# Precision oncolytic viral therapy in colorectal cancer: Genetic targeting and immune modulation for personalized treatment (Review)

MUHAMMAD HARIS SULTAN[1,2], QI ZHAN[1], YIGANG WANG[1], YULONG XIA[2] and XIAOYUAN JIA[1]

[1]College of Life Sciences and Medicine, Xinyuan Institute of Medicine and Biotechnology, Zhejiang Sci-Tech University, Hangzhou, Zhejiang 310018, P.R. China; [2]Center for Translational Medicine and Precision Medicine, Wenzhou Institute, University of Chinese Academy of Sciences, Wenzhou, Zhejiang 325000, P.R. China

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**Abstract.** Colorectal cancer (CRC) is a leading health issue and treatments to eradicate it, such as conventional chemotherapy, are non-selective and come with a number of complications. The present review focuses on the relatively new area of precision oncolytic viral therapy (OVT), with genetic targeting and immune modifications that offer a new future for CRC treatment. In the present review, an overview of the selection factors that are considered optimal for an oncolytic virus, mechanisms of oncolysis and immunomodulation applied to the OVT, as well as new strategies to improve the efficacy of this method are described. Additionally, cause-and-effect relationships are examined for OVT efficacy, mediated by the tumor microenvironment, and directions for genetic manipulation of viral specificity are explored. The possibility of synergy between OVT and immune checkpoint inhibitors and other treatment approaches are demonstrated. Incorporating the details of the present review, biomarker-guided combination therapies in precision OVT for individualized CRC care, significant issues and future trends in this required area of medicine are highlighted. Increasingly, OVT is leaving the experimental stage and may become routine practice; it provides a new perspective on overcoming CRC and highlights the importance of further research and clinical work.

*Correspondence to:* Dr Xiaoyuan Jia, College of Life Sciences and Medicine, Xinyuan Institute of Medicine and Biotechnology, Zhejiang Sci-Tech University, 5 Second Avenue, Xiasha Higher Education Zone, Hangzhou, Zhejiang 310018, P.R. China
E-mail: iamjiaxiaoyuan@163.com

Dr Yulong Xia, Center for Translational Medicine and Precision Medicine, Wenzhou Institute, University of Chinese Academy of Sciences, 1 Building, 7th Floor, 1 Jinlian Road, Longwan, Wenzhou, Zhejiang 325000, P.R. China
E-mail: ylxia@wiucas.ac.cn

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**Contents**



## 1. Introduction

Colorectal cancer (CRC) is one of the most prevalent cancer types worldwide, and was observed to affect 1.9 million individuals in 2020 and led to ~930,000 deaths in the same year, constituting ~10% of cancer-related deaths (1,2). In particular, CRC incidence seems to be increasing in the younger population; early-onset CRC cases have been growing and doubling between 1990 and 2019, which emphasizes the importance of increasing CRC screening and implementing culturally appropriate interventions (3,4). CRC incidence is most elevated in Oceania, including Australia and New Zealand, Europe and in at least some African areas (2).

At present, CRC treatments are based on the disease stage and include surgery, systemic chemotherapy, targeted therapy and radiotherapy. The most distinctive cases of CRC are those that present with hepatic, pulmonary or peritoneal metastases, for which radical surgery, hepatic/pulmonary resections and cytoreductive surgery associated with hyperthermic intraperitoneal chemotherapy are helpful (5-7). Furthermore, bevacizumab and cetuximab as systemic therapies add only 3-6 months of survival to patients with metastatic CRC, and high rates of relapse are still observed (8,9). Single-agent kinase inhibitors selective for pathways such as PI3K/Akt/mTOR have proven suboptimal due to cross-talk between these pathways and poor safety profiles (10). The specificity of traditional treatments, such as chemotherapy and radiotherapy, is very low; they cause significant side effects, and despite treatment with neoadjuvant therapy, there is a chance of recurrence in

54% of patients (11-16). Such issues indicate the importance of a new approach, such as oncolytic viral therapy (OVT). OVs specifically target and destroy tumor cells and stimulate strong antitumor immune responses (17-19). One of the most promising developments is the Food and Drug Administration-approved oncolytic herpes simplex virus type 1 (HSV-1) for treating melanoma. Additionally, there is growing interest in the consistent and clinically significant data reported for the use of vaccinia virus in treating solid carcinomas (20).

The ability to directly lyse tumors and modulate the immune response makes OVT worthwhile. For instance, recent advancements in OVT have shown significant promise in treating CRC. Notably, the oncolytic vaccinia virus, Pexa-Vec, has been evaluated in clinical trials specifically targeting patients with refractory metastatic CRC (21). Early detection through saliva biomarkers can enhance patient selection for OVT, allowing timely intervention and improved treatment success. Understanding oral microbiota may also help optimize OVT protocols for personalized care (22).

An improved matrix stiffness stress in the TME restrains immune cell infiltration and immunotherapy responsiveness; however, it is poorly understood in CRC (23-26). Increasing the understanding of the mechanisms behind extracellular matrix (ECM) biomechanics could improve the effectiveness of immune checkpoint blockade (ICB) therapy and adoptive cell therapy (ACT) (27,28). OVT boosts the upregulation of tumor-associated antigens (TAAs) and cytokines, thereby enhancing the effectiveness of ICB. This is especially valuable in tumors with challenging immune microenvironments, such as microsatellite-stable (MSS) CRC, which typically shows limited response to immunotherapy (28-30). Engineered OVs, such as OH2, demonstrate enhanced specificity and oncolytic potency due to targeted gene manipulations, particularly when compared with unmodified or naturally occurring OVs (29). Together with immunotherapy, OVT has been reported to produce only mild side effects and high rates of $CD8^+$ T-cell infiltration in other cancer types such as melanoma and epithelial tumors, including colorectal, bladder and renal cancer (30,31). Recent research has revealed new approaches to treating CRC by teasing out the potential interactions between OVT, immunotherapy and the mechanical properties of tumors (32). More specifically, it has shed light on several general strategies to possibly optimize ICBs, ACTs and tumor cell vaccines for the treatment of CRC, including the deconstruction of ECM-related hurdles that prevent OVT strategies from reaching target cancer cells and improving the existing OVT strategies tailored to each patient (33).

However, in MSS CRC, the aforementioned improvements have not yielded long-lasting responses, and the highly immunosuppressive TME presents unique obstacles that warrant novel solutions. OVT has been identified as a promising strategy as the oncolytic capabilities are congruent with the immunomodulatory properties. However, there are several obstacles to improve virus delivery, tumor escape mechanisms and the incorporation of OVT into combined treatment strategies (34). The present review fills the gap regarding the lack of a comprehensive synthesis of how recent innovations in genetic engineering, biomarkers and immune modulation are being translated into clinical applications for OVT in CRC. This includes addressing barriers such as tumor heterogeneity, delivery limitations and the integration of OVT with other therapies, which have hindered broader adoption and optimization of this treatment modality. A graphical overview of precision OVT in CRC is provided in Fig. 1. The present review will also address key questions to develop a roadmap for precision OVT in CRC, highlighting technological advancements that could transform the current treatment landscape. The present review discusses not only the application of engineered OVs in the treatment of immune suppression and drug resistance but also underscores the potential of OVT to transform cancer therapy by investigating its value in becoming a fundamental anticancer weapon.

## 2. Genetic targeting of OVs in CRC

*Strategies for identifying optimal OVs*. The selection of optimal OVs relies on key criteria including tumor specificity, replication efficiency and immune-stimulating capabilities. Genetic engineering approaches, such as receptor targeting and promoter-driven tropism, enhance viral selectivity for cancer cells while sparing healthy tissues. Preclinical screening further refines candidates based on safety profiles, oncolytic potency and synergy with existing therapies (35).

*Selection criteria for OVs in CRC*. For precision OVT in CRC, a virus is designed to infect and kill cancer cells but not healthy cells. One of the criteria for selection is replication preference in tumor cells. For example, TG6002, which was manufactured using the genetically modified vaccinia virus, has selected replication in cancer cells due to viral gene deletion. TG6002 converts 5-fluorocytosine to 5-fluorouracil at the tumor site, improving localized therapeutic effects (36). Similarly, different types of adenoviruses, such as Ad-PE, int and ins-GCV, with fabricated integrin-targeting peptides have improved transduction efficiency and therapeutic activity compared with controls (37).

In addition to replication preference, other critical criteria should be considered when selecting OVs for CRC. First, tumor specificity is paramount; OVs must selectively target cancer cells while sparing normal tissues to minimize off-target effects (38). This can be achieved through genetic modifications that exploit unique TME features, such as hypoxia or altered receptor expression (39). Second, the ability of OVs to modulate the immune system is essential. Beyond direct oncolysis, OVs can stimulate both innate and adaptive immune responses, generating long-term antitumor immunity (40). Third, safety remains a key concern, particularly for systemic delivery, where risks of neutralization by circulating antibodies or off-target infection must be mitigated (41). Finally, the potential for combination therapies, such as pairing OVs with immune checkpoint inhibitors (ICIs) or chemotherapy, should be evaluated to enhance therapeutic efficacy and overcome resistance mechanisms (42).

*Mechanisms of action: Oncolysis and immunomodulation*. OVs cause tumor cell shedding and immunogenic death, releasing TAAs into the immune system. This immune activation combines synergistically with checkpoint inhibitors or T-cell engagers, as illustrated in preclinical models of CRC (43). The gut microbiome plays a significant role in CRC progression, and its modulation can influence the efficacy of immunotherapies, including OVs. Emerging evidence suggests

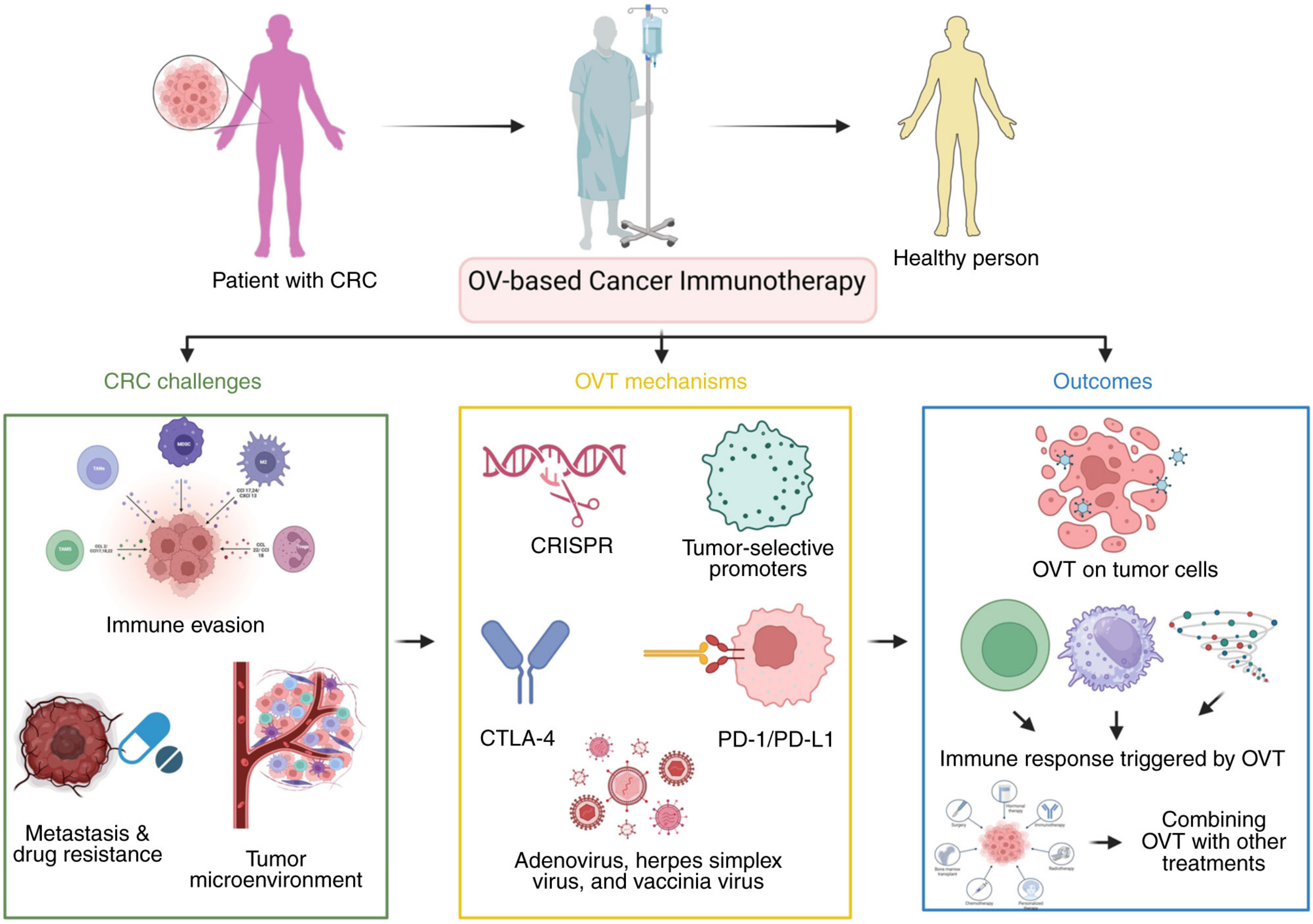


Figure 1. Graphical abstract. This graphical abstract summarizes the role of OVT in CRC treatment, highlighting key challenges such as immune evasion, metastasis and drug resistance, and showcasing strategies such as CRISPR/Cas9 modifications, tumor-selective promoters and checkpoint inhibitors to enhance therapeutic efficacy. The outcomes illustrate OVT's ability to induce tumor cell lysis, release TAAs and stimulate antitumor immunity via CTLs, ultimately improving patient outcomes. This figure was created using BioRender (BioRender Inc.). CRC, colorectal cancer; OVT, oncolytic virus therapy; TAAs, tumor-associated antigens; CTLs, cytotoxic T lymphocytes; CRISPR, clustered regularly interspaced short palindromic repeats; CTLA-4, cytotoxic T-lymphocyte-associated protein 4; PD-1, programmed cell death protein 1; PD-L1, programmed death-ligand 1.

that specific gut microbiota compositions may predict treatment response and modulate the anticancer immune response, although the exact mechanisms remain an active area of research (44).

*Implementation approaches for augmenting effectiveness*. OVT has emerged as a promising modality for cancer treatment, particularly in CRC, where OVs selectively infect and lyse tumor cells while sparing normal tissues (45). To enhance the efficacy of OVT, localized delivery systems such as imaging-guided or catheter-based approaches can be employed to achieve optimal viral density within the TME while minimizing systemic toxicity (46). Carrier cells, including mesenchymal stem cells and cytotoxic immune cells such as T cells, have been explored as 'Trojan horse' delivery vehicles to transport OVs to tumor sites, leveraging their innate homing capabilities (47). However, challenges such as rapid clearance by the reticuloendothelial system and neutralization by circulating antibodies highlight the need for innovative strategies, such as engineering OVs to regulate abnormalities in the TME (such as neovascularization and ECM stiffness) or combining them with ICIs to amplify antitumor immunity (21). Recent advances in genetic editing, viral retargeting and nanotechnology platforms further underscore the potential of OVs to overcome barriers to systemic delivery and improve therapeutic outcomes (48). Non-invasive imaging techniques also play a pivotal role in monitoring viral kinetics and ensuring safety during treatment (49). Together, these strategies provide a comprehensive framework for advancing OVT in CRC.

*TME remodeling*. OVs alter the tumor-associated landscape to enhance the infiltration of immune cells and reduce the presence of immunosuppressive cells. This promotes antitumor immune responses and, more specifically, boosts the effects when used alongside toxic therapies such as chemotherapy or radiotherapy. For example, the engineered vaccinia virus, coxsackievirus B3, plus FOLFOXIRI enhance immunogenicity and CRC survival (50-52).

*Enhancing tumor selectivity and safety*. The selectivity and safety of OVs are critical to CRC management. Viruses are designed to interact with receptor molecules upregulated in CRC cells, including CD46 and intercellular adhesion molecule-1, improving the affinity and viral replication (53-55). Other sites in CRC tumors, including specific metabolic pathways and immune checkpoint suppression, enhance viral survival and replication within an immunosuppressive environment (56). Genetic modifications make the OVs more selective, allowing them to target tumor cells instead of normal cells, thereby enhancing the therapeutic ratio (21).

*Genetic engineering approaches for enhanced specificity in CRC*. Genetic engineering approaches have enabled the fine-tuning of OVs to improve tumor specificity, replication efficiency and immunogenicity. For example, talimogene laherparepvec (T-VEC) is an engineered oncolytic HSV-1 designed to preferentially replicate in tumor cells while inducing antitumor immune responses (57).

*Enhancing tumor tropism and selectivity*. The addition of a heterologous receptor binding domain) to OVs improves their ability to recognize a greater number of receptors. This modification significantly enhances the tropism of these viruses, concentrating on increasing their effectiveness against tumor cells (58). When it comes to therapeutic applications, engineered viruses with an expanded ability to recognize receptors can target and eliminate a wider range of cancer cells, enhancing their capacity to kill tumors that might be less susceptible to viral-mediated cell destruction; it also reduces the dissemination of the OV to the tumor site and enhances the overall effectiveness of the treatment process in combating cancer (59). The regulation of transcription factors has also been studied to selectively enhance viral replication in cancerous tissues (60).

*Incorporating immunomodulatory and anti-angiogenic genes*. Anti-angiogenic genes can prevent tumor angiogenesis, limiting tumor growth and metastasis (61,62). Additionally, immunomodulatory genes such as interleukin-12 (IL-12) and C-X-C motif chemokine ligand 11 (CXCL11) notably enhance the effect of the immune system on the tumor, improving the therapeutic index of OVs. IL-12 promotes T cells producing interferon-$\gamma$ and improves the recognition of tumor cells by cytotoxic T lymphocytes (CTLs) and the intrinsic cytotoxic activity of CTLs. Furthermore, CXCL11 is a potent chemokine that induces immune cells, including CTLs and T helper type 1 (Th1), to infiltrate the TME and strengthen the effective antitumor immune response (63). By combining immunomodulatory tactics with anti-angiogenic methods that disrupt tumor angiogenesis and deprive tumors of their blood supply, it is possible to design a cohesive treatment strategy that addresses both tumor growth and enhances the immune defense against cancer (64,65).

*CRISPR/Cas9 applications in OVT*. CRISPR/Cas9 technology offers a precise and efficient method for editing viral genomes to improve tumor selectivity (66). For example, a tissue-specific HSV-1 has been designed by knocking in the murine IL-12 and CXCL11 cassettes using the ICP34.5 coding region and knocking out the immunomodulatory gene, ICP47. This double alteration improves the selectivity of viral replication in tumor cells and boosts antitumor immunity against CRC. Additionally, the armed HSV-1 features enhanced tumor selective replication and initiates an immune response, resulting in potent antitumor effects. IL-12 favors the Th1 cell development and CTL response, and CXCL11 has been credited for attracting effector T cells and natural killer (NK) cells to the tumor site (67). This coaction greatly optimizes the functional capabilities of immune effector cells and enhances therapeutic efficacy against tumor cells. This approach may be considered a promising strategy for improving OVT in the treatment of CRC (67,68).

*Tumor-selective promoters*. The incorporation of telomerase and CEA gene promoters into OVs ensures targeted viral replication and therapeutic gene expression exclusively in cancer cells (69). This strategy significantly reduces the likelihood of side effects by sparing normal tissues, thereby improving the safety and efficacy of OVT for CRC (54). As a tumor marker with significantly increased expression in colon cancer, CEA serves as an ideal target for constructing oncolytic adenoviruses (OAVs) (70). These viruses are designed to selectively replicate in and lyse tumor cells while sparing normal tissues. The CEA promoter has been successfully used to regulate the expression of therapeutic genes in OAVs, enhancing their specificity and efficacy against CRC (70,71,72).

*Advances in safety and delivery mechanisms*. OVs represent a novel antitumor strategy that selectively targets CRC cells while leaving surrounding healthy tissue intact. However, their clinical application is limited by challenges related to the safety and efficacy of delivering OVs to the target tumor tissue. To address these issues, researchers have developed innovative methods. One approach involves engineering OVs to carry natural microRNAs (miRNAs), which are potent regulators of gene expression. By incorporating sequences upregulated in CRC, miRNAs guide OVs specifically toward cancer cells, minimizing side effects and enhancing safety (73). Another promising strategy is the virosomal administration of interferon, where viral particles are encapsulated in liposomes to protect them from destruction by antibodies and complement proteins. The lipid membrane of these liposomes can also be modified with targeting molecules, such as antibodies or ligands, to direct the OVs to the tumor site. This improvement significantly enhances the efficiency and effectiveness of OV delivery (74) Key strategies for engineering OVs are illustrated in Fig. 2. Although those strategies have shown promising outcomes in preclinical studies, a number of improvements and clinical trials are required to confirm their safety and effectiveness for treating CRC (75,76).

*Comparison of viral platforms*. Several types of OVs for CRC treatment are in use today, which includes the adenoviruses, oHSV2, vaccinia viruses, reoviruses and the Newcastle disease virus. Adenoviruses have been highlighted for their biosafety and potential to selectively infect and lyse cancer cells with therapeutic protein expression, augmented by immunomodulation when combined with checkpoint inhibitors (77). Other OVs include oHSV2, derived from HSV-2, which has exhibited broad potency in a study inducing CRC cell necrosis and enhancing adaptive immune responses, improving the survival time of tumor-bearing mice (78). The vaccinia virus and reovirus exhibit unique characteristics, including a heightened ability to infect tumor tissues and selectively replicate within cancer cells. These properties make them promising candidates for advanced virotherapy strategies aimed at improving tumor targeting and therapeutic outcomes (79,80). Newcastle disease virus is pinpointed for its enhanced effects when applied in conjunction with other methods, thereby proving the capability of OVs in improving the treatment of CRC (81,82).

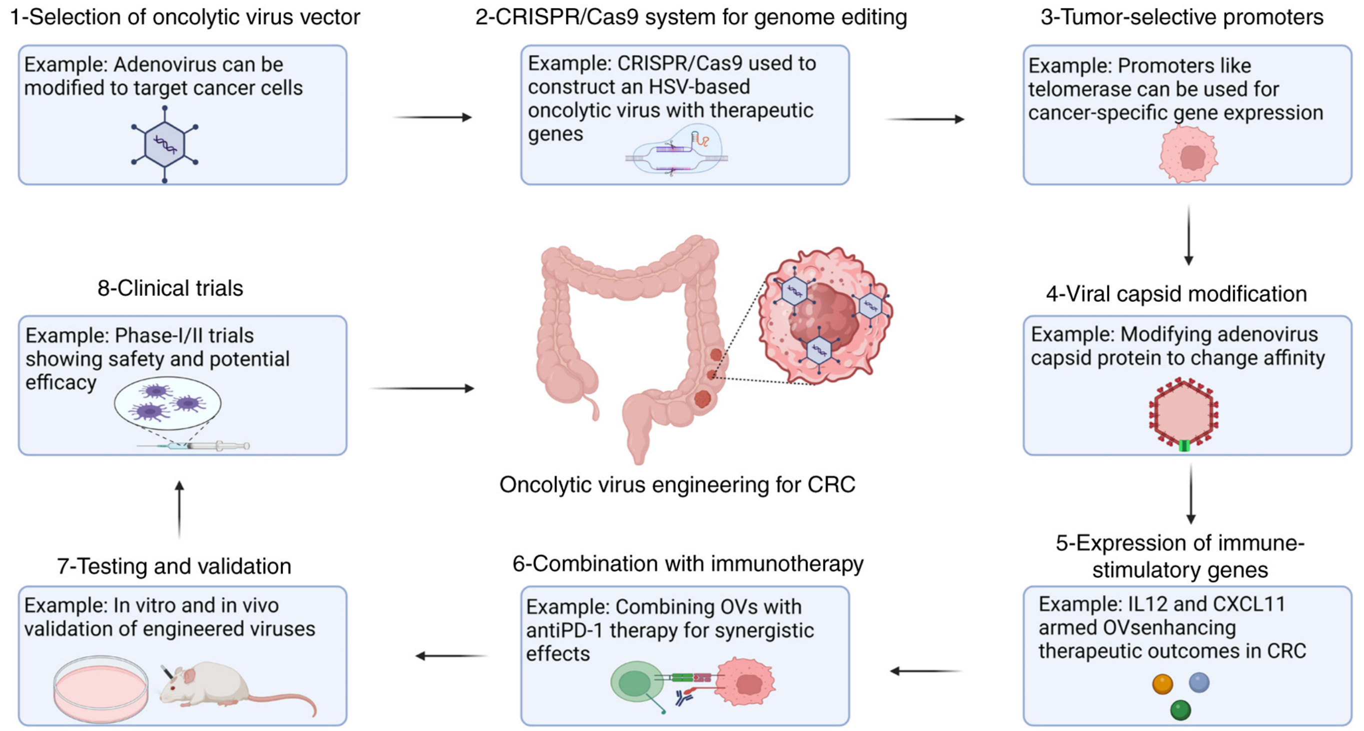


Figure 2. Strategies for engineering OVs for CRC therapy. This figure outlines the key steps in developing OVs for CRC therapy. It begins with selecting a suitable viral vector, such as adenovirus, followed by genetic modifications using tools such as CRISPR/Cas9 to enhance tumor specificity. Tumor-selective promoters (including telomerase-driven promoters) ensure targeted gene expression, while viral capsid modifications improve receptor targeting and reduce off-target effects. The integration of immune-stimulatory genes, such as IL-12 or CXCL11, boosts antitumor immunity. Finally, the figure highlights the importance of rigorous preclinical testing and validation through *in vitro* and *in vivo* studies as well as clinical trials to confirm safety and efficacy. These steps collectively illustrate the engineering and optimization process of OVs for CRC treatment. The material for this figure has been adapted from references (29-31,67,230-237). This figure was created using BioRender (BioRender Inc.). CRC, colorectal cancer; OV, oncolytic virus; CRISPR/Cas9, clustered regularly interspaced short palindromic repeats/CRISPR-associated protein 9; IL-12, interleukin-12; CXCL11, C-X-C motif chemokine ligand 11.

The benefits of adeno-derived oncolytic therapy in CRC arise from the selective replication of the virus in cancer cells, sparing normal cells. This selectivity is achieved by incorporating cancer cell-specific promoters, such as cyclooxygenase-2, which drive viral replication specifically in cancerous cells. Consequently, OAVs can selectively infect and kill cancer cells, with the relative sparing of normal neighboring tissues (83). Moreover, OAVs may recode the TME and induce an intensive immune reaction, which may cooperate with immunotherapies such as anti-programmed cell death protein 1 (PD-1) (84). Nevertheless, drawbacks include difficulties in applying high local concentrations to distant metastatic tumors since conventional methods may not always be sufficient. In addition, OAVs can prompt immunogenic cell death (ICD), but the immune responses can sometimes hinder their effectiveness. Thus, immune clearance strategies are needed (85).

The safety of HSV, particularly the genetically modified T-VEC, is favorable. T-VEC has been shown to have low toxicity in a clinical trial, and side effects, if any, are controllable. Risks and complications are frequent, but severe adverse events are rare. The HSV OVT has been shown to explicitly target tumor cells without affecting normal cells, which has greatly enhanced the safety of the virus (86). HSV can be administered for patients with advanced cancer, such as CRC (87). The vaccinia virus, including modified strains such as JX-594, also demonstrates fairly good safety indicators. In certain co-housing investigations, it has been administered with fewer reported serious side effects. JX-594 does not cause severe local reactions even when used with other chemotherapeutic agents and is compatible with standard clinical treatment regimens (88,89). Certain research has shown that HSV can produce systemic tumor regression in CRC-bearing animals (90). Current clinical trials are testing its utility when combined with other immunotherapies and when administered as a monotherapy. Initial findings indicate that HSV immunomodulatory capabilities can increase antitumor immunity and have implied the effective treatment of patients with CRC (86). Since the antitumor activity is well-known, the vaccinia virus has shown promising results in phase I clinical trials. For instance, Pexa-Vec has been demonstrated to be effective in decreasing tumor size and improving immune effects on CRC cells. Its potential in stimulating the immune system and directly lysing cancer cells provides enough merit to warrant more developments regarding CRC therapy (88,89).

Continually propelling the research effort on CRC vaccines, Ad5GUCY2C-PADRE is a non-replicating plasmid-based adenoviral vector encoding the GUCY2C antigen fused with the helper T-cell epitope, PADRE. This adenoviral vector has been shown to elicit vigorous cytotoxic and humoral immune responses, directly targeting CRC cells with upregulated GUCY2C expression with little side effects (91). However, it was revealed that antibodies targeting the core adenoviral vector could neutralize adenoviral vectors, which may be an issue in the immunology of adenoviral vaccines (91). Furthermore, although the adenoviral vector is known to elicit preexisting immunity in the patient population, a significant drawback, the oncolytic adenovirus Ad5 [E1-, E2b]-CEA(6D) holds promise due its ability to induce immunogenicity against CEA (92). Intratumor influenza vaccines that increase $CD8^+$ T cells in proficient mismatch repair (pMMR) CRC are being launched to treat patients (NCT04591379) (93). Recombinant adenoviruses can transduce antigens and present oncolytic properties that activate CTLs (94). There is also evidence that the Sendai virus and vesicular stomatitis virus have oncolytic abilities in other preclinical CRC models, preferring to infect and kill cancer cells (95). Table I summarizes various OVs, including vaccinia, adenovirus, HSV and Newcastle disease virus, detailing their engineered constructs, immune responses and efficacy in preclinical CRC models. These viruses demonstrate potent anti-tumor effects through mechanisms such as immune activation, cytokine induction and targeted cytotoxicity.

*Targeting key genetic mutations in CRC for enhanced efficacy.* The advancement of CRC and the continual alteration of its state depend on mutations disrupting or impairing critical genes that regulate cell turnover. For instance, KRAS mutations result in the constitutive activation of RAS proteins, driving enhanced cell division and survival, thereby promoting tumorigenesis (96). However, studies have also shown that APC mutations affect the regulation of WNT signaling and hence encourage increased division of tumor cells. Both tropism mutation types are highly associated with CRC pathogenesis (97) and the alteration is applicable irrespective of the stage or location of the tumor. Additionally, loss of p53 function through mutational inactivation of the tumor-suppressor gene TP53 hampers facets of the CRC cell's ability to respond to DNA damage, thereby causing the cells to continue to proliferate (96). SMAD4 loss increases tumor invasiveness, enhances metastatic properties, induces chemo-resistance and is associated with a poorer survival, decreasing the overall survival of patients (98,99).

*KRAS mutations as targets for OVT.* Due to its high incidence rate in CRC (estimated at 45%), KRAS mutations are considered suitable target candidates for OVT (100). These mutations are often associated with resistance to chemotherapy and standard EGFR-directed therapies, the ability of cancer cells to adapt to treatment and a poor prognosis (101). KRAS mutations play a critical role in reprogramming cancer cell metabolism to support rapid growth and survival. These mutations increase the dependency of cancer cells on glutamine, an essential nutrient for energy production and biosynthesis (102). Additionally, KRAS mutations upregulate nuclear factor erythroid 2-related factor 2 (Nrf2) signaling, a pathway that regulates antioxidant responses and metabolic adaptations. By enhancing Nrf2 activity, cancer cells can manage oxidative stress, maintain redox balance and sustain metabolic pathways such as glycolysis and glutaminolysis. Targeting these metabolic vulnerabilities, including glutamine metabolism and Nrf2 signaling, offers potential therapeutic strategies to combat KRAS-driven cancer (103,104). For example, Pelareorep (also known as REOLYSIN), an OVT, can target and kill KRAS-mutated cells, or any tumor cell. Furthermore, these viruses can induce autophagy in cancer cells, which may enhance the therapeutic efficacy of this approach compared with other treatments (105).

*APC and TP53 genetic alterations in CRC and OVT.* Genetic changes in APC and TP53 are significant in CRC progression. Most of the APC gene abnormalities are primary to CRC tumorigenesis, whereby the overproduction of β-catenin stimulates cell proliferation. During the later phases of multistep carcinogenesis, alterations in TP53 affect critical cellular functions such as the cell cycle and apoptosis and thus promote malignancy (106). These genetic changes elucidate the molecular pathogenesis of CRC and highlight their potential as effective targets for OVT (107). Adenoviruses with deletions in the E1B 55kDa gene are OAVs that selectively infect cancer cells with mutations or deletions in the TP53 gene. The E1B 55kDa protein, under normal circumstances, binds to and inactivates the p53 tumor suppressor to enhance replication. The loss of this gene means OAVs are able to replicate only in cells where p53 function is compromised, an attribute of numerous types of cancer (71,108,109).

*BRAF mutations in deficient mismatch repair (dMMR)/microsatellite instability-high (MSI-H) CRC.* Another critical target is the BRAFV600E mutation, prevalent in dMMR or MSI-H CRC. While BRAF and MEK inhibitors have shown success in melanoma (105) and non-small cell lung cancer (110), CRC requires additional EGFR blockade due to compensatory EGFR upregulation (111). Integrating targeted therapies for BRAF mutations with OVT has the potential to improve outcomes in these subsets of patients with CRC (112).

*HER2 amplification and upregulation.* HER2 (also known as erbB2) amplification is frequently detected in CRC and can serve as a prognostic and therapeutic gene; its upregulation is related to tumor progression and poor prognosis, as well as in breast cancer (113-115). The synergy between HER2-targeted treatments with OVT has been noted, especially in HER2-positive CRC subtypes (116).

*MSI-H, dMMR and high tumor mutational burden (TMB).* Since MSI-H, dMMR and TMB are related to sensitivity and prognosis, these biomarkers are essential for identifying patients appropriate for immunotherapy. Patients with such features show improved outcomes when administered ICIs common for CRC (117). For instance, it has been identified that CRC tumors with high TMB show increased responsiveness to immunotherapy, as higher mutational loads generate more neoantigens, enhancing the immune system's ability to recognize and attack cancer cells (118). Future research on OVT biomarkers could contribute to developing individualized therapeutic strategies, potentially leading to higher effectiveness in treatment outcomes.

*Inflammatory microenvironment and PIK3CA mutations.* The pro-inflammatory microenvironment of CRC also yields potential targets for OVT. At present, options for molecular prognostic markers for CRC, including serum IL-6, IL-8, programmed cell death ligand 1 (PD-L1), CEA (71,119), CA19-9 and MMP-9, are still being explored regarding their potential use in immunotherapy (120). Additionally, PIK3CA mutations are associated with the growth and survival of CRC, making them effective targets for OVT. These mutations can improve the vulnerabilities of the tumor to viral infection, which provides a new direction for the treatment of CRC (121).

Table I. Oncolytic viruses in CRC: Virus constructs, immune responses and therapeutic efficacy.

| First author/s, year | Virus type | Virus construct name | Characteristics | *In vivo* model | Immune response | Efficacy | (Refs.) |
|---|---|---|---|---|---|---|---|
| Wang *et al*, 2020 | Vaccinia virus | VVLΔTKΔN1L-mIL-21 | Expresses pleiotropic cytokine IL-21. | Mouse CMT93 subcutaneo us CRC model. | Mainly mediated by$CD8^+$ T cells. | The superior antitumor effect prevents disease recurrence. | (238) |
| Chen *et al*, 2021 | Vaccinia virus | VV-IL-23 (vvDD-IL-23) | Oncolytic vaccinia virus expressing IL-23 variants. | Multiple tumor models including murine colon cancer MC38-luc, ovarian cancer ID-8-luc and mesothelioma AB12-Luc. | Modulates the TME by increasing Th1 chemokines, antitumor factors (IFN-γ, TNF-α, perforin and IL-2, Granzyme B) and activating T cells. | Elicits potent antitumor effects, a systemic antitumor effect dependent on $CD8^+$ and $CD4^+$ T cells and IFN-γ, transformation of 'cold' tumors into 'hot' tumors. | (239) |
| Deng *et al*, 2021 | Vaccinia virus | VG9-IL-24 | A replication-competent vaccinia virus armed with IL-24, designed to infect, replicate within and kill CRC cells. | Human HCT116 CRC model in athymic nude mice and murine CT26 CRC model in BALB/c immune-competent mice. | VG9-IL-24 stimulated multiple antitumor immune responses, including the induction of specific and lasting immune responses against CRC, as evidenced by CTL activity and the secretion of cytokines such as IFN-γ, TNF-α, IL-4 and IL-6. | VG9-IL-24 inhibited tumor growth and prolonged survival in human and murine CRC models. It also demonstrated a potent 'bystander' antitumor effect, eradicating primary and distant tumors. | (240) |
| Li *et al*, 2012 | Vaccinia virus | VV-CCL19 | Oncolytic vaccinia virus expressing murine CCL19 (chemokine) under the control of the pSE/L promoter. | C57BL/6 mice bearing MC38 subcutaneous tumors. | Increased T cell and dendritic cell infiltration into the tumor; selective attraction of lymphocytes expressing CCR7. | The therapy showed enhanced antitumor effects and improved safety, with rapid clearance from normal tissues compared to the control virus (vvDD). | (241) |
| Flanagan *et al*, 2004 | Recombinant vaccinia virus | rVmSLC | Recombinant vaccinia virus expressing murine SLC. | BALB/c mice with established CT26 colon cancer tumors. | Enhanced infiltration of CD4 T cells, correlation with inhibition of tumor growth, CD4 T-cell dependent antitumor response. | Local injection of rVmSLC resulted in significant inhibition of tumor growth, improved tumor weight and survival. | (242) |

Table I. Continued.

| First author/s, year | Virus type | Virus construct name | Characteristics | *In vivo* model | Immune response | Efficacy | (Refs.) |
|---|---|---|---|---|---|---|---|
| Bereta *et al*, 2004 | Recombinant vaccinia virus | rV-CD40L | Recombinant vaccinia virus expressing murine CD40L | Not explicitly mentioned in the provided text. | Stimulates IL-12 secretion by DC, proliferation of B cells and $DX5^+$ (NK/NKT) cells, and IFN-γ synthesis by $DX5^+$ cells in a CD40/CD40L-dependent manner. | Highlights the complex immune regulatory effects of rV-CD40L, suggesting the potential for immunological effects as therapeutic vaccines. | (243) |
| Warner *et al*, 2019 | Chimeric Poxvirus | CF33-hNIS | Chimeric poxvirus encoding the hNIS at a redundant tk locus, allowing for imaging via PET/CT with I-124 and synergy with radioiodine (I-131) therapy. | Nude mice with established HT29 and HCT116 flank xenografts. | Induces caspase-independent immunogenic cell death with translocation of calreticulin, secretion of ATP and release of HMGB1, suggesting activation of antitumor immunity. | Demonstrates tumor regression *in vivo* in colon cancer xenograft models. Systemic delivery of radiotherapeutic I-131 isotope following CF33-hNIS infection enhances and sustains tumor regression compared with virus treatment alone in HCT116 xenografts. | (244) |
| Xing *et al*, 2016 | Adenovirus | AdC68-CTB | Genetically modified for enhanced immunogenicity, suitable for oncolytic virotherapy, minimally neutralized by anti-AdHu5 immunity. | Nude mouse model with NCI-H508 and DiFi tumor cell lines. | Triggers anti-EGFR antibodies and stimulates $CD8^+$ T cell proliferation. | Significantly suppresses tumor growth, effective in reducing tumor size *in vivo*. | (245) |
| Xing *et al*, 2016 | Recombinant adenovirus | Hu5-CTB | A recombinant adenovirus engineered to express full-length cetuximab exhibits tumor-specific replication and diminished EGFR signaling activation, utilizing double-stranded DNA as part of conditional oncolytic therapy. | Nude mouse models were used to evaluate the efficacy of Hu5-CTB *in vivo*, particularly for CRC therapy. | The adenovirus triggers immune responses characterized by T cell activation and significant cytokine release, such as type I interferons, which are crucial in enhancing the immune response against tumor cells. | A single dose of Hu5-CTB induced significant tumor reduction (suppressed tumor growth) in mouse models used for CRC evaluation. | (245) |

Table I. Continued.

| First author/s, year | Virus type | Virus construct name | Characteristics | *In vivo* model | Immune response | Efficacy | (Refs.) |
|---|---|---|---|---|---|---|---|
| Luo *et al*, 2020 | Adenovirus | Ad-RGD-Survivin-ZD55-miR-143 | Triple-regulated oncolytic adenovirus carrying the therapeutic gene miR-143 targeting KRAS. | HCT116 xenograft model. | Activation of the immune system with potential induction of inflammatory cytokines. | Reduced tumor growth as measured in a HCT116 xenograft model. | (74) |
| Rong *et al*, 2024 | Recombinant adenovirus | rAd.mDCN.mCD40L | Expresses mDCN and mCD40L. | CT26 subcutaneous tumor model. | Increased $CD8^+$ T effector cells and $CD4^+$ memory T cells; reduced MDSCs and Tregs. | Significantly inhibited tumor growth and liver metastasis. | (246) |
| Nie *et al*, 2012 | Non-replicative adenovirus | Ad5GUCY2C-PADRE | Non-replicative adenoviral vector encoding GUCY2C antigen. | Not provided. | Induces strong cytotoxicity against CRC cells with upregulated GUCY2C expression. | Induces potent cytotoxic and humoral immune responses but challenges with pre-existing neutralizing antibodies. | (247) |
| Huang *et al*, 2022 | Adenovirus vector | Ad5 [E1-, E2b]-CEA(6D) | Engineered for enhanced immunogenicity against CEA. | Not provided. | Elicits CEA-specific immune responses. | Shows potential to induce CEA-specific immune responses despite pre-existing immunity. | (85) |
| Hecht *et al*, 2023 | HSV-1 | T-VEC | Modified HSV-1 expresses GM-CSF to enhance immune response. | Mouse models of CRC. | Induces both local and systemic immune responses. | Limited efficacy in clinical trials for CRC; shows promise in melanoma. | (248) |
| Chai *et al*, 2022 | Herpes virus | Pseudorabies virus (PRV Bartha K61) | Attenuated live vaccine strain lacks gE and gI genes. | BALB/c nu mice. | Not explicitly mentioned. | Inhibited tumor growth, but caused death in mice due to toxicity, indicating potential safety concerns. | (51) |
| Chai *et al*, 2022 | Herpes virus | Pseudorabies virus (PRV HB98) | Attenuated live vaccine strain lacksTK, gG, and gE genes. | BALB/c nu mice. | Not explicitly mentioned. | Showed higher safety and efficacy than the Bartha K61 strain, with no adverse reactions observed in mice and significant tumor growth inhibition. | (51) |

Table I. Continued.

| First author/s, year | Virus type | Virus construct name | Characteristics | *In vivo* model | Immune response | Efficacy | (Refs.) |
|---|---|---|---|---|---|---|---|
| Tian *et al*, 2023 | NDV | rNDV-mOX40L | Expresses murine OX40L. | CT26 animal model. | Boosts anti-tumor immunity response by delivering a potent costimulatory signal to $CD4^+$ and $CD8^+$ T cells. | Increased tumor inhibition rate and intense infiltration of tumor-specific T cells. | (249) |
| Vigil *et al*, 2007 | Recombinant NDV | rNDV/F3aa-IL-2 | Expresses IL-2. | CT26 colon cancer mouse model. | Enhances antitumor immunity through T-cell activation. | Significantly reduced tumor growth and prolonged survival. | (250) |

CRC, colorectal cancer; TME, tumor microenvironment; SLC, secondary lymphoid chemokine; hNIS, human sodium iodide symporter; mDCN, murine decorin; mCD40L, murine CD40 ligand; HSV, herpes simplex virus; T-VEC, talimogene laherparepvec; IL, interleukin; IFN-γ, interferon-γ; TNF-α, tumor necrosis factor-α; Th1, type 1 helper T cells; CTL, cytotoxic T lymphocyte; CCL19, chemokine (C-C motif) ligand 19; CCR7, C-C chemokine receptor type 7; EGFR, epidermal growth factor receptor; GM-CSF, granulocyte-macrophage colony-stimulating factor; KRAS, Kirsten rat sarcoma viral oncogene homolog; MDSCs, myeloid-derived suppressor cells; Tregs, regulatory T cells; GUCY2C, guanylyl cyclase C; PADRE, Pan DR epitope; CEA, carcinoembryonic antigen; TK, thymidine kinase; gE, glycoprotein E; gI, glycoprotein I; gG, glycoprotein G; PRV, pseudorabies virus; NDV, Newcastle disease virus; OX40L, OX40 ligand; PET/CT, positron emission tomography/computed tomography; I-124, iodine-124; I-131, iodine-131; HMGB1, high mobility group box 1; ATP, adenosine triphosphate; NK, natural killer cells; NKT, natural killer T cells.

## 3. Immune modulation strategies in OVT for CRC

*Mechanisms of immune evasion in CRC*. In a number of cases, the CRC TME is marked by the infiltration of immunosuppressive immune cells, including regulatory T cells (Tregs), MDSCs and M2 macrophages. These cells can negatively regulate effector T cells and NK cells, reducing the antitumor immune response (122). Tumor hypoxia, or low oxygen tension, is a recurrent phenomenon in the TME of CRC. Hypoxia can stimulate the release of hypoxia-inducible factor 1-α (HIF-1α), which is involved in the formation of immunosuppressive factors and therapy resistance (123-125). ECM components in the TME can be degraded by enzymes such as MMPs, and this degradation can prevent immune cells from infiltrating the tumor and promote immunosuppressive activity (126). CRC cells can also release PD-L1, which binds to PD-1 on the surface of T cells, thus suppressing their functioning. The mechanism of this interaction is one of the primary significant ways the tumor can escape the immune response in the TME (127,128). The CRC TME can secrete cytokines such as TGF-β, IL-10 and VEGF, which promote cancer progression by supporting tumor growth, facilitating the formation of new blood vessels and enabling immune system evasion. Furthermore, metabolic changes, including glycolysis and glutaminolysis, produce immunosuppressive metabolites and consume nutrients required for immune cells (129).

In CRC, immune cells such as Tregs and MDSCs are particularly relevant for immune evasion and avoidance (130). Tregs can secrete adenosine and transfer cAMP to effector T cells, thereby suppressing their activity. Tregs also outcompete effector T cells for IL-2, metabolize IL-2 into its biologically inactive form, and inhibit the production of IL-2 by dendritic cells, which are crucial for activating effector T cells. Additionally, Tregs can suppress effector T cells through the induction of apoptosis (131). Both Tregs and MDSCs enhance each other's proliferation, creating a symbiotic circuit of immunosuppression within the TME (132,133). Immune cells, such as Tregs and MDSCs, in the microenvironment of CRC decrease the immune response needed for the virus to act upon the cancer cells and destroy them during OVT (134). These cells can help neutralize the virus and suppress the initiation of antitumor immunity, lowering the effectiveness of the therapy (32).

*Synergy between OVT and ICIs*. ICIs disrupt the binding between the immune checkpoint proteins, such as PD-1/PD-L1, CTLA-4 and sTim-3 (135) and their receptors on the tumor or immune cell surface (134). Cancer cells typically use these interactions to subvert the immune response system in the body. ICIs function by blocking inhibitory pathways, such as PD-1/PD-L1 or CTLA-4 interactions, thereby releasing the suppressive signals on T cells. This enhances T-cell activation and enables them to more effectively recognize and eliminate cancer cells (136).

Combining OVT and ICIs, the immune response to CRC is improved via several mechanisms. OVT employs viruses capable of infecting and killing malignant cells and, at the same time, disseminating TAAs and stimulating antitumor immunity (55). This can enhance the visibility of the tumor to the immune system, thus increasing its immunogenicity (137).

Furthermore, OVT can increase the levels of immune checkpoint proteins on cancer cells, allowing ICIs to bind and enhance the immune response. In addition, OVT can alter the tumor-related stroma and make it less immunosuppressive to cancer cells, thus enabling improved immune cell infiltration and effector functions (55). This pro-additive synergy increases the possibility of a more profound and longer lasting immunological response to CRC (137).

Experimental and clinical data have demonstrated the synergism between OVT and anti-PD-1/PD-L1 and CTLA-4 immunotherapies in CRC. Previous *in vivo* investigations have revealed that OVT may enhance the immunogenicity of tumors, making tumors more conspicuous to the immune system, and raising the levels of immune checkpoint proteins that can be modulated by ICIs (138). According to clinical trials, these combinations have resulted in objective responses and disease control with reasonable toxicity (139,140). CTLA-4 and PD-1/PD-L1, together with the blockade, upregulate T cell activation and treatment effectiveness, improving the antitumor results (141,142).

To maintain the effectiveness of OVT, studies have highlighted the importance of engineering OVs with immune-modulatory genes. For instance, incorporating genes for cytokines such as granulocyte-macrophage colony-stimulating factor (GM-CSF) or checkpoint inhibitors such as anti-PD-1 can enhance antitumor immunity and extend the duration of viral activity (143). Recent findings suggest that OVs equipped with costimulatory molecules, such as VALO-D102 encoding CD40L and OX40L, can significantly improve tumor growth control and boost the infiltration of tumor-specific $CD8^+$ effector T cells, as demonstrated in melanoma models (54). Enhancing the efficacy of OVT can also be achieved by targeting the TME through strategies such as remodeling the ECM or inhibiting immunosuppressive pathways, particularly those involving PI3Kγ in macrophages (144). Additionally, combining OVT with targeted therapies, such as anti-angiogenic agents (including regorafenib) (145) or EGFR inhibitors (including cetuximab), enhances viral delivery and counteracts resistance mechanisms in the TME (146). The synergistic mechanism of OVT and ICIs is depicted in Fig. 3.

CRC develops resistance to ICIs through mechanisms such as altered PI3K/AKT/mTOR signaling, often due to PIK3CA mutations, and interactions within an immunosuppressive TME (147). To overcome this resistance, combining OVT with PI3K-γ inhibitors such as copanlisib or histone deacetylase inhibitors (HDIs) can effectively suppress oncogenic pathways, enhance viral replication and synergistically improve T-cell infiltration alongside ICIs (148). Nevertheless, there are problems with this approach. Despite ICI therapy, 30-50% of patients with MSI-H CRC develop drug resistance, while single-agent ICIs have been reported to have little impact in patients with pMMR or MSS metastatic CRC (149,150). Furthermore, the effectiveness and safety of PD-1/PD-L1 and CTLA-4 ICIs on advanced CRC are still inconclusive in clinical studies, and the outcomes are paradoxical (139,151). By contrast, previous findings suggest that NK-1R antagonists induce apoptosis in CRC cells through endoplasmic reticulum stress and calcium release, activating the PERK/eIF2α/ATF4/CHOP pathway, which enhances chemotherapy sensitivity and reveals potential biomarkers and therapeutic targets (152). Moreover, the heterogeneity of CRC or the TME can modulate the response to therapeutic intervention, and there may be shortcomings in identifying patients likely to benefit from this combined treatment strategy (153).

*Enhancing viral immunogenicity for an improved immune response*. Viral immunogenicity is essential in the mechanism of action of OVT for CRC since it establishes the capacity of the virus to elicit an immune response against cancer cells (154). When OVs infect and lyse cancer cells, they display antigens that the immune system can see and thus stimulate T cells and other immune factors (155). This process is termed ICD and is crucial for priming and reinforcing antitumor responses (57). OVs can be engineered genetically to boost this immunogenicity. These changes may concern the deletion of viral genes that inhibit the host immune response, the addition of other genes that encode immune-stimulating products, such as cytokines (IL-12 and IL-15) or antibodies (such as against PD-1), and the presence of molecules enhancing viral replication in tumor cells and their spread (156,157). For example, oncolytic HSV-1 containing the humanized anti-PD-1 antibody gene has been reported to strengthen immune response and suppress tumor progression in models of CRC (158). Moreover, cytokine preconditioning parental cells can increase the release or the antitumor activity of exosomes originating from such parent cells, thus strengthening the immune response (159). These genetic changes seek to transform the 'cold', difficult-to-treat tumors that are less sensitive to ICIs and enhance their ability to stimulate an immune response (154).

Immune adjuvants play a pivotal role in enhancing the effectiveness of OVT in CRC by amplifying the immune response. They stimulate the innate immune system, promoting the recruitment and maturation of antigen-presenting cells, which is crucial for initiating adaptive immunity against cancer cells (160). For example, T-VEC produces GM-CSF, which improves antigen-presenting cell function and elicits a copious T-cell response at the tumor site (161,162). However, genetic modifications can be made to increase immunogenicity by endowing OVs with 'micrometals', CD40L and OX40L, which have been found to improve tumor control and $CD8^+$ effector T cell functions (163).

New strategies to enhance viral immunogenicity for OVT include the use of OVs with cytokine-gene or immune-checkpoint inhibitors (54). For instance, VALO-D102, an adenovirus that encodes CD40L and OX40L, has a more robust efficacy on tumor suppression if used with an anti-PD-1 antibody (163). Additionally, there is evidence of the employment of bispecific T-cell activators and other antibody formats to increase oncolytic viral productivity (164,165). These strategies aim to counteract the suppressive immunological characteristics of tumors in OVT, thereby enhancing therapeutic effects on CRC.

Furthermore, the emerging therapeutic applications of exosomes are gaining attention due to their ability to modulate epithelial-mesenchymal transition (EMT) and other tumor-promoting processes. Recent advances highlight their potential as effective delivery platforms for miRNAs or drugs to reverse EMT or enhance treatment efficacy (166). By integrating exosome technology with existing OVT

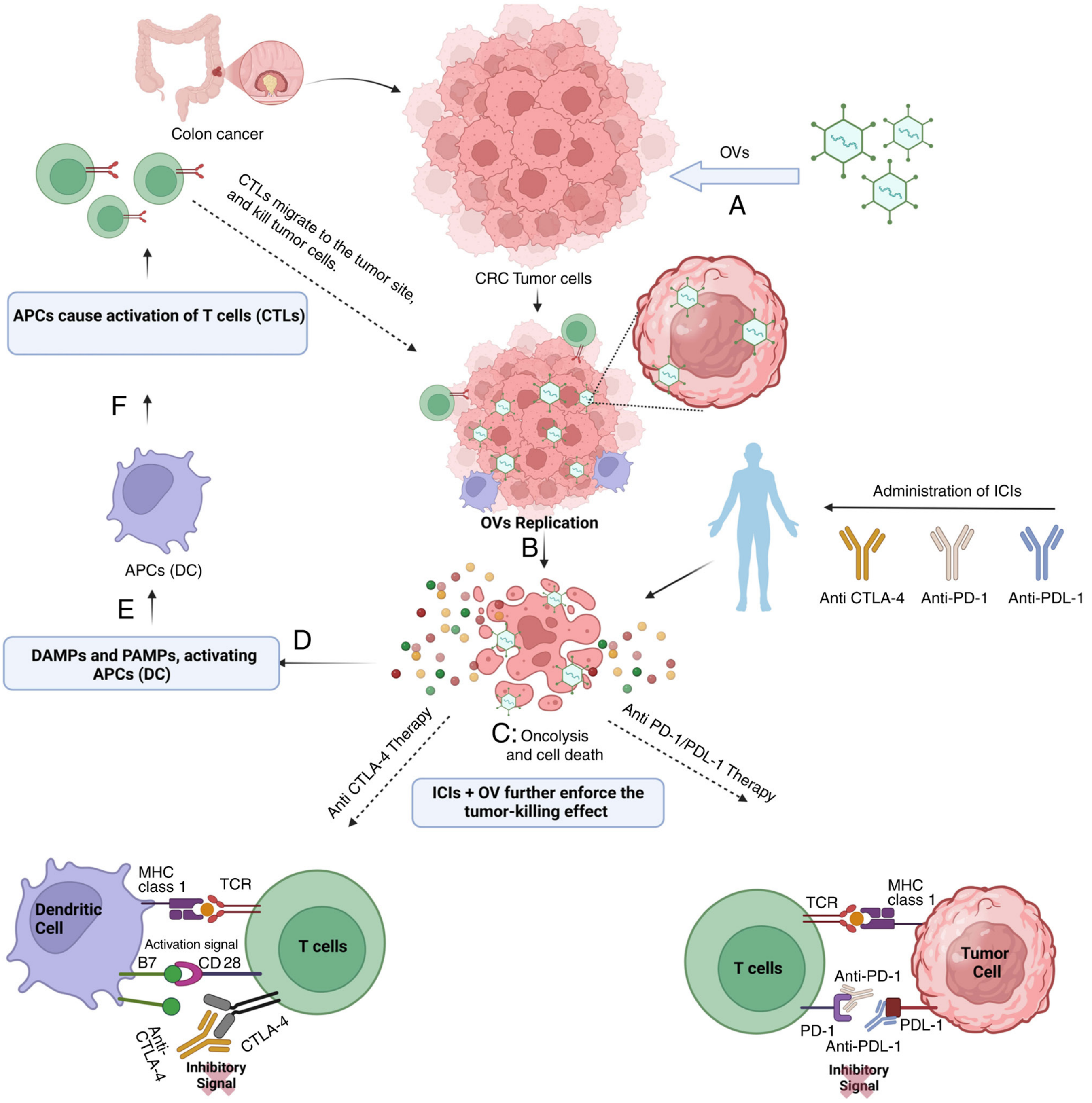


Figure 3. Synergistic mechanism of OV therapy and ICIs in CRC treatment. OVs are antitumor agents used in CRC treatment that infect and destroy CRC tumor cells through oncolysis and release TAAs, DAMPs and PAMPs. These molecules activate antigen-presenting cells, including dendritic cells, to identify and present TAAs to CTLs. CTL activation results in their subsequent recruitment to the tumor stroma and the identification and destruction of target tumor cells. The ICIs, which include anti-CTLA-4 and anti-PD-1/PD-L1, amplify the antitumor immune response by checkpoint blockade. Anti-CTLA-4 treatment and anti-PD-L1 therapy/reformatory strengthen T-cells, while anti-PD-1/PD-L1 therapy counterbalances the immunosuppressive tumor microenvironment that causes immune tolerance/escape. The integrated use of OVs and ICIs results in enhanced immune response and persistent tumor elimination, thus improving the therapeutic results. This figure was created using BioRender (BioRender Inc.). CRC, colorectal cancer; OV, oncolytic virus; DAMPs, damage-associated molecular patterns; PAMPs, pathogen-associated molecular patterns; ICIs, immune checkpoint inhibitors; TAAs, tumor-associated antigens; CTLs, cytotoxic T lymphocytes; CTLA-4, cytotoxic T-lymphocyte-associated protein 4; PD-1, programmed cell death protein 1; PD-L1, programmed death-ligand 1; TCR, T cell receptor.

strategies there is potential for improved treatment outcomes in CRC. Additionally, exosomes play a crucial role as essential mediators of intercellular communication, regulating EMT. This enables tumor cells to acquire invasive and metastatic properties, which poses challenges for effective treatment strategies (167).

*Role of the TME in modulating OVT efficacy.* The TME in CRC is replete with immunosuppressive cells such as Tregs and tumor-associated macrophages (TAMs) that form a massive hurdle to immune effector cells (161). OVT can change this TME by using ICD, which will release other TAAs and recruit immune cells, including macrophages and T cells. These immune cells play a crucial role as they can recognize both the viral infection and tumor antigens, thereby generating a significantly stronger antitumor immune response (30). Macrophages, in particular, when polarized to an M1 phenotype, are known to support the antitumor immune response (162), whereas T cells, especially CD8 cytotoxic T-lymphocytes, directly kill cancer cells (168). Recent developments in OVT have focused on the modification of viral specificity and tropism and the modification of OVs to provide immune-stimulatory molecules to increase viral immunogenicity (169). For instance, viruses can be equipped with cytokines such as GM-CSF, which enhance the antitumor function of lymphocytes, or chemokines, which attract more immune cells into the TME (170). Additionally, incorporating ICIs into the virus can counteract the immunosuppressive state of the TME and enhance the immune targeting of CRC (32).

## 4. Biomarker-driven combination therapies in precision OVT for personalized CRC management

*Chemotherapy and radiation synergy with OVT in CRC*. Chemotherapy and radiation therapy are known treatments for the therapy of CRC with the overall goal of therapeutic mitigation of tumors and the associated symptoms (21). These drugs act by causing permanent defects in the DNA of actively dividing cancer cells, inhibiting growth. Nevertheless, these treatments also target healthy cells and, therefore, cause side effects and may not help all patients, particularly those diagnosed with MSS CRC, since this disease typically does not respond well to immunotherapy (171-173). In this context, the combination of OVT with chemotherapy or radiotherapy has emerged as a promising strategy in CRC treatment. Such combinations aim to enhance therapeutic efficacy by leveraging complementary mechanisms, including the induction of ICD and the modulation of the TME (21). The current research states that integrating these therapies exploits similar pathways, including ICD. OVT, chemotherapy and radiotherapy all release danger associated molecular patterns, which enhance innate and adaptive immunity (174). This improves the overall tumouricidal activity and seems beneficial in permitting lower doses of cytotoxic agents to be deployed in combination with enzymes, thereby diminishing toxicity. For instance, genetically modified oncolytic viruses can be programmed to express enzymes such as cytosine deaminase to transform prodrugs, such as 5-fluorocytosine, into toxic agents wherever the virus is replicating but minimize the side effect on other organs of the body (175).

*Targeted therapies and OVT: Promising drug combinations*. Targeted therapy for CRC is aimed at proteins or molecules that directly impact cancer rather than harming all dividing cells, such as in chemotherapy. These therapies target critical signaling pathways misregulated in CRC, including SHH/Gli, Wnt/β-cat, TGF-β/SMA, EGFR and Notch. For example, vismodegib, a hedgehog (Hh) inhibitor, suppresses the Hh pathway by binding to the Smoothened receptor and thus induces apoptosis in colon cancer cells (176). Similarly, Albring *et al* (177) have demonstrated the ability of berberine and its derivatives to modulate the Wnt/β-catenin signaling cascade.

The combination of targeted agents can improve the outcomes of OVT in managing CRC. When combined with targeted drugs that modulate molecular pathways, OVT generates enhanced toxicity for cancer cells while preserving standard tissue tolerance. In this regard, the use of monoclonal antibodies to EGFR, such as cetuximab and panitumumab, improves the response of patients with CRC to OVT by increasing the sensitivity of cancer cells to the OV (178). The same drugs can positively affect tumor vessels; for instance, bevacizumab can prevent the formation of short and irregular vessels typical for tumor-related vasculature, increasing the effectiveness of OV (179). This implies that there is an opportunity to improve the effectiveness of OVT using targeted therapies that make cancer cells sensitive to the treatment while at the same time lowering the general toxicity. When used together, there is the potential for drug candidates such as anti-angiogenic agents for CRC and EGFR inhibitors to enhance the effectiveness of OVT by making cancer cells more sensitive to treatment while reducing overall toxicity. Certain anti-angiogenic agents, such as regorafenib (an oral multikinase inhibitor), combined with immunotherapies such as nivolumab, have been shown to be beneficial in patients with CRC, especially those with MSS tumors, which are less sensitive to immunotherapies (180). This combination demonstrated an overall response rate of ~44% in the REGONIVO study (181).

Bevacizumab is an anti-angiogenic agent recently licensed in CRC and has demonstrated increased overall survival and progression-free survival (PFS) in metastatic CRC (182). Furthermore, research has shown that combining chemotherapy with bevacizumab in patients with metastatic CRC is influenced by PD-L1 expression, suggesting that PD-L1 status plays a crucial role in the effectiveness of this therapeutic approach (183,184). Cetuximab target the EGFR signaling cascade, which is frequently mutated in CRC, and have shown efficacy in KRAS wild-type metastatic CRC (185). These potent agents have been evaluated in several clinical trials for the first-line, second-line or third-line treatment of metastatic CRCs.

OVT combined with targeted therapies in CRC also poses certain issues, such as resistance, toxicity and dosage. Crossover and bypass can result in innate and acquired resistance due to existing interactions between pathways (186). One major issue with combining these therapies is that, as the number of treatment sessions increases, the rate of complications also rises (187). The dosage needs to be carefully fine-tuned to allow for effective targeted therapy while avoiding toxicity due to the druggability of targets in the TME, which have not been entirely elucidated (188). Some factors that should be weighed include prophylactic considerations, cost/benefit considerations, patient selection and follow-up, given the inter-compliant variation results (189,190).

Table II summarizes various therapeutic agents, including targeted therapies, ICIs and chemotherapy drugs, and their mechanisms of action in enhancing OVT efficacy. These combinations leverage synergistic effects to improve viral replication, immune activation and tumor-specific cytotoxicity.

*Novel biomarker-based strategies for personalized OVT*. Biomarkers are critical in precision medicine in CRC due to

Table II. Combinatorial approaches to enhance OVT efficacy in CRC.

| Therapy type | Drug name | Mechanism of action | How it enhances OVT efficacy | (Refs.) |
|---|---|---|---|---|
| Targeted therapy | Ruxolitinib | JAK-1/2 inhibitor that enhances the replication and activity of VSV-IFN-β by antagonizing antiviral JAK/STAT signaling. | Enhances viral replication and activity, increasing sensitivity to OVT. | (251,252) |
| Targeted therapy | Bortezomib | Proteasome inhibitor used with oHSV, strongly inducing necroptotic cell death and activating NK cells. | Enhances NK cell-mediated antibody-dependent cellular cytotoxicity. | (253-255) |
| Targeted Therapy | Bevacizumab | An anti-angiogenic drug used with OVT inhibits angiogenesis and improves the virus distribution and survival of infected tissues. | Improves virus distribution and survival, enhancing the efficacy of OVT. | (256) |
| Targeted therapy | EGFR inhibitors (cetuximab) | Monoclonal antibody targeting EGFR, inhibiting EGFR-mediated signaling. | Enhances OVT efficacy by inhibiting EGFR signaling. | (185,257) |
| Targeted therapy | Kinase inhibitors (BKM120) | Inhibits the PI3K/AKT signaling pathway. | Promotes viral replication, enhancing OVT efficacy. | (55,258,259) |
| Targeted Therapy | A2a Receptor Antagonists | A2a receptor antagonists block the adenosine A2a receptor, which inhibits T cell activation and function in the tumor microenvironment. | By inhibiting the A2a receptor, these drugs enhance T cell activation and proliferation, counteracting the immunosuppressive effects of adenosine in CRC and thereby improving overall tumor response to OVT. | (260) |
| Immune checkpoint inhibitor | Avelumab (anti-PD-L1) | Combined with cetuximab and FOLFOX6 chemotherapy regimen, it showed a 75% objective response rate and 95% disease control rate in patients with mCRC. | Enhances immune response, improving the efficacy of OVT. | (261-263) |
| Immune checkpoint inhibitor | TIM-3 Inhibitors | TIM-3 is an immune checkpoint receptor that inhibits T cell activation and function upon binding to its ligand, galectin-9. TIM-3 inhibitors block this interaction, promoting T-cell responses. | By blocking TIM-3, these inhibitors enhance T cell activation and effector function, reducing the immunosuppressive effects in the tumor microenvironment of CRC, which can improve the effectiveness of OVT. | (260) |
| CAR-T Cell Therapy | BiTEs | Redirects T cells to tumors, killing both virus-infected and non-infected tumor cells, achieving a 'bystander effect.' | Improves antitumor T cell responses. | (264,265) |
| Immunotherapy | G47Δ-mIL12 | IFNγ and T cell killing inducers promote M1-like polarization ($iNOS^+$ and $pSTAT1^+$) in TAMs. | Enhances synergy with immune checkpoint inhibitors, curing glioblastoma and inducing immune memory. | (266,267) |
| Chemotherapy drugs | 5-FU | A chemotherapeutic agent that inhibits DNA and RNA synthesis by being metabolized into active forms such as FdUMP and FUTP, leading to tumor cell death. | Enhances OVT efficacy by producing chemotherapy drugs locally, reducing systemic side effects. | (268,269) |

CRC, colorectal cancer; OVT, oncolytic virus therapy; JAK, Janus kinase; STAT, signal transducer and activator of transcription; VSV, vesicular stomatitis virus; IFN-β, interferon-β; NK, natural killer; oHSV, oncolytic herpes simplex virus; EGFR, epidermal growth factor receptor; PI3K, phosphoinositide 3-kinase; AKT, protein kinase B; A2a, adenosine A2a receptor; PD-L1, programmed death-ligand 1; mCRC, metastatic colorectal cancer; TIM-3, T-cell immunoglobulin and mucin-domain containing-3; BiTEs, bispecific T-cell engagers; CAR-T, chimeric antigen receptor T-cell; TAMs, tumor-associated macrophages; iNOS, inducible nitric oxide synthase; pSTAT1, phosphorylated signal transducer and activator of transcription 1; 5-FU, 5-fluorouracil; FOLFOX6, combination chemotherapy regimen (folinic acid, fluorouracil and oxaliplatin).

their guiding role in identifying various aspects of the tumor molecular profile that define its management. The American Society of Clinical Oncology, European Society for Medical Oncology and National Comprehensive Cancer Network recognize mutations within these genes as essential pharmacogenomic biomarkers (191-193). Notably, BRAF mutation is considered to be adverse for survival, highlighting the role of biomarkers as a guide for the selection of treatments based on tumor characteristics (194). In particular, PD-L1, TMB and MSI are established as possible indicators of ICIs. PD-L1 is currently used to predict response to anti-PD-1/anti-PD-L1 therapy, while TMB and MSI have some predictive roles in numerous cancer types, including colon cancer (195,196). Another molecular biomarker associated with improved clinical outcomes in ICI therapy for patients with CRC is MSI status, particularly in tumors that are MSI-H or dMMR (197). However, the predictive value of PD-L1 has been criticized since some patients with low or undetectable PD-L1 expression respond to this targeted therapy (198). This indicates that there is a requirement for improved predictive biomarkers for ICI therapy.

Abnormalities in the RAS, BRAF and EGFR genes are considered valid prognostic indicators in CRC (199). More specifically, anti-EGFR receptor therapies fail to show efficiency when KRAS mutations exist in the malignant cells. Detecting individuals with these genetic changes is recommended as these treatments can be unhelpful and expensive (200). In locally advanced rectal cancer, patients harboring wild-type KRAS have improved pathological complete response rates with cetuximab, a targeted agent (201). These biomarkers may be utilized in individualized treatment planning, which will benefit patients by reducing toxicities and misguided treatment costs.

## 5. Overcoming challenges in precision OVT for CRC

*Addressing virus delivery and tumor targeting limitations.* Delivering OVs into CRC tumors is challenging since CRC tumors are developed and heterogeneous. Although the concept of systemic delivery of OVs is thought-provoking due to its capability to target disseminated metastases, the approach is also faced with challenges such as the short biological half-life OVs in the circulatory system and the poor ability to target as well as sediment at the tumor site, thereby reducing the possibility of delivering OVs to the metastatic deposits. Intratumoral delivery, while less versatile as it is restricted to accessible tumors, can garner higher viral shed-load and produce vigorous local antitumor effects (42). Furthermore, other factors, such as the secretion of stromal cell-derived factor-1, can also worsen the mobilization nadir as well as greatly enhance the number of circulating progenitor cells, but the responses are typically relatively weak at untreated distal sites (202).

To enhance the efficiency of systemic delivery, a new method involving nanoparticle encapsulation and engineered vesicles has been proposed. Nanoparticles improve the resistance, solubility, crossover ability and circulation time of OVs; in other words, they make viral vectors safer and more effective in clinical applications (203). A recent innovation in nanoparticle construction has incorporated more hierarchical structures, bio-feedback guidance components and conjugates, which make OVs reach tumor-specific locale more efficiently (204,205). For example, biomimetic nanoparticles coated by cell membranes can use immune evasion, prolonged circulation and disease-specific targeting to enhance the oncolytic effect of OVs at the tumor site (206). The systemic and intratumoral delivery methods are compared in Fig. 4.

In summary, different delivery systems of OVs possess certain limitations, namely, immune clearance and insufficient tumor accumulation, which can be resolved by nanoparticle encapsulation and engineered vesicles. Modifications to nanoparticles, such as surface functionalization with targeting ligands or the incorporation of stimuli-responsive elements, have been shown to significantly enhance their ability to deliver OVs specifically to tumor lesions while minimizing off-target effects (207). These strategies will likely enhance the clinical use of OVT in CRC.

*Resistance mechanisms and OVT.* Understanding the mechanisms of cancer drug resistance can guide the optimization of existing targeted therapies, identify therapeutic targets valuable to discovering new and improved agents and form the basis of therapeutic advances in cancer treatment (208). As with other cancer treatments, CRC cells may interfere with or become resistant to OVT via several processes. This mechanism involves the activation of the PI3K-$\gamma$/AKT pathway in tumor-associated myeloid cells (TAMCs), which fosters an immunosuppressive environment by downregulating cytotoxic $CD8^+$ T lymphocytes and inhibiting their activity (209-212). Additionally, CRC cells may have upregulated expression of immune checkpoint molecules, such as PD-L1, and thereby inhibit T cell function. The TME is also involved and the mechanical property of the tumor tissue and matrix stiffness hampers immune cell infiltration, which is essential for the effectiveness of OVT (213,214).

Understanding mechanisms of resistance is needed to outline how to counter this type of adaptive resistance. However, when the current OVTs are combined with players such as HDIs, the viral replication, oncolytic activity and recognition of NK cell activating ligands and TAAs are all boosted (215-217). Inhibition of PI3K-$\gamma$ can overcome immuno-suppression in TAMCs and improve the therapeutic effect of OVT (218). The aberrant activation of the PI3K/AKT/mTOR signaling pathway in CRC promotes therapeutic resistance by enhancing cancer cell survival, metabolic reprogramming and immune evasion through mechanisms such as PTEN loss or PIK3CA mutations (219). Targeting this pathway with PI3K inhibitors (such as alpelisib and copanlisib) or mTOR inhibitors (such as everolimus) may counteract resistance by suppressing downstream oncogenic signaling and restoring apoptotic sensitivity (220). Combining these inhibitors with immune checkpoint blockers (such as pembrolizumab and nivolumab) could further enhance efficacy by reversing PI3K/AKT-mediated immunosuppression and reinvigorating antitumor immunity (221). Additionally, epigenetic therapies targeting DNA methyltransferases or histone deacetylases may reactivate tumor suppressor genes silenced by PI3K/AKT-driven hypermethylation, while miRNA-based strategies (such as with miR-34a) could downregulate pathway components (222). Thus, it can be stated that using selective

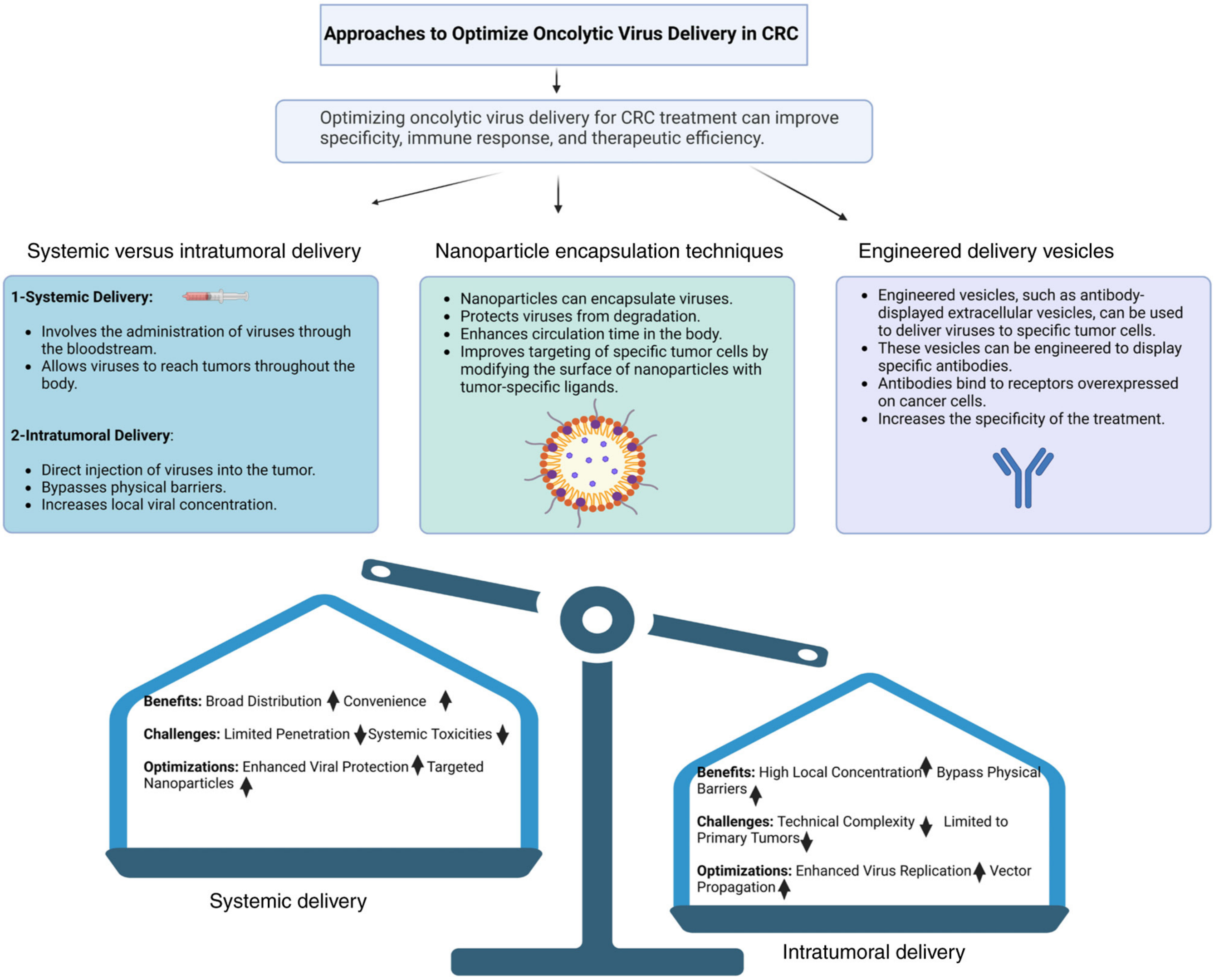


Figure 4. Comparison of systemic and intratumoral OV delivery methods for CRC. This figure illustrates the comparison between systemic and intratumoral delivery methods for OV therapy in CRC, highlighting their respective benefits, challenges and optimization strategies. Systemic delivery involves administering viruses through the bloodstream, enabling broad distribution and convenience but facing limitations such as limited penetration and potential systemic toxicities. By contrast, intratumoral delivery entails direct injection into the tumor, offering high local viral concentration and bypassing physical barriers, though it is technically complex and mostly limited to primary tumors. To enhance efficacy, nanoparticle encapsulation techniques protect viruses from degradation, extend circulation time and improve targeting by modifying the surface with tumor-specific ligands. Additionally, engineered vesicles, such as antibody-displayed extracellular vesicles, can deliver viruses specifically to tumor cells, increasing treatment specificity through targeted binding to upregulated receptors on cancer cells. These optimizations aim to improve specificity, immune response and therapeutic efficiency in CRC treatment. This figure was created using BioRender (BioRender Inc.). CRC, colorectal cancer; OV, oncolytic virus.

targeting of specific resistance mechanisms and combining OVT with other treatment approaches, it is possible to effectively overcome the adaptive resistance of CRC cells to OVT.

*Managing safety and toxicity in precision OVT.* Potential issues connected with OVT for colon cancer are related to uncontrolled proliferation of the virus, the reaction of the immune defense mechanisms, other types of infections as well as unpredictable side effects. Furthermore, combined interventions of OVT with other treatments may increase such possibilities, posing threats to the patient's well-being and thus mandating close supervision (175). Further studies are being conducted to determine the correct dosage of OVs. Specific indicators, such as biomarkers or patient characteristics, are used to select the most suitable candidates for OVT and to anticipate possible side effects linked to this innovative treatment protocol (175).

Measures that help to avoid contact with the OV, including strict measures for transportation and administration of the OV, are necessary to minimize the potential exposure. Moreover, the training of clinicians and information for patients on how to take care of the area where injections are administered is also required. Adverse effects surveillance in patients administered OVT faces challenges such as under-reporting and bias in data collection, which can obscure the true incidence of adverse events. Additionally, distinguishing between genuine product-related effects and coincidental events complicates accurate surveillance and assessment (57). As effective as OVT may be for precision therapy in CRC, it must also be regulated for safety due to possible unintended consequences and viral transmission. Genetic engineering for specificity, containing the virus or making the treatment highly supervised, and monitoring the treatment closely and strictly can guarantee the use of OVs in cancer therapy.

**6. Clinical trial insights and challenges**

*Challenges*. The gaps in the patient selection criteria for current OVT clinical trials for CRC are multifaceted. One significant gap is the historical underrepresentation of specific populations in clinical trials, limiting the generalizability of results and affecting the diversity of eligible participants. Specifically, the strict eligibility criteria often exclude a large proportion of patients, particularly those who are older, female or non-Latinx Black, and those with a lower socioeconomic status, thus limiting the pool of eligible candidates. The lack of broadened eligibility criteria may potentially exclude patients who could benefit from the treatment (223). Furthermore, the challenges in validating combination treatment strategies using patient-derived organoids highlight the need for more refined and representative models to guide patient selection in clinical trials (153). Addressing these gaps is crucial for optimizing patient selection, ensuring more inclusive trials and improving the clinical applicability of OVT in CRC.

In OVT clinical trials for CRC, selecting predictive endpoints is crucial for determining success. According to the latest research, endpoints such as PFS and immune reactivity are considered highly predictive of success (224). PFS is a critical secondary endpoint that measures the time from trial randomization to the occurrence of disease progression or death, and it is a standard measure of the effectiveness of a treatment in delaying disease progression. However, immune reactivity is assessed through the objective response rate, which measures the proportion of patients achieving a complete or partial response, and the disease control rate, which includes patients with stable disease for a defined period (225). These endpoints are integral to evaluating the immunotherapeutic effects of OVT.

*Clinical trials*. Recent progress in OVT for CRC has occurred through efforts to enhance the viral agents' specificity and carcinoid efficacy by gene engineering and distinct delivery system methods in the last decade. New strategies include elements of viral genomes that promote antitumor immune responses and the integration of OVT with ICIs to overcome TME (226). To illustrate this progress, Table III summarizes ongoing and completed clinical trials investigating OVT in CRC, highlighting the use of engineered viruses, immune-modulating therapies and innovative delivery systems.

*Regulatory, ethical and practical aspects of precision OVT*. Precision oncology, mainly with the use of virtual trials and OVT, opens new possibilities in clinical practice. However, their implementation should also consider compliance with regulatory requirements concerning the safety and efficacy of applied approaches, data correctness and ethical standards. Additionally, patient privacy, data security measures and consent are essential for successfully approving and developing precision OVT (227). The application of precision to OVT for patients has notable challenges, such as restraints in

Table III. Ongoing clinical trials.

| First author/s, year | Trial identifier | Therapy/agent | Mechanism/approach | Phase | Focus | (Refs.) |
|---|---|---|---|---|---|---|
| Qi *et al*, 2024 | NCT05228119 | RT-01 (oncolyticadenovirus) + nivolumab (PD-1 inhibitor) | Boost immune response to CRC via combination therapy. | Ongoing | Investigates toxicity, tolerability and therapy efficacy of combination to remodel the TME. | (226) |
| Qi *et al*, 2024 | NCT05354102 | Live bacterial consortium + nivolumab | Novel immunotherapy for advanced malignancies, including CRC | Open-label | Assesses toxicity, tolerability and efficacy of combination therapy in the immunosuppressive TME. | (226) |
| Zhang *et al*, 2024 | NCT04755543 | OH2-based oncolytic virus + LP002 (PD-L1 antibody) | Combined therapy for patients with advanced CRC resistant to standard treatments. | Phase I | Assesses safety, tolerance and efficacy of combination therapy | (32) |
| Shebbo *et al*, 2024 | NCT03740256 | CAdVEC (oncolytic virus)+ HER2-specific CAR-T | Combines oncolytic virus with CAR-T therapy for HER2+ CRC. | Phase I/II | Evaluates survival of modified cells and tumor impact, with outcomes expected by the end of 2024. | (270) |

CRC, colorectal cancer; TME, tumor microenvironment; PD-1, programmed cell death protein 1; PD-L1, programmed death-ligand 1; HER2, human epidermal growth factor receptor 2; CAR-T, chimeric antigen receptor T-cell; OH2, oncolytic herpes simplex virus type 2;CAdVEC, conditionally replicating adenovirus; RT-01, oncolytic adenovirus; FOLFIRI, folinic acid, fluorouracil, irinotecan; FOLFOX, folinic acid, fluorouracil and oxaliplatin.

safety and efficacy, Good Manufacturing Practice standards and patient consent. Long-term monitoring is necessary for continuous post-market surveillance (226). The germline genetic profile, comorbidities and age-specific mutational processes should inform the creation of these regimens. The modified form of OVT can increase the efficacy of treatments due to the targeted receptor contributing to immune evasion, thus leading to improved patient results (228). Researchers hope that adjusting the therapy according to the properties of the tumor will enhance the antitumor immune response and overall survival. Additionally, the synergistic effects of OVT with other immunotherapies may be apparent, improving the overall therapeutic advantage (55).

The use of genetically modified OVs introduces unique ethical challenges, particularly concerning patient safety and potential long-term risks. While these viruses are engineered to selectively target cancer cells and minimize off-target effects, unintended consequences such as viral shedding, transmission to non-target tissues or unforeseen immune reactions could pose significant risks (229). Ensuring rigorous preclinical testing, transparent informed consent processes and long-term post-market surveillance is critical to mitigating these concerns. Additionally, equitable access to precision OVT must be considered, as high costs and complex logistical requirements may limit availability in low-resource settings. Addressing these ethical dimensions will not only safeguard patients but also foster public trust in this transformative therapeutic approach.

## 7. Conclusions

OVT represents a new paradigm in the field of CRC treatment, as it has the potential to circumvent the drawbacks associated with conventional therapies while utilizing targeting and immune-stimulating properties. During the last decade, genetic engineering and biomarkers have opened the door for targeted treatment options, a style that remains novel in CRC due to intersquamous tumor heterogeneity. Additionally, combining ICIs with other conventional methods such as chemotherapy and targeted therapy produces synergistic effects on treatment outcomes.

Nevertheless, the OV technique has certain limitations, which have posed the following challenges: How best to deliver viruses, what strategies to counter viral resistance and the question of safety when the viruses will be used clinically. These include integrating new, efficient delivery systems (nanoparticle encapsulation) and creating new biomarkers. Although OVT holds significant promise, its implementation in low- and middle-income countries is hindered by notable challenges, such as inadequate healthcare infrastructure, prohibitive treatment costs and limited access to advanced therapeutic technologies. To bridge these gaps, it is crucial to develop innovative strategies, including cost-effective viral production techniques, simplified and scalable delivery systems as well as fostering international collaborations aimed at promoting equitable access to OVT-based treatments. Such efforts could pave the way for broader global adoption of this transformative cancer therapy.

Future directions for enhancing translation involve incorporating artificial intelligence into these findings and utilizing well-established clinical trials for patient selection. By addressing these issues through a behavioral/precision OVT approach, CRC treatment may undergo a radical transformation, establishing a new standard in the oncology process. With ongoing research, this innovative treatment modality could eventually transition from experimental to routine practice, offering millions of patients a renewed chance at life worldwide.


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## Authors' contributions

Conceptualization was conducted by MHS and YW; MHS, XJ and YW collected and organized the relevant literature; YX and QZ conducted a systematic review of the literature to identify key themes and trends; MHS, QZ, YX, XJ and YW critically evaluated the sources and synthesized the findings; project administration was conducted by YW; the original draft was written by MHS; reviewing and editing the manuscript was performed by MHS, YW and XJ. All authors read and approved the final version of the manuscript. Data authentication is not applicable.

## Ethics approval and consent to participate

Not applicable.

## Patient consent for publication

Not applicable.

## Competing interests

The authors declare that they have no competing interests.

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