Oncolytic viruses for the treatment of cancer: current strategies and clinical trials

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Tumor-selective replicating viruses offer appealing advantages over conventional cancer therapy and are a promising new approach for the treatment of human cancer. The development of virotherapeutics is based on several strategies that each provides a different foundation for tumor-selective targeting and replication. Results emerging from clinical trials with oncolytic viruses demonstrate the safety and feasibility of a virotherapeutic approach and provide early indications of efficacy. Strategies to overcome potential obstacles and challenges to virotherapy are currently being explored and are discussed here. Importantly, the successful development of systemic administration of oncolytic viruses will extend the range of tumors that can be treated using this novel treatment modality.

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▼ The goal in developing new therapies for the treatment of cancer is to design agents that have a large therapeutic index (i.e. high potency against malignant cells), but only limited pathogenicity to normal tissue. Naturally occurring lytic viruses have evolved to infect, replicate in and lyse human cells. It is evident that the replication cycle of many viruses exploits the same cellular pathways that are altered in cancer cells [1,2]. Recent advances in our understanding of the molecular biology of cancer, as well as the availability of technologies to genetically engineer viruses, have led to the concept of oncolytic viruses. These biological agents are thought to hold the toxic and discriminatory power that is expected from an efficacious therapy against human cancer and they have several appealing properties. Whereas conventional chemotherapeutic drugs distribute relatively uniformly throughout the human body and follow the typical log kinetics of cell killing, the local replication of administered oncolytic viruses amplifies the input dose and creates a high concentration of therapeutic agent at the target site. This accumulation helps to spread the agent to adjacent tumor cells and limits potentially toxic side effects within normal tissue. Because direct cell killing caused by viruses is an active and highly complex process that involves many cellular pathways, the occurrence of drug resistance appears unlikely. Furthermore, additional mechanisms, such as the stimulation of the humoral and cellular immune response of the host [3], could potentially enhance virus-induced tumor regression. Finally, by arming the oncolytic viruses with therapeutic genes, their antitumor toxicity could be increased [4].

Strategies to generate tumor-selective viruses

There are several strategies that achieve tumor-selectivity of replication-competent viruses, some of which are discussed here (Table 1 and Figure 1).

Inherent tumor-selectivity

One approach to achieve viral tumor-selectivity is to use viruses that possess inherent tumor-selectivity. Several RNA virus species are tumor-tropic, which is partly the result of their ability to grow exclusively in cells with defective antiviral response systems [e.g. Newcastle-disease virus (NDV) and vesicular stomatitis virus (VSV)] [5,6]. In the case of NDV and VSV, these RNA viruses are sensitive to inhibition by interferon, and thus normal cells are almost completely protected from infection and replication while tumor cells that lack a functional interferon response are rapidly lysed. Replication of reovirus, which is another RNA virus with inherent tumor-selectivity, is restricted by activation of the double-stranded

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Table 1. Strategies to achieve tumor-selectivity

Strategy to achieve tumor-selectivity	Advantages	Disadvantages	Virus species	Stage of development	Refs or source
Inherent tumor-selectivity	Viral modifications are not required No foreign DNA elements required	Specificity can not easily be modified Depends on natural lytic strength of virus Targeted modifications are technically challenging	Reovirus	Clinical	[7], Oncolytics Biotech[a]
			Newcastle-disease virus	Clinical	[66,67]
			Vesicular stomatitis virus	Preclinical	[5,6]
			Autonomous parvovirus	Preclinical	[10]
Attenuation by deletion of viral genes or gene fragments	Attenuation based on general tumor biology Broad mechanism of selectivity (targets signaling pathways) No foreign DNA elements or genes required	Risk of revertants to wild-type Multiple functions of viral genes	Adenovirus	Clinical	[19,29]
			Herpes simplex virus	Clinical	[11], MediGene AG[b]
			Vaccinia virus	Preclinical	[13]
			Poliovirus	Preclinical	[14]
			Measles virus	Preclinical	[5]
Transcriptional targeting	Technology well established Targeting based on known tumor biology	Insertion of foreign DNA elements Tumor escape by promoter shut-off	Adenovirus	Clinical	[58], Cell Genesys[c]
			Herpes simplex virus	Preclinical	[41]
Cellular targeting	Specific infection of tumor cells Reduced attenuation required Potential reduction in toxicity Potential reduction in dose size required	Technically challenging Detargeting and targeting required Tumor escape by mutation of receptor Insertion of foreign DNA elements	Adenovirus	Preclinical	[43]
			Herpes simplex virus	Preclinical	[44]

[a]http://www.oncolyticsbiotech.com; [b]http://www.medigene.com; [c]http://www.cellgenesys.com.

RNA-activated protein kinase (PKR) by early viral transcripts [7]. However, increased levels of Ras activity, as is frequently observed in a wide variety of human tumors, counteract this inhibition by activating a phosphatase that antagonizes the PKR effects, which consequently enables virus replication [8]. Autonomously replicating parvoviruses (e.g. B19 or H1) that efficiently lyse transformed fibroblasts while sparing normal cells have been reported [9,10]; the mechanism of this process of differentiation has yet to be elucidated.

Attenuation of wild-type viruses through the targeted deletion of viral genes

Another strategy is based on restricting virus replication to malignant cells, which involves deletions of viral gene regions or entire genes that are crucial for efficient replication in normal tissue but that are dispensable in tumor cells ('attenuation') (Figure 1a). Genetically modified, tumor-selective mutants have been described for a variety of virus species, including herpes simplex viruses (HSV), adenoviruses, vaccinia viruses and polioviruses [11–14]. Tumor cells and cells that have been infected by viruses exhibit significant similarities in their abilities to interfere with signal transduction pathways, for example, promoting the transition from the prereplication (G1) to replication (S) stage of the cell cycle [1,2]. In particular, p53- and retinoblastoma protein (Rb)-governed checkpoints must be passed before cellular proliferation can be initiated. Several viral gene products that interact with cellular

components, and thereby influence the cell cycle and cell survival, have been identified [1,2].

To target HSV replication to malignant cells, a variety of mutants have been designed with functional inactivation of the viral genes that encode for thymidine kinase, ribonucleotide reductase and infected cell protein 34.5 (ICP34.5) (e.g. HSV1716, dlsptk and hrR3) [11]. The cellular forms of viral ribonucleotide reductase and thymidine kinase are not expressed in quiescent cells, but are upregulated during the G1 and S phases of the cell cycle because they generate deoxynucleoside triphosphates (dNTPs), which are needed for DNA synthesis. This limits replication of HSV that is defective in such gene functions to rapidly proliferating cells, such as tumor cells [15]. The neurovirulence factor ICP34.5 has been characterized as an inhibitor of PKR. Therefore, PKR-induced shutoff of cellular protein synthesis following infection with HSV is circumvented by ICP34.5 [16]. However, Ras activity directly inhibits PKR-mediated effects, and thus the action of ICP34.5 is not required in cell lines that have a constitutively activated Ras signaling pathway [17]. To reduce the probability of the occurrence of wild-type revertants, and to increase the safety level, some viruses contain multiple mutations within their genomes (e.g. G207) [18]. In addition to HSV, other tumor-selective adenoviruses generated using this approach have been reported [19].

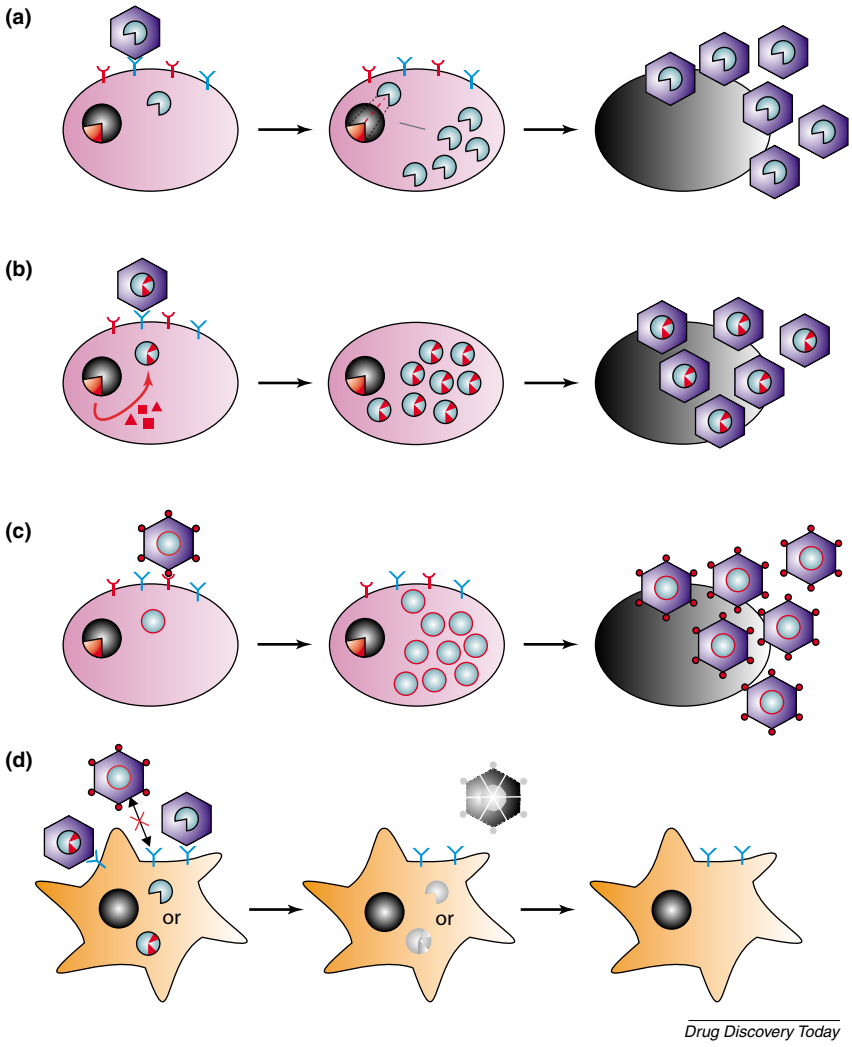

Figure 1. Strategies to achieve tumor-selectivity of oncolytic viruses. **(a)** Attenuation. Viral gene regions or entire genes that are crucial for the efficient replication of the virus in normal tissue are deleted from the viral genome. The genetic setting of tumor cells can complement those missing functions. **(b)** Transcriptional targeting. The expression of specific viral genes that are essential for viral replication is restricted to tumor cells through the use of tumor-specific promoters that are incorporated into the viral genome. **(c)** Cellular targeting. The natural cell tropism of the virus has been changed through genetic modification of the viral attachment proteins in such a way that only tumor-specific surface proteins are recognized as viral binding receptors. This strategy limits the entry of oncolytic viruses to tumor cells. **(d)** Effect of oncolytic viruses on normal cells. Although cellular-targeted oncolytic viruses (c) are unable to enter normal cells, the infection of normal cells with oncolytic viruses produced using strategies (a) and (b) leads to abortive infection.

The adenoviral early gene 1 A (E1A) proteins are potent transactivators that induce the entry of quiescent cells into the cell cycle and direct their progression to the S-phase; this sequence is predominantly initiated by the binding of the E1A proteins to the Rb protein, which triggers the release of the E2F transcription factor that is important for regulation of expression of cellular genes that control cellular DNA synthesis and proliferation [20]. However, the uncontrolled release of E2F and entry of quiescent cells into the cell cycle induces the accumulation of active p53 in the nucleoplasm, which causes growth arrest or apoptosis before the virus can replicate productively [21]. Therefore, adenoviruses encode another set of proteins, the E1B proteins (E1B55K and E1B19K), that counteract the p53-mediated effects triggered by E1A [22–24]. The E1B55K-deficient adenovirus dl1520 (Onyx-015) was the first replication-selective adenovirus to undergo rigorous

clinical trials as a cancer treatment [25]. Although the exact mechanism of action of Onyx-O15 is not completely understood [26–29], results from animal models and clinical trials indicate that this compound is a promising anticancer agent.

Another mutation approach is based on the deletion of the E1A conserved region 2; this deletion prevents interaction of E1A with the Rb protein. Several deletion mutants, including some mutants in combination with cell cycle-dependent promoters (e.g. Onyx-411, Δ24 and dl922-947), have been reported [30–32]. Based on the attenuation approach, several oncolytic versions of vaccinia viruses that comprise mutations in the genes encoding thymidine kinase and/or vaccinia growth factor (VGF), which render the viruses highly tumor-selective, have been described [13]. Although preclinical results with these mutants are promising, the clinical utility could be limited by premature immunologic clearance of the virus and inadequate bystander effects. Poliovirus mutants are also available that selectively replicate in cell lines that are derived from human glioblastomas; this selectivity is the result of an altered internal ribosomal entry site (IRES) [33].

Transcriptional targeting

A third approach to restricting viral replication to malignant cells involves the engineering of tumor- or cell type-specific promoters and enhancers into viruses to limit the expression of the genes that are essential for viral replication in tumors ('transcriptional targeting') (Figure 1b). Replication-competent adenoviruses that have restricted expression of the *E1A* and *E1B* genes have been produced for prostate carcinomas using prostate-specific promoters, such as the prostate-specific antigen (PSA) promoter, the probasin promoter and combinations of both (e.g. CV706 and CG0787) [34,35]. Selective expression of E1A has been attempted in specific carcinomas, such as hepatocellular carcinoma (α-fetoprotein promoter) and breast carcinoma (mucin-1 promoter and estrogen-receptor promoter) [36–40]. In addition, the general characteristics of tumor cells (e.g. telomerase promoter and hypoxia-inducible factor responsive elements) have been used to design oncolytic adenoviruses [36–40]. As well as adenoviruses, replication-selective HSVs have been designed and investigated. The expression of the essential immediate-early *ICP4* gene, which is under control of the albumin-enhancer-promoter, principally restricts replication of HSV to the liver and to hepatocellular carcinoma [41]. In a similar approach, the calponin promoter has been used to generate HSV mutants that replicate selectively in malignant human soft tissue and bone tumors.

Cellular targeting

Tumor-selective uptake of replication-competent viruses can be achieved by modifications of the viral coat in a process that is known as 'cellular targeting' (Figure 1c). In theory, this strategy offers the advantage that more virulent viruses can be used: because these virus mutants would not infect normal cells, there should be a reduction in toxicity. Adenoviruses have been extensively studied using the cellular targeting approach, and there have been several attempts to modify the fiber proteins to redirect the natural vector tropism away from normal cells towards malignant cells [42,43]. More recently, HSV vectors with modified vector tropism have been produced. For example, engineered HSV-1 vectors have been designed that can only enter cells that express the interleukin-13 (IL-13) receptor α2, such as malignant brain tumors [44]. In these mutants, the natural binding sites for sulfated proteoglycans in the viral glycoproteins B and C have been ablated, and IL-13 has been inserted [44]. Interestingly, blood-borne Sindbis vectors that specifically target and kill tumor cells after systemic administration have been recently reported [45]. Tumor regression could be achieved in a variety of different tumor models, including syngeneic and spontaneous tumors. Although the exact mechanism of tumor-selective infection has yet to be elucidated, it seems possible that Sindbis vectors infect tumor cells via interaction with both heparan sulfate and the 67 kDa high-affinity laminin-receptor (LAMR), which is substantially upregulated in numerous human cancers and is related to increasing invasiveness and malignancy [45]. Furthermore, in contrast to normal cells, the majority of LAMRs on malignant cells are not occupied by laminin, which appears to render Sindbis vectors able to infect tumor cells specifically [45].

Escape from the immune system

In a recent study, Yu *et al*. [46] systemically administered vaccinia virus mutants to mice and demonstrated that these mutants selectively enrich and amplify in gliomas, as well as prostate, bladder and metastatic mammary carcinomas. The authors proposed that a small number of systemically applied microorganisms could enter tumors through leaky vasculature. Because the immune system is largely suppressed in tumors, microorganisms that are distributed to a tumor escape the immunosurveillance system of the host. By contrast, the immune system of the host clears the remaining circulating viral particles shortly after intravenous delivery.

Clinical trials with oncolytic viruses

As replication of human viruses is typically restricted to human cells, issues of tumor specificity, replicative efficacy

Table 2. Clinical trials with oncolytic viruses

Virus species	Virus	Administration	Cancer type	Stage of clinical development	Status	Refs or source
Adenovirus	Onyx-015	Intratumoral injection	Head and neck cancer	Phase II	Completed	[49]
		Intratumoral injection	Pancreatic cancer	Phase II	Completed	[51]
		Mouthwash	Oral dysplasia	Phase I	Completed	[52]
		Intraperitoneal injection	Ovarian cancer	Phase I	Completed	[53]
		Hepatic intraarterial infusion	Liver metastases of colorectal	Combined Phase I and Phase II	Completed	[54,55]
		Intravenous	Metastatic colorectal cancer	Phase II	Completed	[57]
	CV706	Intraprostatic injection	Prostate cancer	Phase I	Completed	[58]
	CG7870	Intraprostatic injection	Prostate cancer	Combined Phase I and Phase II	Ongoing	Cell Genesys[a]
	Ad5-CD/TKrep	Intraprostatic injection	Prostate cancer	Phase I	Completed	[59,60]
Herpes simplex virus	G207	Intratumoral injection	Glioma	Phase I	Completed	[61]
	NV1020	Hepatic intraarterial infusion	Liver metastases of colorectal cancer	Combined Phase I and Phase II	Ongoing	[62], MediGene AG[b]
	HSV1716	Intratumoral injection	Glioma	Phase I	Completed	[64]
		Intratumoral injection	Metastatic melanoma	Pilot study	Completed	[65]
	OncoVEX	Injection into skin metastases	Skin metastases of solid cancers	Phase I	Ongoing	BioVex[c]
Newcastle disease virus	MTH-68	Intravenous	Glioma	Pilot study	Completed	[66]
	PV701	Intravenous	Advanced solid cancers	Phase I	Completed	[67]
Reovirus	Reolysin	Intratumoral	Skin metastases of solid cancers	Phase I	Completed	Oncolytics Biotech[d]
		Intratumoral	Prostate cancer	Phase I	Completed	
		Intratumoral	Glioma	Phase I	Ongoing	
		Intravenous	Advanced solid cancers	Phase I	Ongoing	

[a]http://www.cellgenesys.com; [b]http://www.medigene.com; [c]http://www.biovex.com; [d]http://www.oncolyticsbiotech.com.

and viral distribution cannot be fully addressed in animal models of cancer. Therefore, several research groups and companies have rapidly moved into clinical testing. Initial testing began with intratumoral injection and proceeded to intracavitary (such as intraperitoneal) and intravascular administration (such as hepatic artery infusion). More recently, systemic (i.e. intravenous) applications have been studied. Several clinical trials on the use of replication-competent viruses have now been published (Table 2).

Adenovirus

Of all the viruses that are undergoing clinical development, the adenovirus Onyx-015 (also known as dl1520 and CI-1042) has made the most progress, and has proven to be a safe single agent in Phase I and II trials for the treatment of patients with squamous cell carcinoma of the head and neck (SCCHN) [47,48]. SCCHN patients were chosen for initial trials because head and neck tumors harbor p53 mutations, the tumors can be injected directly, the clinical response can be assessed and biopsies can be obtained. Onyx-015 was injected intratumorally and treatment was well tolerated, with the main toxicity being mild flu-like symptoms (in particular, fever and chills). Viral replication was detected in 20% of patients and, although an antitumor response was seen in 14% of patients, a clinical benefit was not seen in the majority of the patients. However, when combined with chemotherapy, intratumoral injection of Onyx-015 demonstrated an impressive clinical

response rate in 63% of the evaluated patients, with 27% (eight patients) demonstrating full response to therapy (i.e. complete disappearance of all tumor manifestations) and 36% indicating partial responses (i.e. tumor shrinkage by over 50% of initial tumor volume) [49]. This response rate is far in excess of the expected response rates of patients that were heavily pretreated with chemotherapy alone [49]. Six months after the end of the study, none of the responding tumors had progressed, whereas all non-injected tumors that were treated with chemotherapy alone had advanced. Tumor biopsy specimens obtained after treatment showed tumor-selective viral replication and necrosis induction [49]. Based on these encouraging results, Onyx Pharmaceuticals (http://www.onyx-pharm.com) initiated a randomized Phase III clinical trial with Onyx-015 for recurrent SCCHN. These trials have currently been stalled and their progression has yet to be sanctioned.

Onyx-015 was also administered intratumorally to patients with unresectable pancreatic cancer through computed tomography (CT)-guided injection [50] or endoscopic ultrasound injection in combination with intravenous gemcitabine [51] in a Phase I and a combined Phase I and Phase II clinical trial, respectively. Two patients incurred duodenal perforations from the rigid endoscope tip, but the treatment was generally well tolerated. In the combination trial, two partial regressions and two minor responses were observed, but these were not clearly attributable to Onyx-015 administration [51]. Another tested indication of Onyx-015 includes local mouthwash therapy for premalignant oral dysplasia [52]. Histological resolution was seen in seven (37%) out of 19 patients, but these effects were transient in the majority of patients. To test intracavitary applications of Onyx-015, a Phase I trial of intraperitoneal injection of Onyx-015 was undertaken in patients with recurrent and/or refractory ovarian cancer [53]. No significant toxicity was observed at the maximum dose of 10^{11} plaque-forming units (pfu). However, no clinical or radiographic evidence of a tumor response was observed in any of the patients [53].

Intravascular applications have proved particularly promising. Hepatic arterial infusion of Onyx-015 in combination with 5-fluorouracil (5-FU) and leukovorin for liver metastases of gastrointestinal (GI) tract tumors was tested in a combined Phase I and Phase II clinical trial [54,55]. Although increased levels of liver enzymes and hyperbilirubinemia were transiently observed in a subset of patients, the regimen proved to be safe and feasible. Delayed secondary peaks of viremia at 72 h post-inoculation were detected and interpreted as an indication of viral replication. Out of 27 patients, three (11%) had a partial response, while 13 (48%) had a minor response or stable disease. However, an antitumoral activity that could be directly attributed to Onyx-015 was difficult to assess because most patients were not refractory to 5-FU and leukovorin chemotherapy [54,55].

To test systemic administration, Onyx-015 was given by intravenous infusion to patients with metastatic solid tumors (Phase I) [56], and in a Phase II clinical trial to patients with metastatic colorectal cancer [57]. Toxicity was manageable and consisted primarily of flu-like symptoms (including chills, rigors and fever); however, one patient out of 18 was hospitalized for severe lethargy. No liver toxicity was observed and viral DNA was detectable for as long as 72 h in 36% of the patients, all of which developed neutralizing antibodies. Three out of the 18 treated patients had minor reductions of carcinoembryonic antigen (CEA) levels and seven patients were assessed as having stable disease for 11 to 18 weeks. However, a progression in disease was ultimately observed in all patients [57].

Early clinical trials with adenoviruses that are transcriptionally targeted to replicate in prostate cancer cells have demonstrated promising results. A Phase I clinical trial was carried out in patients suffering from locally recurrent prostate cancer with adenovirus CV706 (PSA-restricted expression of E1A and/or E1B) [58]. Intraprostatic injection of this virus caused mild flu-like symptoms and hematuria. However, 13 out of 20 patients (65%) experienced a reduction in serum PSA of ≥30% from pre-treatment levels [58]. Patients receiving the highest intraprostatic dose of CV706 showed a reduction of PSA ≥50%, which underlines the biological activity of this compound, and post-CV706 treatment prostate biopsies revealed viral replication in individual patients [58].

In two Phase I clinical trials, the efficacy of the 'armed' adenovirus Ad5-CD/TKrep as a treatment for prostate cancer was tested as an intraprostatic injection, either alone [59] or in combination with radiation therapy [60]. Two days after adenovirus injection, the prodrugs 5-fluorocytosine and valganciclovir were administered and no dose-limiting toxicity was observed. Seven out of 16 (44%) patients demonstrated a ≥25% decrease in PSA levels and 19% showed a ≥50% decrease in PSA [54]. As expected, the response rates were increased when the course of treatment was supplemented with radiation [60].

Herpes simplex virus

The HSV-1 variant G207 was tested in a Phase I dose-escalation study that was designed to determine the safety of stereotactic inoculation for the treatment of recurrent malignant glioma [61]. Twenty-one patients received between 10^6 and 3×10^9 pfu. There were no serious side effects and, importantly, no patient developed HSV encephalitis.

In several individual patients, radiographic imaging suggested an antitumor response [61].

Early clinical data suggested a safe toxicity profile for the virus NV1020, which is in ongoing combined Phase I and Phase II trials as a therapy for liver metastases of colorectal cancer [62]; NV1020 is currently being developed by MediGene AG (http://www.medigene.com). OncoVex® (BioVex; http://www.biovex.com/index.html), which expresses the immunostimulatory granulocyte–macrophage colony-stimulating factor (GM-CSF), is currently in Phase I clinical testing for skin metastases of solid tumors, but no clinical data has as yet been reported.

HSV1716 was administered by intratumoral injection to a small group of nine patients suffering from relapsed malignant glioma [63]. The study demonstrated the therapeutic feasibility of HSV1716 with viral doses up to 10^5 pfu, with no signs of encephalitis. These findings were confirmed in another trial in which 12 glioma patients underwent intratumoral injection with 10^5 pfu, followed by surgery [64]. The data documented intratumoral replication within high-grade gliomas without causing toxicity in both HSV-seropositive and HSV-seronegative patients [64]. In addition, HSV1716 was injected intralesionally into subcutaneous nodules of metastatic melanoma of five patients [65]. As an internal control, a second nodule was injected with sterile saline. A flattening of previously palpable tumor nodules and microscopic evidence of tumor necrosis were observed. Although patient numbers are too small to draw definite conclusions, the observed effects do warrant further investigation [65].

Newcastle disease virus

MTH-68 and PV701 are two attenuated, non-recombinant strains of NDV, which is an avian paramyxovirus that causes flu-like symptoms in humans. Csatary *et al*. [66] examined the oncolytic potential of NDV by treating four cases of advanced high-grade glioma with daily intravenous injections of live attenuated NDV MTH-68 [66]. Two of the four patients had near complete disappearance of their gliomas, while the remaining two patients experienced stabilization of their disease. Although these four cases were treated with different dosing regimens, these data are nevertheless encouraging and provide grounds for further development [66]. Intravenous administration of PV701 was tested in a large Phase I clinical trial as a single agent in 79 patients with advanced cancers [67]. Flu-like symptoms, fever and hypotension were recorded, but no serious side effects were observed. Interestingly, with repeated cycles, the occurrence and intensity of flu-like symptoms decreased. The majority of patients developed antibodies to NDV. After intravenous treatment, 14 patients had stable disease for four to >30 months, seven demonstrated minor responses, two had a partial response and one patient with chemotherapy-refractory tonillar (squamous cell) carcinoma had a complete response but then relapsed seven months after treatment began [67].

Reovirus and vaccinia virus

No clinical data on the use of reovirus or vaccinia virus (expressing the immunostimulatory GM-CSF protein) have been published. As summarized in Table 2, several Phase I trials have been completed or are ongoing.

Challenges of oncolytic virotherapy

Oncolytic viruses can rapidly replicate in and spread through 2D cell cultures that are derived from a variety of different tumor types. However, there are several factors that could hamper the efficient spread of oncolytic viruses within a solid tumor mass [68]. Physical barriers such as necrotic areas within the infected tumor, normal stroma cells and extracellular matrix, or the presence of the basal membrane, could limit the distribution and infection of the diffuse virus. Mathematical models of viral replication have underlined the importance of diffuse tumor inoculation for the control of tumor growth and for the initiation of a self-perpetuating process of intratumoral viral replication [69,70]. In addition, the physical size of the administered virus particles and their interaction with the receptors that are present on normal cells could be crucial. New delivery technologies, such as convection-enhanced delivery (CED) of drugs to the brain, will need to be explored for oncolytic viral therapy of brain tumors to achieve an even virus distribution. CED enables potent drugs, which would otherwise be too toxic to the body, or drugs that are not capable of passing through the blood–brain barrier, to be slowly and continuously infused into particular brain tumors through small plastic catheters. After surgery, the drug is administered via the catheters using an infusion pump over a period of several days, and then the catheters are removed. Tools have been developed that facilitate enhanced virus administration within the tumor using several simultaneous needle injections. Importantly, the immune system of the host could limit ongoing viral replication within the tumor, and rising antibody titers could neutralize repeatedly administered viruses before the tumor has been successfully eradicated [12]. Although these neutralizing antibodies do not appear to be a major limitation for the intratumoral injection of oncolytic viruses that spread through direct cell–cell contact (e.g. HSV and measles virus), they could become a crucial factor for systemic therapy that uses intravenous administration [68]. The complement system might be another impediment

to effective delivery via the intravenous route [71]. By contrast, immune responses could also enhance the efficacy of oncolytic viral therapy, as suggested by a significant number of studies investigating viruses that express immunostimulatory proteins [4,72]. Cytotoxic T-cell responses that are directed against the tumor have been identified as a potentially important therapeutic factor [72]. Therefore, several methods that eliminate undesired immune effects while preserving beneficial properties have been proposed. The production of neutralizing antibodies could be transiently ablated by administration of anti-CD20 antibodies (rituximab) against B-lymphocytes before oncolytic virotherapy. Alternatively, the exchange of blood plasma (plasma pheresis) will enable the elimination of antibodies that are directed against viruses from the bloodstream [68]. To prevent inactivation of administered viruses by the complement, the complement could be transiently neutralized by administration of cobra venom factor or cyclophosphamide (CPA) [73]. In addition, the use of virus mutants that incorporate complement resistance factors into the outer membrane, which therefore conveys resistance to the complement, has been suggested [68]. A major factor that could potentially lead to the rapid clearance of viruses from the bloodstream could be uptake into Kupffer cells, which are extremely active phagocytic cells that line the walls of the sinusoids of the liver [68]. However, preclinical experiments with several vectors have shown that systemic metastases can be targeted following intravenous administration, despite a level of clearance by the liver. Finally, sufficient expression of viral receptors on malignant cells is required for therapeutic efficacy [68] – a factor that has been identified as a potential limitation for oncolytic adenoviruses. Expression of the key receptor for adenoviral uptake (CAR) appears to be downregulated during tumor progression, presumably by activation of oncogenic signaling pathways that render highly malignant cells less susceptible to therapy [74]. However, strategies have been suggested to overcome these prospective obstacles, such as the pharmacological inhibition of the Ras-signaling pathway [e.g. by using inhibitors of the mitogen-activated protein kinase kinase (MEK)], which increases the expression of CAR on the cell surface and consequently restores the permissiveness of these cells to viral uptake [75]. In addition, retargeted viruses are being explored that comprise new receptor-binding motifs within their coat that enable CAR-independent infection [43].

Conclusion and future perspectives

Replication-competent oncolytic viruses, either naturally occurring or genetically engineered, represent a promising new class of agents that are in development for the treatment of human cancer. Potential hurdles have been identified, and solutions to these problems are currently being explored in preclinical studies and clinical trials. To date, clinical experience indicates that these agents are safe. To increase potency, two key strategies are being pursued. Combination of oncolytic virotherapy with traditional chemotherapy and radiotherapy significantly enhances the efficacy of virotherapy, frequently on a synergistic basis [76]. In parallel, additional antitumor mechanisms are applied to oncolytic viruses to arm them with therapeutic transgenes (e.g. prodrug-converting enzymes and antiangiogenic or immunomodulatory proteins) that induce bystander effects that are capable of eliminating tumor cells that are not directly killed through viral oncolysis [4].

Randomized Phase III clinical trials are urgently needed to assess the clinical efficacy of these promising biological agents. The availability of systemic therapy in conjunction with oncolytic viruses will enhance the potential of oncolytic viruses to become a viable new therapeutic approach for the treatment of cancer.

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