Computer-aided designing of oncolytic viruses for overcoming translational challenges of cancer immunotherapy

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Wild-type and genetically engineered oncolytic viruses (OVs) represent powerful therapeutic agents in cancer immunotherapy. Several OV species are in clinical trials for cancer treatment. Preclinical and clinical trials revealed several issues related to OV therapy in terms of viral delivery, spread, antiviral immune response, and tumor resistance. Here, we suggest some promising computational strategies that can overcome these issues. The strategies include predicting and prioritizing tumor-homing peptides, anticancer peptides, neoantigens, and miRNA response elements in the viral genome. The combination of computational approaches with genetic engineering could enhance the safety, delivery, oncolysis, and antitumor immune responses of OVs.

Preclinical and clinical studies indicate that tumor microenvironment (TME) suppresses antitumor immunity [1]. The regulation of TME is crucial for the treatment of cancer. In this regard, immunotherapy is currently one of the most important therapeutic strategies for fighting cancer. In contrast to conventional therapies, immunotherapy activates the host immune system against localized and metastatic tumors [2].

Since the US Food and Drug Administration (FDA) approval of T-VEC, the use of OVs as a new class of immunotherapeutic drugs to treat cancer has gained momentum [3]. More than 60 OVs are in clinical trials as a monotherapy or in combination with other therapies, supporting the importance of OVs in cancer treatment. The superiority of oncolytic virotherapy (OVT) over other approaches relies on its ability to selectively replicate and overcome transcriptional and mutational resistance of cancer cells, its manufacturing flexibility, and potentiation of existing therapies [4]. In addition to their natural oncolytic ability, virus-mediated cancer cell death also activates the immune response via multiple mechanisms. OVs disrupt the immunologically suppressed TME by inducing the expression of cytokines and chemokines. This recruits innate/adaptive immune cells locally and systemically in TME, subsequently causing immunogenic cell death (ICD) [5].

In this review, we specifically focus on the mechanism of oncolysis, and the therapeutic and translational challenges associated with developing successful OVTs as immune adjuvants for the induction of antitumor immunity. We also discuss possible innovative computational strategies to enhance the oncolytic ability of viruses for cancer treatment.

Mechanism of oncolysis

OVs achieve their therapeutic efficacy by the selective killing of tumor cells and the establishment of systemic antitumor immunity. Selective replication of OVs in tumor cells causes oncolysis, which releases cell debris and tumor antigens into TME. The released debris and immunosuppressed TME attract immune cells, such as dendritic cells (DC) and antigen-presenting cells (APC), towards the tumor site, which engulf the tumor antigen and cell debris. These antigens are then processed and expressed by immune cells, which contributes to the priming of anticancer T and B cell responses [6]. Consequently, this leads to the development of specific antitumor immunity with long-term protection against cancer recurrence. Tropism of OVs towards cancer cells is achieved by multiple factors: (i) a receptor-mediated mechanism where receptors for viral entry are often highly expressed on tumor cells

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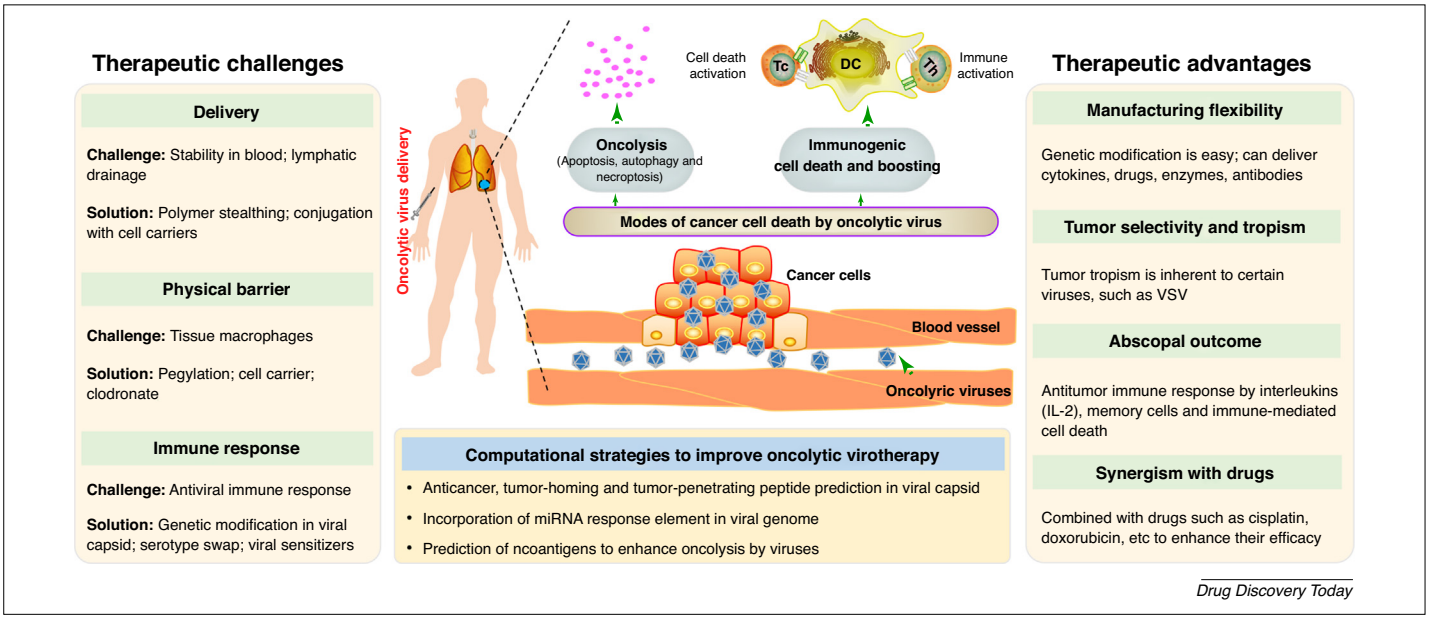

FIGURE 1

Generalized overview of the mechanism of action of oncolytic viruses (OVs), their therapeutic advantages, challenges, and computational strategies to improve oncolytic virotherapy (OVT). OVs not only induce tumor cell death, but also simultaneously stimulate innate and adaptive immunity via the presentation of tumor and viral antigens to major histocompatibility complex (MHC) molecules, respectively. Oncolysis is achieved by apoptosis, necrosis, and autophagy cell death processes that release viral and tumor antigens. These released tumors and viral antigens are captured by antigen-presenting cells (APCs), such as dendritic cells (DCs), which leads to the generation of an active immune response by stimulating the maturation of immature T cells to become either mature CD4+ T helper (Th) cells or mature cytotoxic CD8+ T cells. The generated mature CD4+ T cell boosts the immune system and the CD8+ T cells start infiltrating tumor cells to specifically eliminate them. Abbreviations: Tc, cytotoxic T cells; VSV, vesicular stomatitis virus.

(e.g., the measles virus (MV) utilizes CD46 cancer cell surface receptors for entry); (ii) the virus exploits the abnormal signaling pathway adopted by cancer cells for sustained growth to make their targeted entry (e.g., the AKT signaling pathway is utilized by myxoma virus [7]); (iii) viruses, such as vesicular stomatitis virus (VSV), utilize the hypoxic environment resulting from rapid cell proliferation for their entry and replication inside tumor cells [8]; and (iv) many tumors have deficiencies in antiviral type-1 interferon signaling, thus supporting selective virus replication [9] (Fig. 1). In addition to the above-mentioned mechanisms, tumor-driven mutational changes in signaling pathways also support the selectivity of viruses towards tumor cells [10].

Challenges and current strategies to improve oncolytic virotherapy

The successful execution of OVT requires the handling of several crucial limitations. These limitations range from virus safety, delivery, replication, and physical barriers, to the immune response.

Safety

Published results from preclinical and clinical studies highlights potential concerns about the safety of OVs, primarily including toxicity, environmental shedding, and reversal to wild-type strains [11]. Genetic engineering was used to modify the genomes of OVs for marked increases in both efficacy and safety. Examples include the deletion of *UL39* to limit the replication of the virus to cancer cells only, and counteracting the PKR response by deleting *Y1 34.5* in herpes simplex virus (HSV) [8]. Table 1 provides details of genetic modifications performed to improve OV safety.

Delivery

The efficiency of OVT is heavily influenced by the route of delivery into tumor mass. Data from published literature and clinical studies indicate that preferred modes of delivery of OV are via direct injection or intratumorally (IT) for solid malignancies and intravenously (IV) for other cancer types [12]. Systemic IV administration allows the bloodstream to carry OVs to metastatic sites. A very low dose of IV-administered OV reaches the target sites owing to factors such as lymphatic drainage, and sequestration of the virus in the capillaries of spleen and liver, and OV stability in blood. In recent years, cell-based carriers, including tumor, immune, and stem cells (SCs) have been developed to improve the targeted delivery of OVs [13].

Viral replication and physical barriers

IT or IV delivery of OVs shows transient and localized effects in tumors. This limits the use of OVT to solid tumors and, thus, poses a problem in their development as a successful anticancer agent. Experimental studies demonstrated that the localized effect of OV results from tissue-resident macrophages and extracellular matrix (ECM) proteins [14]. The ECM of cancer cells limits the intratumoral spread of OVs, similarly to that found with Semliki forest virus (SFV). The main approach to overcome the problem imposed by ECM is the use of matrix-degrading enzymes, such as proteases, which allows the safe passage of anticancer agents to cancer cells [15]. ICOVIR-17 is a modified OV that was armed to express a matrix-degrading enzyme and showed improved oncolytic properties [16]. Another modified OV, ICOVIR-5, is in a clinical trial for the treatment of advanced melanoma (Table 1).

TABLE 1

Representative clinical trials of OVs for cancer therapeutics[a]

Drug name	Modification and combination	NCT number; Phase of trial	Cancer type; route; no. of patients	Study outcome
HSV: genome size 240 Kb; linear ds DNA; packaging capacity 150 kb				
Talimogene Laherparepvec (T-VEC)	Deletion of *ICP34.5, ICP47, US11;* insertion of *GM-CSF*	NCT00769704; Phase III	Advanced melanoma; intralesional T-VEC, and subcutaneous GM-CSF; 436	Higher DRR and longer median OS in patients with stage IIIB, IIIC, or IVM1a; antitumor immunity by increasing T cell and decreasing Tregs
	Deletion *ICP34.5, ICP47, US11;* Insertion *GM-CSF*; T-VEC plus ipilimumab	NCT01740297; Phase II	Advanced melanoma; intralesional T-VEC and intravenous ipilimumab; 198	Combination improved ORR; >50% decrease in size of tumor; no toxicity; improved safety; improved antitumor immune response
Seprehvir (HSV1716)	Deletion of *RL1*	NCT01721018; Phase I and II	Malignant pleural mesothelioma; intrapleural; 12	Tumor shrinkage; well tolerated and stable; necrosis induction
TBI-1401 (HF10)	Deletion of both copies of *UI56*, single copy of *UL52*	NCT02428036; Phase I	Pancreatic cancer; intratumoral; 6	Well tolerated; stable disease; increase in antitumor T cell reactivity and cytokines
Adenovirus: genome size 26–46 kb; linear ds DNA; packaging capacity >8 kb				
ONCOS-102 (CGTC-102)	Deletion of *E1A*; insertion of serotype 3 knob and *GM-CSF*; ONCOS-102 plus cyclophosphamide	NCT01598129; Phase I	Solid tumors; intratumorally ONCOS-102 and orally cyclophosphamide; 12	ONCOS-102 safe; no DLT; activation of Th1 immune profile in patients
CG0070	Deletion of *E2F* promoter; insertion of *GM-CSF*	NCT02365818; Phase II	Non-muscle invasive bladder carcinoma; intravesical; 66	Overall, 47% of patients showed CR and 50% patient with CIS grade
ICOVIR-5	E2F-1 promoter and delta 24 deletions; expressing PH20	NCT01864759; Phase I	Advanced melanoma; Intravenous; 14	Fails to induce tumor regression; Induce antitumoral immune response
MV: genome size 15 kb; nonsegmented RNA; packaging capacity 6–8 kb				
MV-CEA	Insertion of *CEA* gene and sodium iodide importer gene in MV genome	NCT00408590; Phase I	Ovarian cancer; primary peritoneal cavity cancer; intraperitoneal; 37	Well tolerated; longer PFS; evidence of immune stimulation
VV: genome size 190 kb; linear ds DNA; packaging capacity 25 kb				
Pexastimogene-devacirepvec (PexaVec) (JX594)	*TK* deletion and *GM-CSF* insertion	NCT00554372; Phase II	Advanced hepatocellular carcinoma; intratumoral; 30	Dose-dependent response; no toxicity and MTD; ten patients had stable disease; immunotherapeutic effect seen
	TK deletion and *GM-CSF* insertion	NCT01387555; Phase IIa,b	Sorafenib therapy-failed hepatocellular carcinoma; intravenous; 129	Well tolerated; did not improve OS as second-line therapy after sorafenib failure; induced T cell response
Prostvac-V (rilimogene galvacirepvec); Prostvac-F (rilimogene glafolivec)	Genetically modified to express *PSA*, *TRICOM* and *GM-CSF*; subsequent Prostvac-F boost	NCT00108732; Phase III	Castration-resistant prostate cancer; subcutaneous; 1297	Well tolerated; improved survival; stimulated T cell response
GL-ONC1	Insertion of *Ruc-GFP*, β-glucuronidase, β-galactosidase gene	NCT01443260; Phase I	Peritoneal carcinomatosis; intraperitoneal; 43	Well tolerated; increased tumor cell destruction; induction of humoral immune response in all patients
Parvovirus (H-1PV); genome size 5–6 kb; linear ss DNA; packaging capacity 5 kb				
ParvOryx01	Wild-type	NCT01301430; Phase I and II	Glioblastoma multiforme; firstly, intratumoral and peritumoral and, secondly, intravenous and peritumoral; 18	None reported
Reovirus: genome size 16–27 kb; linear ds RNA				
Reolysin (paratrooper)	Wild-type; reolysin plus carboplatin, paclitaxel, and drugs alone	NCT01280058; Phase II	Pancreatic acinar carcinoma, PDAC, stage IV pancreatic cancer; intravenous; 73	Did not improve PFS when given in combination compared with drug alone; combination increased T cell, IL-6,8, NK cells, and 14 proinflammatory plasma cytokines

[a]Abbreviations: CR, complete response; ds, double-stranded; MTD, maximum tolerated dose; ORR, overall response rate; OS, overall survival; PFS, progression-free survival.

The availability of tumor-associated macrophages (TAMs) in the tumor mass, their genomic instability, and fast response to outside stimuli highlight their potential as therapeutic targets for cancer. The delivery of clodronate liposome is among one of the widely used approaches for TAM deletion [17]. Another strategy is to use modified oncolytic vaccinia virus (VV) expressing an antagonist of CXCR4, which showed a marked increase in viral replication and reduced the infiltration of tumor-promoting cells [18] (Table 1).

Host antiviral immune response against the virus

Generation of innate and adaptive immune responses against the delivered OVs are major factors affecting their use in clinics. Consequently, the delivered OVs are neutralized by antibodies and other complement components [19]. Rapid virus neutralization by host immune cells requires the increased delivery of OVs to patients, which ultimately affects the dose, and presents safety and toxicity concerns. Serotype swapping, genetic modification, polymer-coated viruses, and combinations with inhibitors and drugs are therapeutic approaches currently used to overcome these limitations [13]. Ad5/35, an adenovirus expressing the fiber protein Ad35, shows reduced toxicity and antiviral immune response [20]. Genetic modification of the surface glycoproteins of the MV was carried out to reduce antiviral immunity [21]. In other strategies, the use of polymer-coated viruses increased the virus circulation time and also helped the virus to escape antibody neutralization [22].

Another commonly used therapeutic strategy in clinical trials is the use of OVs in combination with drugs, such as cyclophosphamide, temozolomide, and paclitaxel. The combination reduces the antiviral response and the activity of regulatory T cells (Tregs). The use of a histone deacetylase inhibitor (HDACi) along with OVs is another therapeutic strategy to overcome the host antiviral immune response (Table 1).

Immunosuppressive tumor microenvironment

The TME comprises a heterogeneous population of neoplastic and nontransformed cells, such as cancer-associated fibroblasts (CAFs), vascular endothelial cells (ECs), adipocytes, stromal cells, and various resident or migratory immune cells. Nontransformed cells of TME secrete immunosuppressive cytokines, chemokines, soluble factors, and various matrix-remodeling enzymes that support tumor growth and the maintenance of immunosuppression in TME [23].

The immunosuppressive TME is another main obstacle that prevents OVs from reaching their full therapeutic potential. Novel strategies using recombinant OVs to deliver therapeutic genes, such as those encoding cytokines (GM-CSF, IL-2, IL-12, IL-15, and IL-24), chemokines (CCL5 and 6), immune checkpoint inhibitors [24] (PD-1 and CTLA4), suicide genes (HSV-TK and cytochrome P450), tumor suppressor genes [25] (p53, p16, and PTEN) and proapoptotic genes (apoptin and TRAIL), are under development and some are in clinical trials (Table 1) for overcoming the obstacles posed by TME.

Oncolytic viruses in current clinical trials

The main focus of OVT is to recruit active immune cells to constantly attack tumor cells. Successful demonstration of this in various *in vitro* and experimental models [26] paved the way for testing OVs in clinical trials for safety, toxicity, clinical, and immunological outcomes [27]. Currently, there are several viral species in different stages of investigation for immune-oncolytic use. Some of the virus species are in their wild-type forms, such as reovirus and parvovirus, whereas others have been engineered to improve tumor cell selectivity and oncolysis (Table 1). The HSV-based drug, T-VEC, is a modified OV in which the genes *ICP34.5* (for neurovirulence) and *ICP6* (a ribonucleotide reductase gene) have been deleted. *ICP6* is necessary for viral replication in normal cells; thus, its deletion results in selective replication in cancer cells. To improve its immune-oncolytic properties, the gene encoding GM-CSF was incorporated into the HSV genome [28]. Replication of adenoviruses occurs in S-phase of the cell cycle [29] and the deletion of the *E1A* gene from the viral vector promotes its safe replication. Table 1 lists natural or modified OVs in current clinical trials.

Novel computational strategies to enhance the efficacy of OVT

Despite various successes, the above-mentioned strategies have one or more issues associated with them: for example: (i) toxicity of the cell-based carrier vehicle to virus progeny [30]; (ii) serotype switching is not possible for each virus type; (iii) mutating surface residues and polymer coatings can alter virus tropism [21]; (iv) genetic modifications can affect viral efficacy [31]; and (v) immunosuppressive drugs cannot be given to patients who are immunocompromised, thus limiting tumor cell infection and safety.

If the full potential of OVs is to be realized in the clinic, future therapeutics approaches must overcome the above-mentioned limitations. Certain OV platforms based on HSV, reovirus, and VV have shown success in clinical trials for treating advanced cancers. Yet, these OV species required extra modifications to make them more potent for cancer immunotherapy. There are several aspects of interactions between OVs and tumor host cells that can have a clear value as a target to improve therapeutics. At the level of OVs, these include improving delivery to the tumor, increasing infectivity towards tumor cells, improving oncolysis, and persistence. At the host level, these include overcoming physical barriers, tumor escaping the antiviral immune response, and manipulating the immunosuppressive TME.

Computational aided design (CAD) has emerged as a big player in the drug discovery process in recent years. By harnessing the power of information technology, big data, and CAD, we can build and design new OVs. Here, we suggest several computational strategies that can be used to manipulate the above-mentioned parameters to make OVs safer, more potent, and with a high therapeutic index.

Anticancer, tumor-homing, and tumor-penetrating peptides for improvement in OVT

Anticancer and tumor-homing peptides have immense potential for developing therapeutics in terms of high specificity, cell permeation, affinity, and minimal drug interactions [32]. Anticancer peptides attach to the cancer cell membrane and can induce cell death via multiple mechanisms, such as apoptosis, autophagy, pyroptosis, and necrosis. Tumor-homing peptides can also serve as a basis for carrying payloads specifically to the tumor site. Currently, many tumor-homing peptide-based therapies for cancer treatment and diagnosis are being tested in

various phases of clinical trials. We suggest the use of computational methods, such as TumorHPD, AntiCP, ACPred, MLACP, ACPP, and ACPred-FL to design anticancer and tumor-homing peptides that can be used to genetically engineer OVs for improved cancer management (Table 2).

In addition, the prolonged maintenance of sufficient amounts of OV in the tumor site is vital for successful cancer therapy because the delivered viruses are constantly being attacked and cleared by host immune cells. In solid malignancies, OVs have to penetrate the tough ECM of the tumor mass. Various genetic engineering approaches have been used to insert the peptide and protein domains in viral capsids to increase their infection and transduction efficiency [33]. Inserting tumor-penetrating peptides (TPP) within the capsid of OVs offers several advantages, including: enhanced binding between virus and target cell receptor; enhanced transduction efficiency,

TABLE 2

. Overview of open-source computational methods for the advancement of OVT

Method	Brief description and website	Refs
CellPPD	Predicts and designs cell-penetrating peptides; In addition, allows computation of peptide analogs and physicochemical properties (http://webs.iiitd.edu.in/raghava/cellppd/)	[39]
SkipCPP-Pred	Predicts cell-penetrating peptides using adaptive k-skip-n-gram algorithm (http://server.malab.cn/SkipCPP-Pred/Index.html)	[40]
CPPpred	Developed on a nonredundant data set for predicting cell-penetrating peptides (http://bioware.ucd.ie/~compass/biowareweb/Server_pages/cpppred.php)	[41]
CPPred-RF	Identifies cell-penetrating peptides and their uptake efficiency (http://server.malab.cn/CPPred-RF/)	[42]
CellPPDMod	*In silico* model for predicting cell–penetrating potential of chemically modified peptides (http://webs.iiitd.edu.in/raghava/cellppdmod/)	[43]
KELM-CPPpred	Application of kernel-extreme learning machine for predicting cell-penetrating peptides (http://sairam.people.iitgn.ac.in/KELM-CPPpred.html)	[44]
ccSOLomics	Web server for solubility prediction of proteins and peptides (http://s.tartaglialab.com/static_files/shared/tutorial_ccsol_omics.html)	[45]
PROSO II	Computational tool to evaluate protein solubility and identify sequence features that have strongest impact on protein solubility (http://mbiljj45.bio.med.uni-muenchen.de:8888/prosoII/prosoII.seam)	[46]
TEPITOPEpan	Pan-specific method for predicting binding of a peptide against 700 HLA-DR alleles (www.biokdd.fudan.edu.cn/Service/TEPITOPEpan/)	[47]
HLArestrictor	Predicts HLA binders for patient based on their HLA alleles (www.cbs.dtu.dk/services/HLArestrictor/)	[48]
Rankpep	Position-specific scoring matrix-based algorithm for predicting MHC class I and II binders (http://imed.med.ucm.es/Tools/rankpep.html)	[49]
ProPredI	Web server for predicting promiscuous binders for large numbers of HLA Class I alleles (https://webs.iiitd.edu.in/raghava/propred1/)	[50]
ProPred	Web server for predicting promiscuous binders that can bind large numbers of HLA Class II alleles (https://webs.iiitd.edu.in/raghava/propred/)	[51]
IEDB	Contains extensive collection of experimentally measured immune epitopes and a suite of tools for predicting and analyzing epitopes (www.iedb.org/)	[52]
pVAC-Seq	Genome-guided *in silico* approach for identifying tumor antigens (https://github.com/griffithlab/pVAC-Seq)	[53]
Cancertope	Genome-based approach for identification of personalized epitopes for designing vaccines against cancer (https://webs.iiitd.edu.in/raghava/cancertope/)	[54]
MiRanda	Identification of potential miRNA target sites in genomic sequence (http://cbio.mskcc.org/microrna_data/miRanda-aug2010.tar.gz)	[55]
miRmap	Identifies potential miRNA target sites in a genome sequence and repression strength of miRNAs (https://mirmap.ezlab.org/)	[56]
IL4Pred	Web server for predicting IL-4-inducing MHC class II binders and generating peptide derivatives (https://webs.iiitd.edu.in/raghava/il4pred/)	[57]
IL17eScan	Tool for predicting IL-17-inducing peptides (http://metagenomics.iiserb.ac.in/IL17eScan/)	[58]
CancerIN	Web server developed for predicting anticancer activity of small molecules (https://webs.iiitd.edu.in/oscadd/cancerin/)	[59]
CDRUG	Tool for predicting anticancer activity of a chemical compound (http://bsb.kiz.ac.cn/CDRUG/)	[60]
TumorHPD	Method for predicting tumor-homing peptides with ability to deliver drugs to tumor site (https://webs.iiitd.edu.in/raghava/tumorhpd/)	[60]
CancerPred	Predicts cancer lectins with important roles in tumor cell differentiation and metastasis (https://webs.iiitd.edu.in/raghava/cancer_pred/)	[61]
AntiCP	Web server to design and predict anticancer potential of a peptide or protein sequence (https://webs.iiitd.edu.in/raghava/anticp/)	[62]
ACPred	Tool for prediction and analysis of anticancer peptides (https://github.com/chaninlab/acpred-webserver)	[63]
ACPred-Fuse	A method for identification of peptides that have anticancer activities (http://server.malab.cn/ACPred-Fuse/Server.html)	[64]
ACPred-FL	Computational method for identification of anticancer peptides (http://server.malab.cn/ACPred-FL)	[65]
HemoPI	Web server for identification of peptides with hemolytic potency and of peptide analogs with minimum hemolytic potency (https://webs.iiitd.edu.in/raghava/hemopi/)	[66]
ToxinPred	Classification of toxic and nontoxic peptides and to design nontoxic peptides with minimum mutation in a peptide (https://webs.iiitd.edu.in/raghava/toxinpred/)	[67]
AlgPred	Method for predicting protein allergens based on similarity of known epitopes with any region of protein and for locating position of epitope in the protein (https://webs.iiitd.edu.in/raghava/algpred/)	[68]
AllerTOP	Tool for prediction of allergens based on main physicochemical properties (www.pharmfac.net/allertop/)	[69]

and promoting internalization of the virus. Targeted delivery of TPP can specifically increase the aggregation of drugs, nanotherapeutics, and antibodies in cancerous mass [34]. Thus, tumor-homing peptides that have also tumor and/or cell-penetrating capability could serve as a better mechanism for the targeted delivery of OVs. We suggest building a random peptide display library by predicting TPP sequences within the viral capsid to develop vectors with enhanced tumor selection and penetration. In this regard, various computational tools, such as CellPPD, SkipCPP-Pred, CPPpred, KELM-CPPpred, and CPPred-RF, can be used for *in silico* prediction, designing, and prioritization of cell-penetrating peptides (Table 2). The bioavailability of these peptides is increased if they are more water soluble. The process of optimizing the aqueous solubility is mainly empirical and cumbersome but several bioinformatics tools have been developed to accelerate this process. ccSOL and PROS II are two such tools to predict the solubility of the proteome based on the physicochemical properties of the primary sequence (Table 2).

Tumor-specific neoantigens for improvement of OVT

Oncolysis results in the release of tumor-associated antigens (TAA)/neoantigens; recognized by the immune system, and which serves the basis of the generation of the antitumor immune response via CD4+ and CD8 + T cells. These TAAs often arise from mutated proteins of cancer cells and, thus, are nonself in nature. These are ideal therapeutic molecules for cancer immunotherapy. Preclinical data show promising results in cancer management using neoantigen-based vaccine approaches because they not only boost the antitumor immune response, but also reduce the risk of autoimmune reactions [35]. Neoantigens that can be expressed and processed via major histocompatibility complex (MHC) alleles provide a novel way to boost cancer immunotherapy. The genomic profiles of the cancer cells can be exploited to identify the neoantigens that can be processed in the proteasome and further bind to human leukocyte antigens (HLAs). Thus, accurate identification and prediction of neoantigens that can specifically bind to MHC molecules are vital for developing epitope-based vaccines and improving immunotherapy. The predicted tumor-specific neoantigens can also be combined with OVs and DCs for the advancement of *in situ* vaccination. There are several tools available for epitope prediction, such as TEPITOPEpan, HLArestrictor, Rankpep, ProPred-I, ProPred, and IEDB analysis resources. Several pieces of open-source software are also available for computing neoantigens, such as Vaxrank, ProTECT, Epidisco, and pVAC-Seq (Table 2).

Predicting miRNA response elements in the viral genome for enhanced oncolysis and reducing off-targets

Dysregulated expression of genes and miRNAs has a main role in tumorigenesis. The dysregulated miRNAs and genes have been used to provide selectivity of OVs toward cancer cells [36]. OVs engineered to express miRNA response element (MRE) in the 3′-untranslated region (UTR) of their genome to handle the downregulated miRNAs of host cancer cells were found to be effective in suppressing the growth of tumors. MREs can reduce the occurrence of OVs in nontumoral tissue and, therefore, can minimize the undesired toxicity associated with viral tropism when injected systemically [37]. By utilizing the genomic expression of cancer samples, and using computational strategies to design MRE for downregulated genes and miRNA, the specificity and safety of OVs can be increased. Virologists can use tools, such as MiRanda and miRmap, for predicting the MRE within the viral genome and their target site repression strength, respectively (Table 2). Thus, this strategy can be used to develop cancer tissue-specific OV-based therapy.

Immunomodulators and targets to overcome cancer mutational resistance

Advanced tumors have a natural tendency to acquire mutations and develop resistance towards a variety of therapeutic agents. Overcoming mutational resistance is the major challenge for the success of any new therapy. The traditional 'one drug one ligand' concept is inadequate to overcome the ever-advancing mutational cancer process. Thus, a modern-day approach to combat cancer drug resistance seeks multifunctional compounds that can efficiently interact with multiple targets simultaneously. OVs can selectively attack tumor cells in multiple ways and, therefore, tumors acquire less resistance towards OVT [38]. This property of OVs makes them attractive as a suitable carrier for developing multifunctional compounds for cancer immunotherapy. Computational strategies can be utilized for predicting and prioritizing improved immunomodulators, such as cytokines, proteasome inhibitors, viral sensitizers, immunosuppressive compounds, proapoptotic ligands, and molecules, which can activate the antitumor immune response. Exploiting the genetic engineering approach, multifunctional OVs can be developed that bind to several targets in cancer cells and could provide a novel means to overcome tumor resistance. Computational methods, such as IL-4pred and IL17escan, can be utilized for predicting the cytokine peptide-based immunomodulators, whereas tools such as cancerIN and CDRUG, can be used to detect the immunosuppressive anticancer properties of compounds (Table 2).

Safety concerns with OVT

OVs offer a promising therapeutic option for cancer treatment owing to their favorable risk:benefit ratio. Researchers are continuously searching for strategies that can exploit the advantageous properties of OVs, such as increased circulation time, reduced off-target effects, enhanced immune response, and improved oncolysis. Data from most preclinical and clinical studies show that OVs are well tolerated in patients. However, some clinical trials reported mild toxicity in patients because of OVT. Sometimes, chemical modulators and peptides are foreign in origin; thus, incorporating them within viruses and their administration can lead to problems such as toxicity and allergies. Therefore, before treating patients with OVs, we advocate other computational tools that can be utilized to predict the above-mentioned adverse effects. We suggest the use of HemoPI, ToxinPred, AlgPred, and AllerTop to predict hemolytic toxicity, pernicious effects and the potential allergenicity of the peptides and/or proteins inserted in the viral genome.

Also, protein–protein interactions (PPIs) with abnormalities can induce signaling changes responsible for various pathological conditions, such as infection, chronic inflammation, cardiovascular diseases, and neurodegeneration. Various computational repository and prediction tools, such as BioGRID, STRING, STRINGdb, TRI_tool, SPRINT, and Ens-PPI, can be used to assess toxicity resulting from disturbances in PPIs caused by OVT. Table 2 provides a brief description of open source software that can be utilized for improving the efficacy of OVT.

Concluding remarks

Data from clinical studies show that immune therapies have significant potential in cancer treatment. However, the ability of tumors to evade the immune system remains a challenge. The approval of T-VEC by the FDA in 2015 was a turning point for the development of OVs as a new class of immunotherapeutics. OVs are unique in their action because they not only kill tumor cells, but also provide long-lasting immune response against tumor. OVT also suffers limitations, such as virus delivery, physical barriers, and sometimes host antiviral immune responses. To strengthen the overall response rate and delivery issues, OVs are being tested in combination with anticancer drugs, immune checkpoint inhibitors, antibodies, and cell-based carriers, among others. Genetic engineering techniques enable researchers to optimize OV delivery, specificity towards tumor cells, and their efficacy by different combination approaches with the aim to achieve maximum therapeutic benefit. This review describes several computational strategies that can be used to further improve OV delivery, antiviral immunity, and oncolysis, as well as to overcome problems posed by TME and physical barriers. Computational strategies could lead to the development of improved OVs with strong therapeutic potential.

To conclude, OVs offer tremendous potential for the treatment of cancer. Although patients who are refractory to the current standard of care could well benefit from this novel approach, eagerness to rush through clinical trials might jeopardize their health as well as the integrity of the OV field. Preclinical fervor should be tempered with caution during this precarious phase, and clinical trials should be carefully designed with rigorous scientific backing. We hope to see the future generation of novel OVs as single agents to combat cancer with minimum repeated doses and low toxicity towards healthy cells.

Author contributions

G.P.S.R. conceived and coordinated the project; A.L. and R.K. collected the data; A.L., R.K., and G.P.S.R wrote the manuscript, which was revised by all authors.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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