analyze and summarize data on the current state of clinical research on OVs.

A PubMed search identified 182 articles from 1997 to 2024 with 154 studies reporting data on 4,850 patients. We found that adenovirus (n = 44) is the most common OV in clinical trials with more than two-thirds (n = 108) using modified or recombinant viral backbones, and granulocyte-macrophage colony-stimulating factor (GM-CSF; n = 40) was the most common transgene. The most common tumors targeted were melanoma (n = 1,997) and gastrointestinal (GI; n = 916) cancers with the most common monotherapy received by intratumoral (n = 3,003) or intravenous (n = 1,318) delivery routes. The most common combination included chemotherapy (n = 54).

Treatment-related adverse events included low-grade constitutional symptoms and local injection site reactions. Measurements of virus shedding were frequently performed, but many studies were limited to blood and tumor tissue analysis, using only polymerase chain reaction (PCR). Although most studies reported antiviral antibody titers (n = 101), only a few reported virus-specific T-cell responses (n = 23). Objective responses were recorded in 458 (9.4%) patients and disease control was achieved in 1,141 (23.5%) patients, although standard reporting criteria were used in only 60.4% of cases.

These data provide an insight into the current state of clinical research on OVs and highlight potential areas requiring further investigation to better define the role of OVs in cancer treatment.

Keywords: oncolytic virus, immunotherapy, virotherapy, clinical research, clinical trials

Conflict of interest. The authors declare the absence of obvious or potential conflicts of interest related to the publication of this article.

Source of financing. The authors state that they received no funding for the study.

For citation: Golovinov I.V., Goncharova A.S., Shulga A.A., Vlasov S.N., Dimitriadi S.N. Clinical trials on oncolytic viruses. *Bulletin of Siberian Medicine.* 2024;23(4):158–168. https://doi.org/10.20538/1682-0363-2024-4-158-168.

✉ *Golovinov Igor V.,* ivgolovinov@yandex.ru

Клинические исследования онколитических вирусов

Головинов И.В.[1], Гончарова А.С.[1], Шульга А.А.[1], Власов С.Н.[2], Димитриади С.Н.[1]

[1] Национальный медицинский исследовательский центр (НМИЦ) онкологии
Россия, 344037, г. Ростов-на-Дону, ул. 14-я Линия, 63

[2] Ростовский государственный медицинский университет (РостГМУ)
Россия, 344022, г. Ростов-на-Дону, пер. Нахичеванский, 29

РЕЗЮМЕ

Онколитические вирусы (ОВ) – это новый класс таргетных противоопухолевых препаратов, обладающих уникальными механизмами действия. Эволюция в области виротерапии прошла от использования штаммов, пассированных *in vitro* (первое поколение), к генно-инженерным вирусам с повышенной селективностью (второе поколение) и, в конечном итоге, к рекомбинантным ОВ, экспрессирующим трансгены (третье поколение).

Цель обзора заключалась в проведении анализа и обобщении данных о текущей ситуации в клинических исследованиях ОВ.

Поиск в PubMed за период с 1997 по 2024 г. выявил 182 статьи, из которых 154 предоставили данные о 4 850 пациентах. Согласно публикациям, аденовирус (n = 44) является наиболее распространенным ОВ в клинических исследованиях, причем более двух третей (n = 108) использовали модифицированные или рекомбинантные вирусные основы с наиболее частым трансгеном в виде гранулоцитарно-макрофагального колониестимулирующего фактора (GM-CSF; n = 40). Среди опухолей в большинстве случаев исследовались меланома (n = 1 997) и рак желудочно-кишечного тракта (ЖКТ; n = 916) с использованием преимущественно монотерапии ОВ через внутриопухолевое (n = 3 003) или внутривенное (n = 1 318) введение. Часто встречающаяся комбинация включала химиотерапию (n = 54).

Нежелательными явлениями, связанными с лечением ОВ, были конституциональные симптомы низкой степени тяжести и местные реакции в месте инъекции. Часто проводили измерения выделения вируса, однако во многих исследованиях ограничивались анализом крови и опухолевой ткани, применяя только полимеразную цепную реакцию. Несмотря на то, что в большинстве работ сообщали о титрах противовирусных антител (n = 101), лишь в некоторых были отмечены вирусспецифические Т-клеточные ответы (n = 23). Объективные ответы (ORR, objective response rate) были зафиксированы у 458 (9,4%) пациентов, а контроль заболевания достигался у 1 141 (23,5%) больного, хотя стандартные критерии отчетности использовались лишь в 60,4% случаев.

Эти данные дают представление о текущем состоянии клинических исследований ОВ и выявляют потенциальные области, требующие дальнейшего изучения для более четкого определения роли ОВ в лечении рака.

Ключевые слова: онколитический вирус, иммунотерапия, виротерапия, клинические исследования, клинические испытания

Конфликт интересов. Авторы декларируют отсутствие явных и потенциальных конфликтов интересов, связанных с публикацией настоящей статьи.

Источник финансирования. Авторы заявляют об отсутствии финансирования при проведении исследования.

Для цитирования: Головинов И.В., Гончарова А.С., Шульга А.А., Власов С.Н., Димитриади С.Н. Клинические исследования онколитических вирусов. *Бюллетень сибирской медицины.* 2024;23(4):158–168. https://doi.org/10.20538/1682-0363-2024-4-158-168.

INTRODUCTION

In the field of cancer treatment, researchers are constantly developing new therapeutic strategies to combat the complex and heterogeneous nature of this serious disease [1, 2]. One innovative approach that is gaining popularity is oncolytic viral therapy, which harnesses the potential of viruses to selectively target and destroy tumor cells while sparing healthy tissue. Oncolytic viral therapy is a promising strategy in the

fight against cancer. It demonstrates multifaceted mechanisms that induce direct tumor lysis, stimulate antitumor immune responses, and improve the effectiveness of conventional treatments [3–5].

Oncolytic viral therapy is based on the use of natural or modified recombinant viruses constructed using genetic engineering methods to infect, replicate, and destroy malignant cells. These viruses are engineered to exploit vulnerabilities and genetic abnormalities present in tumor cells, destroying them while sparing healthy tissue. The selectivity of these viruses against tumor cells is often achieved through genetic modifications that make them unable to replicate in healthy tissues, thereby increasing their safety for clinical use [6].

Prior to conducting studies in humans, it is necessary to evaluate selectivity, cytotoxicity, biodistribution, and replication of the virus in *in vitro* cell lines and animal models [7, 8]. The results obtained from animal models can provide some insight into the response of patients [9].

Over the past few decades, the study of oncolytic viral therapy has progressed from preclinical studies to numerous clinical trials, indicating a transition from theoretical speculation to concrete therapeutic potential. Currently, according to Clinicaltrials.gov, there are 107 ongoing clinical trials, 89 of which are recruiting participants. These trials encompass a variety of cancer types and numerous oncolytic viruses with diverse mechanisms of action and delivery strategies. The results of the studies are crucial in comprehending the safety, efficacy, and challenges related to oncolytic viral therapy as a viable cancer treatment option [10, 11].

The aim of this study was to review the current situation in clinical trials on oncolytic viral therapy.

LITERATURE SEARCH METHODS

A systematic literature search was conducted using the PubMed database with the keywords “oncolytic virus” and “oncolytic viruses”. The search was limited to clinical trials and randomized clinical trials. A total of 182 articles were identified and reviewed, of which 154 contained original reports of clinical trial data using oncolytic viruses.

RESULTS

Oncolytic viruses in clinical trials

The literature search identified 154 clinical trials from 1997 to 2024 that reported on the use of oncolytic viruses (OVs). These studies involved 4,850 patients with various forms of malignant neoplasms (Table). Of the studies conducted, the majority (n = 86; 55.8%) were phase I trials. This suggests that oncolytic virotherapy is a novel approach and indicates that negative results from later-stage studies may not have been published yet, thus hindering complete understanding of the effectiveness of OVs in cancer patients.

We found that out of the total number of studies, 15 (9.7%) were phase I / II trials, 28 (18.2%) were phase II trials, and 20 (13.0%) were clinical trials that were not clearly classified but were mostly early-phase studies or the first clinical trials in humans. Phase III trials accounted for approximately 3% (n = 5) of the selected studies. However, even if a drug successfully completes phase III clinical trials, there is still a risk of failure. For example, on August 2, 2019, Labiotech announced the completion of a phase III clinical trial of Pexa-Vec (JX-594), a genetically modified vaccinia virus expressing *GM-CSF* and lacking the thymidine kinase gene, for the treatment of liver cancer. The interim analysis showed that the efficacy of Pexa-Vec in combination with sorafenib was greater than that of sorafenib alone, but the likelihood of prolonging patient survival was low, so the study was terminated early [12]. Therefore, current literature focuses on early-phase clinical trials.

Table

Patient characteristics in clinical trials of OVs

Characteristics	*n*
Tumor localization	
Brain	377
Breast	156
Gastrointestinal tract	916
Genitourinary system	245
Gynecologic tumors	219
Head and neck	198
Lungs	297
Melanoma	1,997
Sarcoma	148
Other solid tumors	204
Hematologic tumors	93
Delivery method	
Intratumoral	3,003
Intravenous	1,318
Several	122
Other	407
Phase	
I	1,793
I/II	345
II	1,317
III	932
Not specified	463

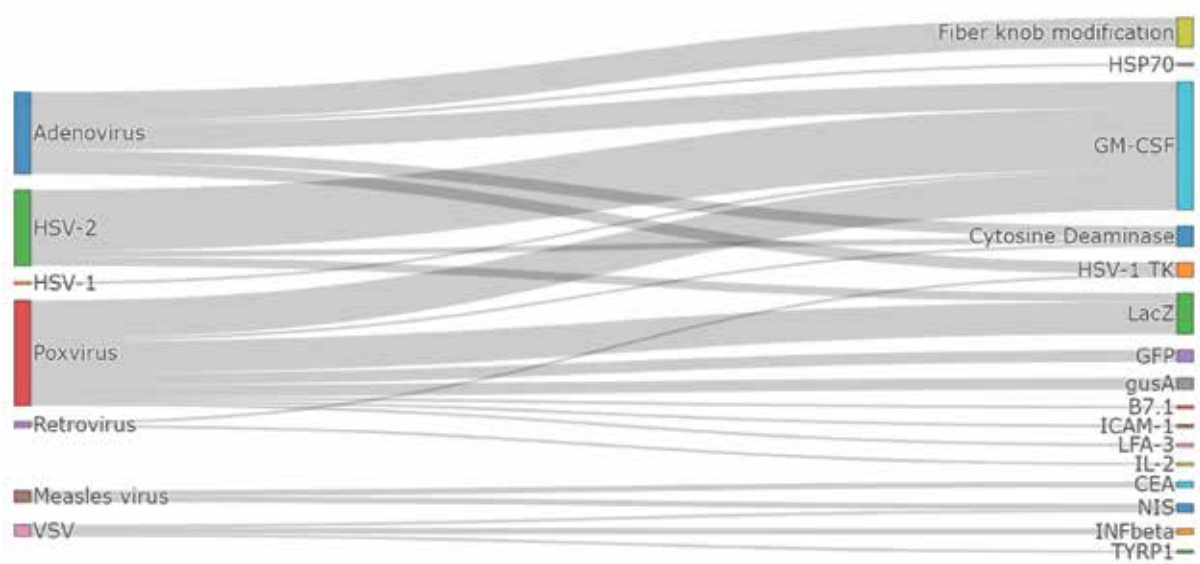

Figure. Transgenes used as a payload for oncolytic viruses

A variety of DNA and RNA viruses can be used as OVs. Most of the clinical trials included in the review used DNA viruses due to the advantages of their larger and more stable genome, which facilitates genetic engineering and the addition of multiple transgenes (Figure) [13]. The most commonly used viruses were adenovirus (n = 44; 28.6%), followed by herpes simplex virus type 1 (HSV-1; n = 37; 24.0%), reovirus (n = 25; 16.2%), and poxviruses (n = 18; 11.7%).

Additionally, six studies (3.9%) utilized Coxsackie virus, while five studies (3.2%) each employed Newcastle disease virus and measles virus. Four studies (2.6%) used parvovirus. Although some clinical trials have mentioned other viruses, such as Seneca Valley virus, Sendai virus, vesicular stomatitis virus (VSV), herpes simplex virus type 2 (HSV-2), retrovirus, and rhinovirus / poliovirus chimera, none of the published studies used more than one type of OVs.

Insertional mutagenesis is a problem that occurs when an exogenous DNA sequence from a virus integrates into the genome of the host organism [14]. This phenomenon can be harmless, but it can also lead to the transformation of host cells and even cause tumorigenesis. The risk of insertional mutagenesis depends on the characteristics of the virus. For instance, RNA viruses without a DNA phase, as well as viruses that replicate in the cytosol, do not pose any risk in this regard. Some viruses, including echoviruses, vaccinia virus, Coxsackie virus, and Newcastle disease virus, are considered safe. Although HSV-1 replicates in the nucleus, it has not been shown to cause insertional mutagenesis [15]. Adenovirus type 5 vectors are also safe due to the episomal nature of DNA [16].

However, retroviruses and lentiviruses are known for their ability to invade the genome of the host cell. Retroviruses are single-stranded RNA viruses that, upon entering the cell cytoplasm, are converted into proviral double-stranded DNA and subsequently translocated into the nucleus. While retroviruses and lentiviruses are popular vectors for gene therapy, the risk of genotoxicity remains a concern [17]. Therefore, it is crucial to thoroughly study the origins of a virus before looking into its development.

Approximately one-third (n = 46) of the clinical studies used wild-type virus, while two-thirds (n = 108) used genetically modified viruses. The modifications primarily consisted of deleting nonessential viral genes to promote selective replication in tumor cells and attenuate viral pathogenicity. In 69 clinical trials, genetic modifications also included the expression of one or more transgenes using 101 recombinant genes (Figure). The most frequently expressed transgene was *GM-CSF* (n = 40; 26.2%). *GM-CSF* stimulates the proliferation, differentiation, and migration of macrophages and dendritic cells, promoting the generation of adaptive immune responses by facilitating the cross-presentation of tumor antigens [18].

The next most commonly expressed transgenes were those used for the selection and identification of recombinant viruses after host infection. The study utilized *LacZ* (n = 16), which encodes bacterial β-galactosidase, and *GUSB* (n = 4), which encodes β-glucuronidase. Additionally, seven viruses were

used, each encoding genes for prodrug enzymes, such as cytosine deaminase (n = 7) and HSV-1 thymidine kinase (n = 7), which convert a nontoxic prodrug into a cytotoxic agent. The transgenes included immune-enhancing genes, such as interleukin-2 (*IL-2*; n = 1), interferon-beta (*IFNβ*; n = 2), lymphocyte function-associated antigen 3 (*LFA-3*; n = 1), costimulatory molecule *B7.1* gene (n = 1), and intercellular adhesion molecule 1 (*ICAM-1*; n = 1) gene.

In addition, several studies have employed different transgenes to monitor viral replication and biodistribution. Specifically, one study utilized heat shock protein 70 (*HSP70*), two studies used the carcinoembryonic antigen (*CEA*) gene, and three studies used sodium iodide symporter (NIS) and the tyrosine kinase-related protein 1 (*TYRP1*) gene. The *NIS* gene has been used to visualize viral biodistribution and replication using CT and sensitize cells to radiation therapy. Finally, ten studies utilized adenoviruses that expressed modified type 5 fibers, which were designed to enhance viral cell entry [19].

The selection of the most suitable virus and transgenes should be based on further biological analysis of tumor cells, host factors, and mechanisms that promote the activation of Th1 and CD8+ effector immune responses in T cells. Studies have shown that intracellular sensors, such as the cGAS-STING complex and Toll-like receptors, play a crucial role in inducing innate immunity by tumor cells [20]. The intracellular sensors used to recognize DNA and RNA viruses are also used for the same purpose in cancer. However, their status in cancer is not yet precisely determined [21].

TYPES OF TUMORS IN CLINICAL TRIALS

Clinical trials on OVs cover a wide range of tumors and focus on a large number of cancer patients (Table). Melanoma and gastrointestinal cancer were the most commonly studied tumors. Melanoma patients accounted for 50 clinical trials with the largest number of patients (n = 1,997), likely due to the relative ease of accessing tumors for local injection. An example of this is a phase III clinical trial of T-VEC, a genetically modified HSV-1-expressing *GM-CSF*, which included 436 melanoma patients [22]. There were 106 clinical trials involving 916 patients with gastrointestinal cancer. Table summarizes various tumor localization targeted in clinical trials, including genitourinary tumors (n = 43), breast and gynecologic cancers (n = 48), sarcomas (n = 27), and head and neck cancers (n = 23).

Based on the number of patients included in clinical trials, melanoma was the most common cancer type, followed by gastrointestinal cancer (n = 916; 18.9%), brain tumors (n = 377; 7.8%), lung cancer (n = 297; 6.1%), genitourinary cancer (n = 245; 5.1%), gynecologic cancer (n = 219; 7.7%), head and neck cancer (n = 198; 4.1%), and breast cancer (n = 156; 3.2%). The study included 204 patients (4.2%) with solid tumors that were not otherwise defined, as well as 93 patients (1.9%) with various hematologic malignancies.

DRUG COMBINATIONS

Of the 154 studies reviewed, 94 (61.0%) clinical trials used OV monotherapy, while 60 (39.0%) studies used OVs in combination with at least one other treatment or anticancer drug. Among the combinations, the most common drugs were cytotoxic chemotherapeutic agents (n = 54; 35.1%) and immune checkpoint inhibitors (n = 16; 10.4%).

Other modalities used in combination OV therapy studies included radiation therapy (n = 9; 5.8%), chemotherapy prodrugs (n = 8; 5.2%), tyrosine kinase inhibitors (n = 2; 1.3%), and immunomodulatory drugs (n = 1; 0.6%). The most common chemotherapy drugs included paclitaxel (n = 9) and cyclophosphamide (n=8), the latter being used in pretreatment chemotherapy to stimulate an antitumor immune response. In addition, gemcitabine was used in six studies. Two studies were unclear about the type of chemotherapy. Eight studies combined OVs with prodrugs, including four studies with the 5-fluorouracil precursor 5-fluorocytosine, three studies with ganciclovir, and one study with valganciclovir. Sixteen studies reported a combination of OVs and immune checkpoint inhibitors. Of these, five studies used ipilimumab and pembrolizumab, two studies evaluated the combination of OVs with bevacizumab, and one study with nivolumab, durvalumab, pucotenlimab, and tremelimumab. Additionally, two studies reported on the combination of OVs and tyrosine kinase inhibitors, specifically bortezomib and erlotinib. Finally, one study used a combination with interleukin-2.

Considering the diversity and heterogeneity of solid tumors, combining OVs with other treatment modalities may enhance their effectiveness. When developing combination therapy, it is crucial to consider drug interactions and the sequence of their use to minimize possible antagonistic effects. Chemotherapy can inhibit DNA synthesis, mitosis, and cell division, and

cause DNA damage. OVs replicate in tumor cells and contribute to the induction of DNA damage. Therefore, combining OVs with chemotherapy may enhance the antitumor effect synergistically [23, 24]. Combination therapy that includes OVs and checkpoint inhibitors is an attractive approach. OVs can attract tumor-infiltrating lymphocytes and stimulate the release of tumor antigens, danger signals, and proinflammatory cytokines, which further increases T cell recruitment and promotes immune cell activation. Viral infection may increase the expression of immune checkpoint molecules, such as CTLA-4 and PD-1, which typically inhibit T cell activation [25–27]. Additionally, the combination of radiation therapy and OVs has a synergistic effect on tumor treatment [28].

In addition to ongoing research that combines OVs with checkpoint inhibitors, viruses are being developed that can produce their own antibodies. For instance, HSV-1, which can express antibodies to PD-1, was developed to treat glioblastoma [29]. Although this construct has only been tested in mouse models so far, it represents a promising example of enhancing OV activity by inserting an antibody gene.

ADMINISTRATION ROUTES

Selecting the optimal route of administration is a controversial issue in the clinical development of OVs. Therefore, we analyzed the routes of administration used in published clinical studies (Table). The most commonly used method was intratumoral injection, which was used in 88 studies (57.1%). OVs are suitable for direct injection into the tumor. However, the number and localization of tumors may restrict the use of this method. Intratumoral injections provide direct tumor access, but the OV may be distributed unevenly within the tumor, reducing its effectiveness.

Intravenous delivery was used in 57 clinical studies (37%). It has the potential to infect metastatic lesions but may be limited by dilution in the blood and clearance from the body. This method avoids challenges of localizing each tumor, but there is a risk of inadequate transmission of the virus to the tumor site, which reduces its effectiveness [30].

Other delivery methods used in the studies included hepatic artery infusion in five studies (3.2%) and intraperitoneal delivery in eight studies (5.2%). Additionally, intravesical injection (n = 3), direct injection into the removed tumor bed (n = 3), convection-enhanced delivery (CED) into the brain tumor bed (n = 2), intradermal injection (n = 2), and infection of tumor cells *ex vivo* (n = 1) were employed. Two studies reported the use of stem cell delivery. No clinical trials using nanovesicle delivery have been reported, although preclinical trials have described such methods [31, 32].

Phase III clinical trials only used intratumoral injections, indicating their primary role in OVs with high commercial potential. This method is safer and ensures that the virus reaches its target directly.

There is interest in discovering new delivery methods that can prevent premature clearance of the virus and improve its biodistribution in tumor sites [33].

According to the Table, the most common delivery routes were intratumoral (n = 3,003; 61.9%) and intravenous (n = 1,318; 27.2%) injections. In the same studies, 122 patients received OVs through multiple routes, mostly combining intravenous with intratumoral administration. Besides, 407 (8.4%) patients received OVs through other routes, as described above.

Intratumoral injections were commonly used for melanoma, prostate cancer, and gliomas [34, 35]. Depending on the tumor localization and accessibility, the virus can be delivered once (for instance, into the glioma cavity during surgery) or several times (as in melanoma) [36].

Intravenous delivery can also take place via peripheral intravenous injection or can be more targeted by hepatic artery infusion for liver metastases [37]. Intravenous administration offers several advantages, including ease of administration, standardized dosage, and the possibility of repeated and prolonged administration [38]. However, the main disadvantage of this method remains the development of neutralizing antibodies and clearance of the virus from the blood.

Biodistribution of the virus depends on the route of administration. Intravenous administration allows the virus to spread through the bloodstream, reaching well-perfused organs, such as the liver, heart, lungs, kidneys, and brain. The spleen is also highly susceptible to circulating particles due to its high blood supply and capillary system. Local administration, on the other hand, results in the virus being mainly concentrated in organs near the injection site [39].

OV SAFETY PROFILE

The reviewed trials primarily assessed the safety of agents used in clinical practice. The adverse events associated with OV treatment were mostly

low-grade constitutional symptoms (CTCAE grade 1–2) and local injection site reactions. Fever was the most frequently reported adverse event, it was noted in 96 studies (grade 1–2 in 80 trials and grade 3–4 in 16 studies). Mild symptoms commonly reported included chills ($n = 83$), nausea and vomiting ($n = 67$), flu-like symptoms ($n = 36$), fatigue ($n = 52$), and pain ($n = 34$). Pain at the injection site was also reported in 43 studies. More severe adverse events (grade 3 or higher) included nausea and vomiting ($n = 12$), pain ($n = 11$), fever ($n = 6$), fatigue ($n = 6$), and flu-like symptoms ($n = 3$).

Clinical trials reported 155 grade 3 and 33 grade 4 adverse events. Many of the events observed were related to disease progression or the effects of other drugs used in combination therapy. The safety profile of OVs appears acceptable, given the large number of early-phase clinical trials that often include late-stage patients. Adverse events were mostly comparable for intratumoral and intravenous administration.

However, there are certain safety issues associated with different administration routes, and risks are present with intra-arterial administration of the agent. Gene therapy can cause a strong immune response, and in rare cases, excessive inflammation can damage organs and lead to death. For instance, in 1999, an 18-year-old patient died after receiving an adenovirus injection into a branch of the hepatic artery. Adenoviral vectors and transgenes were found in all of the patient's organs during autopsy, marking the first report of a death from gene therapy and highlighting its risks and serious side effects. When developing agents for intra-arterial administration, it is crucial to consider their safety and increase the dosage carefully to ensure effectiveness. At present, intravenous OV formulations are primarily used at early clinical stages (phase I and II) and have not yet advanced to phase III.

OV SHEDDING IN CLINICAL TRIALS

Viral shedding from treated patients may pose a risk to the environment and human health. FDA guidelines provide detailed information on viral shedding studies, including clinical trial design, and the collection and analysis of shedding data. The guidelines also note that viral shedding may be dose-dependent, so shedding studies should be performed after phase I when the dosage is well defined [41].

None of the reviewed studies reported transmission of viral infection to family members or healthcare personnel. Out of the 154 studies that were published, 122 (79.2%) assessed viral shedding, while 32 (20.8%) did not.

The presence of the virus in tissues is crucial for delivering the virus to tumor sites and identifying potential shedding sites. Clinical trials on OVs assessed various tissues and fluids, with blood or serum being the most common viral shedding site in 89 (57.8%) studies. Viral shedding in urine was noted in 57 (37.0%) studies and in tumor biopsy specimens – in 41 (26.6%) studies. The next most common was viral shedding in saliva or oral swabs, reported in 28 studies (18.2%), and in sputum samples, reported in 20 studies (13.0%). Other fluids or tissues, including cerebrospinal fluid, peritoneal washings, and injection sites, were collected in 41 studies.

In 122 studies that assessed viral shedding, evidence of the presence of the virus was found. Polymerase chain reaction (PCR) was the most commonly used method for detection in 100 (82.0%) studies. Plaque assays, which measure infectious virus particles, were performed in one study alone and in 21 (17.2%) published studies together with PCR [42].

ANTIVIRAL IMMUNITY IN CLINICAL TRIALS

Antiviral immunity plays a crucial role in clinical trials of oncolytic virotherapy and is a significant correlative biomarker. The presence of neutralizing antibodies is a major obstacle to successful therapy. The agents selected for treatment must be capable of infecting human cells, which has both advantages and disadvantages. One of the primary reasons for limiting the effectiveness of oncolytic virotherapy in humans is their immunity against the virus. Patients may have been previously exposed to or vaccinated against some of the naturally occurring viruses used in OV-based treatment, leading to the formation of neutralizing antibodies [43]. For instance, nearly 90% of people have antibodies against reovirus. The effectiveness of the measles virus, also considered a potential pathogen, is reduced due to the presence of antibodies against it in patients' blood.

Out of the 154 studies analyzed, 101 (65.6%) works measured antiviral antibody titers. Of these, 43 (27.9%) studies assessed neutralizing antibodies, while the remaining studies measured non-neutralizing antibody titers. Virus-specific T cell responses were investigated less frequently and were reported in only 23 (14.9%) clinical studies.

ANTITUMOR ACTIVITY IN CLINICAL TRIALS

Antitumor activity is an important consideration in OV trials, although many of these studies were conducted at early stages of development and were not designed to detect therapeutic responses, which complicates the analysis of clinical endpoints. However, most of them recorded clinical responses. Ninety-three studies (60.4%) used different Response Evaluation Criteria in Solid Tumors (RECIST), including standard RECIST in 74 studies (48.1%), modified RECIST in 12 studies (7.8%), and irRECIST criteria in 7 studies (4.5%). Four additional studies (2.6%) used modified WHO criteria, including the phase III OPTiM T-VEC study. The remaining 57 studies (37%) did not mention specific response criteria.

Out of the 4,850 patients who participated in these studies, the overall objective response rate was 9.4% (n = 458). Complete responses were observed in 3.5% (n = 171) of patients, while partial responses were observed in 5.9% (n = 287) of patients. Additionally, disease stabilization was observed in 14.1% (n = 683) of patients, resulting in disease control in 23.5% (n = 1,141) of patients. It is worth noting that a minor response was recorded in only 0.3% (n = 17) of patients. It is important to note that although the numbers are modest, most of the studies were phase I clinical trials and were not specifically designed to evaluate clinical responses.

CONCLUSION

We conducted a review of clinical experience with OVs over the past two decades. Our analysis provides an overview of different types of OVs used in clinical practice, target tumors, combinations, and the status of ongoing studies. Most clinical trials use large DNA viruses with various modifications, and *GM-CSF* is mainly used as transgenes. Most viruses are administered via intratumoral injection, although there has been an increase in the number of studies using intravenous administration. Monotherapy for osteosarcoma predominates in most studies, and combination therapy most often includes chemotherapy.

Despite the large number of clinical trials conducted, currently only four OVs have received approval for use as a treatment for malignant tumors. The first OV to be approved for the treatment of melanoma was the unmodified picornavirus ECHO-7 (Rigvir) in Latvia in 2004 [44]. In 2005, a modified adenovirus H101 (Oncorine) was registered in China for the treatment of head and neck or esophageal cancer [45]. In 2015, T-VEC (Imlygic) became the first OV to be approved in the United States for the treatment of unresectable advanced melanoma [46]. Subsequently, T-VEC was registered in Europe, Australia, Switzerland, and Israel. The only OV-based drug that has received FDA approval is G47Δ (Delytact), a modified HSV-1 expressing the *E. coli LacZ* gene, which received conditional and time-limited approval in June 2021 in Japan for the treatment of malignant gliomas [47].

Safety concerns for patients and the environment, such as off-target effects, virus mutations, and transmission [48], may be the reason why despite decades of research and numerous clinical trials, only one OV-based drug has been approved. Each time the original virus replicates, there is a high probability of viral evolution, resulting in the proliferation of new viral lineages due to defects in the viral polymerase [49].

The optimal method for administering OVs remains an open question. Developing systemic delivery faces main challenges, such as serum neutralization of the virus and hepatotoxicity. After treatment, individuals may shed live, replicating viruses, increasing the likelihood of transmission to healthy individuals. Due to the high mutation rate of viruses, particularly those containing RNA, there is a risk of infection transmission when they are released into the environment with waste [50].

Based on this, there is a need to conduct additional preclinical trials to better understand the basic biological mechanisms underlying the antitumor activity of OVs. Clinical trials need to standardize methods for assessing viral distribution and implement appropriate biomarkers that will provide information on both antiviral and antitumor immunity. In addition, encouraging publication of research data in this area will help accelerate clinical development and maximize the potential of OVs for the treatment of patients with cancer.

REFERENCES

1. Debela D.T., Muzazu S.G., Heraro K.D., Ndalama M.T., Mesele B.W., Haile D.C. et al. New approaches and procedures for cancer treatment: Current perspectives. *SAGE Open Med*. 2021;9:1–10. DOI: 10.1177/20503121211034366.
2. Kit O.I., Kharagezov D.A., Lazutin Yu.N., Mirzoyan E.A., Milakin A.G., Stateshny O.N., et al. Immunotherapy for epithelial tumors of the thymus. *South Russian Journal of Cancer*.

2023;4(3):56–67. (In Russ.). DOI: 10.37748/2686-9039-2023-4-3-7.
3. Santos Apolonio J., Lima de Souza Gonçalves V., Cordeiro Santos M.L., Silva Luz M., Silva Souza J.V., Rocha Pinheiro S.L. et al. Oncolytic virus therapy in cancer: A current review. *World J. Virol*. 2021;10(5):229–255. DOI: 10.5501/wjv.v10.i5.229.
4. Hemminki O., Dos Santos J.M., Hemminki A. Oncolytic viruses for cancer immunotherapy. *J. Hematol. Oncol*. 2020;13(1):84. DOI: 10.1186/s13045-020-00922-1.
5. Heidbuechel J.P.W., Engeland C.E. Oncolytic viruses encoding bispecific T cell engagers: a blueprint for emerging immunovirotherapies. *J. Hematol. Oncol*. 2021;14(1):63. DOI: 10.1186/s13045-021-01075-5.
6. Cristi F., Gutiérrez T., Hitt M.M., Shmulevitz M. Genetic modifications that expand oncolytic virus potency. *Front. Mol. Biosci*. 2022;9:831091. DOI: 10.3389/fmolb.2022.831091.
7. Silva Lima B., Videira M.A. Toxicology and biodistribution: the clinical value of animal biodistribution studies. *Mol. Ther. Methods Clin. Dev*. 2018;8:183–197. DOI: 10.1016/j.omtm.2018.01.003.
8. Yamaguchi T., Uchida E. Oncolytic virus: regulatory aspects from quality control to clinical studies. *Curr. Cancer Drug Targets*. 2018;18(2):202–208. DOI: 10.2174/1568009617666170222142650.
9. Russell S.J., Peng K.W. Oncolytic virotherapy: a contest between apples and oranges. *Mol. Ther*. 2017;25(5):1107–1116. DOI: 10.1016/j.ymthe.2017.03.026.
10. Li K., Zhao Y., Hu X., Jiao J., Wang W., Yao H. Advances in the clinical development of oncolytic viruses. *Am. J. Transl. Res*. 2022;14(6):4192–4206.
11. Kit O.I., Ignatov S.N., Zlatnik E.Yu., Soldatkina N.V., Rostorguev E.E., Sagakyants A.B., Bondarenko E.S., et al. Oncolytic virotherapy in glioblastoma treatment: progress and challenges in clinical research (literature review). *Siberian Journal of Oncology*. 2020;19(6):133–140. (In Russ.). DOI: 10.21294/1814-4861-2020-19-6-133-140.
12. Abou-Alfa G.K., Galle P.R., Chao Y., Erinjeri J., Heo J., Borad M.J. et al. PHOCUS: A Phase 3, Randomized, Open-Label Study of Sequential Treatment with Pexa-Vec (JX-594) and Sorafenib in Patients with Advanced Hepatocellular Carcinoma. *Liver Cancer*. 2023;1–17. DOI: 10.1159/000533650.
13. Rahman M.M., McFadden G. Oncolytic viruses: newest frontier for cancer immunotherapy. *Cancers (Basel)*. 2021;13(21):5452. DOI: 10.3390/cancers13215452.
14. Aiuti A., Cossu G., de Felipe P., Galli M.C., Narayanan G., Renner M. et al. The committee for advanced therapies' of the European Medicines Agency reflection paper on management of clinical risks deriving from insertional mutagenesis. *Hum. Gene Ther. Clin. Dev*. 2013;24(2):47–54. DOI: 10.1089/humc.2013.119.
15. Kaufman H.L., Kohlhapp F.J., Zloza A. Oncolytic viruses: a new class of immunotherapy drugs. *Nat. Rev. Drug Discov*. 2015;14(9):642–662. DOI: 10.1038/nrd4663.
16. Appaiahgari M.B., Vrati S. Adenoviruses as gene/vaccine delivery vectors: promises and pitfalls. *Expert Opin. Biol. Ther*. 2015;15(3):337–351. DOI: 10.1517/14712598.2015.993374.
17. Sena-Esteves M., Gao G. Introducing genes into mammalian cells: viral vectors. *Cold Spring Harb. Protoc*. 2020;2020(8):095513. DOI: 10.1101/pdb.top095513.
18. Kumar A., Taghi Khani A., Sanchez Ortiz A., Swaminathan S. GM-CSF: a double-edged sword in cancer immunotherapy. *Front. Immunol*. 2022;13:901277. DOI: 10.3389/fimmu.2022.901277.
19. Zhao Y., Liu Z., Li L., Wu J., Zhang H., Zhang H. et al. Oncolytic adenovirus: prospects for cancer immunotherapy. *Front. Microbiol*. 2021;12:707290. DOI: 10.3389/fmicb.2021.707290.
20. Woo S.R., Fuertes M.B., Corrales L., Spranger S., Furdyna M.J., Leung M.Y. et al. STING-dependent cytosolic DNA sensing mediates innate immune recognition of immunogenic tumors. *Immunity*. 2014;41(5):830–842. DOI: 10.1016/j.immuni.2014.10.017.
21. Iurescia S., Fioretti D., Rinaldi M. Targeting cytosolic nucleic acid-sensing pathways for cancer immunotherapies. *Front. Immunol*. 2018;9:711. DOI: 10.3389/fimmu.2018.00711.
22. Andtbacka R.H.I., Collichio F., Harrington K.J., Middleton M.R., Downey G., Öhrling K. et al. Final analyses of OPTiM: a randomized phase III trial of talimogene laherparepvec versus granulocyte-macrophage colony-stimulating factor in unresectable stage III–IV melanoma. *J. Immunother. Cancer*. 2019;7(1):145. DOI: 10.1186/s40425-019-0623-z.
23. Zhang B., Cheng P. Improving antitumor efficacy via combinatorial regimens of oncolytic virotherapy. *Mol. Cancer*. 2020;19(1):158. DOI: 10.1186/s12943-020-01275-6.
24. Soliman H., Hogue D., Han H., Mooney B., Costa R., Lee M.C. et al. Oncolytic T-VEC virotherapy plus neoadjuvant chemotherapy in nonmetastatic triple-negative breast cancer: a phase 2 trial. *Nat. Med*. 2023;29(2):450–457. DOI: 10.1038/s41591-023-02210-0.
25. Ren Y., Miao J.M., Wang Y.Y., Fan Z., Kong X.B., Yang L. et al. Oncolytic viruses combined with immune checkpoint therapy for colorectal cancer is a promising treatment option. *Front. Immunol*. 2022;13:961796. DOI: 10.3389/fimmu.2022.961796.
26. Monge C., Xie C., Myojin Y., Coffman K., Hrones D.M., Wang S. et al. Phase I/II study of PexaVec in combination with immune checkpoint inhibition in refractory metastatic colorectal cancer. *J. Immunother. Cancer*. 2023;11(2):e005640. DOI: 10.1136/jitc-2022-005640.
27. Liu X., Zhang J., Feng K., Wang S., Chen L., Niu S. et al. Efficacy and safety of oncolytic virus combined with chemotherapy or immune checkpoint inhibitors in solid tumor patients: A meta-analysis. *Front. Pharmacol*. 2022;13:1023533. DOI: 10.3389/fphar.2022.1023533.
28. Zhang B., Cheng P. Improving antitumor efficacy via combinatorial regimens of oncolytic virotherapy. *Mol. Cancer*. 2020;19(1):158. DOI: 10.1186/s12943-020-01275-6.
29. Passaro C., Alayo Q., De Laura I., McNulty J., Grauwet K., Ito H. et al. Arming an oncolytic herpes simplex virus type 1 with a single-chain fragment variable antibody against PD-1 for experimental glioblastoma therapy. *Clin. Cancer Res*. 2019;25(1):290–299. DOI: 10.1158/1078-0432.CCR-18-2311.
30. Zhu X., Fan C., Xiong Z., Chen M., Li Z., Tao T. et al. De-

velopment and application of oncolytic viruses as the nemesis of tumor cells. *Front. Microbiol*. 2023;14:1188526. DOI: 10.3389/fmicb.2023.1188526.
31. Ji W., Li L., Zhou S., Qiu L., Qian Z., Zhang H. et al. Combination immunotherapy of oncolytic virus nanovesicles and PD-1 blockade effectively enhances therapeutic effects and boosts antitumour immune response. *J. Drug Target*. 2020;28(9):982–990. DOI: 10.1080/1061186X.2020.1766473.
32. Chen L., Ma Z., Xu C., Xie Y., Ouyang D., Song S., Zhao X. et al. Progress in oncolytic viruses modified with nanomaterials for intravenous application. *Cancer Biol. Med*. 2023;20(11):830–855. DOI: 10.20892/j.issn.2095-3941.2023.0275.
33. Ban W., Guan J., Huang H., He Z., Sun M., Liu F. et al. Emerging systemic delivery strategies of oncolytic viruses: A key step toward cancer immunotherapy. *Nano Res*. 2022;15(5):4137–4153. DOI: 10.1007/s12274-021-4031-6.
34. Fares J., Ahmed A.U., Ulasov I.V., Sonabend A.M., Miska J., Lee-Chang C., Balyasnikova I.V. et al. Neural stem cell delivery of an oncolytic adenovirus in newly diagnosed malignant glioma: a first-in-human, phase 1, dose-escalation trial. *Lancet Oncol*. 2021;22(8):1103–1114. DOI: 10.1016/S1470-2045(21)00245-X.
35. Fujita K., Kato T., Hatano K., Kawashima A., Ujike T., Uemura M. et al. Intratumoral and s.c. injection of inactivated hemagglutinating virus of Japan envelope (GEN0101) in metastatic castration-resistant prostate cancer. *Cancer Sci*. 2020;111(5):1692–1698. DOI: 10.1111/cas.14366.
36. Silk A.W., O'Day S.J., Kaufman H.L., Bryan J., Norrell J.T., Imbergamo C. et al. A phase 1b single-arm trial of intratumoral oncolytic virus V937 in combination with pembrolizumab in patients with advanced melanoma: results from the CAPRA study. *Cancer Immunol. Immunother*. 2023;72(6):1405–1415. DOI: 10.1007/s00262-022-03314-1.
37. Nawrocki S.T., Olea J., Villa Celi C., Dadrastoussi H., Wu K., Tsao-Wei D. et al. Comprehensive Single-Cell Immune Profiling Defines the Patient Multiple Myeloma Microenvironment Following Oncolytic Virus Therapy in a Phase Ib Trial. *Clin. Cancer Res*. 2023;29(24):5087–5103. DOI: 10.1158/1078-0432.CCR-23-0229.
38. Hill C., Carlisle R. Achieving systemic delivery of oncolytic viruses. *Expert Opin. Drug Deliv*. 2019;16(6):607–620. DOI: 10.1080/17425247.2019.1617269.
39. Naumenko V., Van S., Dastidar H., Kim D.S., Kim S.J., Zeng Z. Visualizing oncolytic virus-host interactions in live mice using intravital microscopy. *Mol. Ther. Oncolytics*. 2018;10:14–27. DOI: 10.1016/j.omto.2018.06.001.
40. Bulcha J.T., Wang Y., Ma H., Tai P.W.L., Gao G. Viral vector platforms within the gene therapy landscape. *Signal Transduct. Target Ther*. 2021;6(1):53. DOI: 10.1038/s41392-021-00487-6.
41. Bubela T., Boch R., Viswanathan S. Recommendations for regulating the environmental risk of shedding for gene therapy and oncolytic viruses in Canada. *Front. Med. (Lausanne)*. 2019;6:58. DOI: 10.3389/fmed.2019.00058.
42. Onnockx S., Baldo A., Pauwels K. Oncolytic viruses: an inventory of shedding data from clinical trials and elements for the environmental risk assessment. *Vaccines (Basel)*. 2023;11(9):1448. DOI: 10.3390/vaccines11091448.
43. Berkeley R.A., Steele L.P., Mulder A.A., van den Wollenberg D.J.M., Kottke T.J., Thompson J. et al. Antibody-neutralized reovirus is effective in oncolytic virotherapy. *Cancer Immunol. Res*. 2018;6(10):1161–1173. DOI: 10.1158/2326-6066.CIR-18-0309.
44. Alberts P., Tilgase A., Rasa A., Bandere K., Venskus D. The advent of oncolytic virotherapy in oncology: The Rigvir® story. *Eur. J. Pharmacol*. 2018;837:117–126. DOI: 10.1016/j.ejphar.2018.08.042.
45. Xia Z.J., Chang J.H., Zhang L., Jiang W.Q., Guan Z.Z., Liu J.W. et al. Phase III randomized clinical trial of intratumoral injection of E1B gene-deleted adenovirus (H101) combined with cisplatin-based chemotherapy in treating squamous cell cancer of head and neck or esophagus. *Ai Zheng*. 2004;23(12):1666–1670.
46. Andtbacka R.H., Kaufman H.L., Collichio F., Amatruda T., Senzer N., Chesney J. et al. talimogene laherparepvec improves durable response rate in patients with advanced melanoma. *J. Clin. Oncol*. 2015;33(25):2780–2788. DOI: 10.1200/JCO.2014.58.3377.
47. Frampton J.E. Teserpaturev/G47Δ: first approval. *BioDrugs*. 2022;36(5):667–672. DOI: 10.1007/s40259-022-00553-7.
48. Forbes N.S., Coffin R.S., Deng L., Evgin L., Fiering S., Giacalone M. et al. White paper on microbial anti-cancer therapy and prevention. *J. Immunother. Cancer*. 2018;6(1):78. DOI: 10.1186/s40425-018-0381-3.
49. Lawler S.E., Speranza M.C., Cho C.F., Chiocca E.A. Oncolytic viruses in cancer treatment: a review. *JAMA Oncol*. 2017;3(6):841–849. DOI: 10.1001/jamaoncol.2016.2064.
50. Omole R.K., Oluwatola O., Akere M.T., Eniafe J., Agboluaje E.O., Daramola O.B. et al. Comprehensive assessment on the applications of oncolytic viruses for cancer immunotherapy. *Front. Pharmacol*. 2022;13:1082797. DOI: 10.3389/fphar.2022.1082797.

Authors' contribution

Golovinov I.V., Shulga A.A., Vlasov S.N. – analysis and interpretation of the data. Goncharova A.S., Dimitriadi S.N. – conception and design, final approval of the manuscript for publication.

Authors' information

Golovinov Igor V. – Junior Researcher, Experimental Laboratory Center, National Medical Research Center of Oncology, Rostov-on-Don, ivgolovinov@yandex.ru, https://orcid.org/0000-0003-3011-6904

Goncharova Anna S. – Cand. Sci. (Biology), Head of Experimental Laboratory Center, National Medical Research Center of Oncology, Rostov-on-Don, fateyeva_a_s@list.ru, https://orcid.org/0000-0003-0676-0871

Shulga Anna A. – Junior Researcher, Experimental Laboratory Center, National Medical Research Center of Oncology, Rostov-on-Don, slip.anka96@mail.ru, https://orcid.org/0009-0006-1125-2897

Vlasov Sergei N. – 4th-year Student, Faculty of Medicine and Prevention, Rostov State Medical University, Rostov-on-Don, ser. vl4s0v02@yandex.ru, https://orcid.org/0000-0003-3289-8436

Dimitriadi Sergei N. – Dr. Sci. (Med.), Senior Researcher, Oncourology Department, National Medical Research Center for Oncology, Rostov-on-Don, Dimitriadi.pro@gmail.com, https://orcid.org/0000-0002-2565-1518

(✉) **Golovinov Igor V.,** ivgolovinov@yandex.ru

Received 05.03.2024;
approved after peer review 26.03.2024;
accepted 14.05.2024