Oncolytic virus therapy is a new era of cancer treatment at dawn

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Oncolytic virus therapy has recently been recognized as a promising new therapeutic approach for cancer treatment. An oncolytic virus is defined as a genetically engineered or naturally occurring virus that can selectively replicate in and kill cancer cells without harming the normal tissues. In contrast to gene therapy where a virus is used as a mere carrier for transgene delivery, oncolytic virus therapy uses the virus itself as an active drug reagent.

The concept of oncolytic virus therapy has existed for some time (Fig. 1). Tumor regression has often been observed during or after a naturally acquired, systemic viral infection. In 1949, 22 patients with Hodgkin’s disease were treated with sera or tissue extracts containing hepatitis virus. Between 1950 and 1980, many clinical trials were performed in attempts to treat cancer with wild type or naturally attenuated viruses, including hepatitis. West Nile fever, yellow fever, dengue fever and adenoviruses. However, these viruses were not deemed useful as therapeutics reagents because, in those days, there was no known method to control the virulence and yet retain viral replication in cancer cells.

It is now recognized, because protection mechanisms against viral infection (e.g. interferon-beta signal pathway) are impaired in the majority of cancer cells, that most viruses can replicate to a much greater extent in cancer cells than in normal cells. Therefore, getting a virus to replicate in cancer cells is not a problem: What is difficult is making a virus not replicate in normal cells at all, while retaining its replication capability in cancer cells. Attempts to achieve cancer cell-specific replication have been undertaken either by selecting a virus that is non-virulent in humans or by engineering the virus genome (Fig. 2). Representing the former strategy is Reolysin, a wild-type variant of reovirus that exhibits oncolytic properties in cells with activated Ras signaling with limited virulence in normal human cells. The latter strategy is, however, better suited to achieving strict control of viral replication. In 1991, Martuza et al. demonstrated that a genetically engineered herpes simplex virus type 1 (HSV-1) with a mutation in the thymidine kinase (TK) gene replicated selectively in cancer cells and was useful for treating experimental brain tumors. Their findings opened up a whole new area of oncolytic virus...
development that involves designing and constructing the viral genome. During the past two decades of thriving development, probably the most important finding regarding oncolytic virus therapy was that a systemic tumor-specific immunity is efficiently induced in the course of oncolytic activities. This phenomenon is now widely recognized as the common feature for all oncolytic virus therapy that is expected to play a major role in prolonging the survival of cancer patients (Fig. 3).

To date, two genetically engineered oncolytic viruses have been approved for marketing as drugs. One is Oncorine (H101, the same construct as ONYX-015), an E1B-deleted adenovirus, which was approved in China for head and neck cancer and esophagus cancer in 2005. The use and clinical data of Oncorine is so far limited to China. The other is T-Vec (talimogene laherparepvec, IMLYGIC, formerly OncoVEX GM-CSF), which was approved for melanoma by the FDA in the USA in October 2015 and was subsequently approved in Europe in January 2016 and in Australia in May 2016 (Fig. 1). Many clinical trials using T-Vec are currently performed worldwide by the pharmaceutical company in order to expand its application and also to expand countries for marketing. This review focuses on those oncolytic viruses under development that are likely to become treatment options in the near future (Table 1).

Genetically engineered oncolytic viruses

With the development of modern techniques of genetic engineering and increasing knowledge regarding the functions and structures of viral genes, designing and manipulating the viral genome to create a non-pathogenic virus has become the standard method for oncolytic virus development. Typically, DNA viruses are used for this strategy. T-Vec. T-Vec is a double-mutated HSV-1 with deletions in the γ34.5 and α47 genes, and the human granulocyte-macrophage colony-stimulating factor (GM-CSF) gene inserted into the deleted γ34.5 locus. The deletion in the γ34.5 genes is mainly responsible for cancer-selective replication and attenuation of pathogenicity. Because the γ34.5 genes function to negate the host cell’s shut-off of protein synthesis upon viral infection, inactivation of γ34.5 renders the virus unable to replicate in normal cells. However, because cancer cells are in defect of the shut-off response, γ34.5-deficient HSV-1 can still replicate in cancer cells. The α47 gene functions to antagonize the host cell’s transporter associated with antigen presentation; therefore, the deletion of the gene precludes the downregulation of MHC class I expression, which should enhance the antitumor immune responses. The deletion in the α47 gene also results in immediate early expression of the neighbor US11 gene, which results in enhanced viral replication in cancer cells. The GM-CSF expression was intended to enhance the antitumor immunity induction, although convincing preclinical evidence has not been shown.

The safety of T-Vec was tested in a phase I study in patients with various metastatic tumors, including breast, head/neck and gastrointestinal cancers, and malignant melanoma. Overall, intralesionial administration of the virus was well tolerated by patients. Although no complete or partial responses were...
observed, stable disease was observed in several patients, and most tumor biopsies showed tumor necrosis. T-Vec was further tested in phase II studies in patients with metastatic melanoma. A single arm phase II study resulted in an overall response rate of 26%, with responses in both injected and un.injected lesions, including visceral lesions. An increase in CD8+ T cells and a reduction in CD4+FoxP3+ regulatory T cells were detected in biopsy samples of regressing lesions. A randomized phase III trial was performed in patients with un resected stage IIB–IV melanoma (OPTIM; NCT00769704). A total of 436 patients were randomly assigned in a 2:1 ratio to intralesional T-Vec or subcutaneous GM-CSF treatment arms. T-Vec was administered at a concentration of 10^5 plaque forming units (pfu)/mL injected into 1 or more skin or subcutaneous tumors on Days 1 and 15 of each 28-day cycle for up to 12 months, while GM-CSF was administered at a dose of 125 μg/m2/day subcutaneously for 14 consecutive days followed by 14 days of rest, in 28-day treatment cycles for up to 12 months. At the primary analysis, 290 deaths had occurred (T-Vec, n = 189; GM-CSF, n = 101). The durable response rate (objective response lasting continuously ≥6 months) was significantly higher in the T-Vec arm (16.3%) compared with the GM-CSF arm (2.1%). The overall response rate was also higher in the T-Vec arm (26.4% vs 5.7%). The most common adverse events with T-Vec were fatigue, chills and pyrexia, but the only grade 3 or 4 treatment-related adverse event, occurring in over 2% of patients, was cellulitis (T-Vec, n = 6; GM-CSF, n = 1). There were no fatal treatment-related adverse events. At the time of publication, median overall survival (OS) was 23.3 months for the T-Vec arm versus 18.9 months for the GM-CSF arm (hazard ratio, 0.79; P = 0.051), but the difference in OS became significant (P = 0.049) by the time of drug application. The treatment benefit in OS was more obviously significant when T-Vec was used as the first-line treatment, and in the subgroup of patients with stage IIB, IIC or IVM1. This phase III trial was the first to prove that local intrale sional injections with an oncolytic virus can not only suppress the growth of injected tumors but also prolong the OS, supposedly via induction of systemic antitumor immunity. Based on this observation, several clinical trials of T-Vec in combination with systemic administration with immune check point inhibitors are ongoing.

G47Δ. G47Δ is a triple-mutated third-generation oncolytic HSV-1 that was developed by Todo et al. by adding another deletion mutation to the genome of G207, a second generation HSV-1. G47Δ was developed to strengthen the antitumor efficacy while retaining the safety features of G207, mainly through enhancing the capability to elicit specific antitumor immunity. Two of the mutations of G47Δ are created in the γ34.5 and α47 genes, the same genes that T-Vec utilizes. G47Δ further has an insertion of the Escherichia coli LacZ gene inactivating the ICP6 gene. The ICP6 gene encodes the large subunit of ribonucleotide reductase (RR) that is essential for viral DNA synthesis. When the growth of -deficient HSV-1 strains such as G207 and T-Vec. Furthermore, because the immediate-early expression of US11 caused by the deletion within the α47 gene prevents the premature termination of protein synthesis that slows the growth of γ34.5-deficient HSV-1 strains such as G207, G47Δ shows augmented replication capability in cancer cells, resulting in having a wider therapeutic window than any other oncolytic HSV-1.

G47Δ demonstrated a greater replication efficacy and a higher antitumor efficacy than G207. G47Δ exhibited efficacy in basically all in vivo solid tumor models tested, including glioma, breast cancer, prostate cancer, schwannoma, nasopharyngeal carcinoma, hepatocellular carcinoma, colorectal cancer, malignant peripheral nerve sheath tumors and thyroid carcinoma. G47Δ has been shown to kill cancer stem cells derived from human glioblastoma efficiently. Following the phase I–IIa study in patients with recurrent head and neck cancer, an orphan drug designation from FDA.
A phase I trial of CG0070 was conducted in patients with non-muscle-invasive bladder cancer. Ad5 adenovirus was engineered so that the human E2F-1 promoter drives the E1A gene, and the human GM-CSF gene is inserted. E2F-1 is regulated by the retinoblastoma tumor suppressor protein (Rb), which is commonly mutated in bladder cancer, and a loss of Rb binding results in a transcriptionally active E2F-1.

A phase I trial of CG0070 was conducted in patients with non-muscle-invasive bladder cancer who did not respond to BCG therapy. Single or multiple (every 28 days × 3 and/or weekly six times) dose(s) of up to 3 × 10^13 virus particles (vp) were administered intravesically. No clinically significant serious adverse events related to treatment were reported, and the most common adverse events observed were grade 1–2 bladder toxicities, such as dysuria, bladder pain and frequency. The overall response rate was 48.6% (17 of 35), which increased to 63.6% (14 of 22) in the multi-dose cohort. In the following randomized phase II/III trial in patients with non-muscle-invasive bladder cancer, 15 patients received CG0070 and 7 control patients received other standard intravesical therapies (BOND, NCT01438112). Although there was no apparent difference in the initial CR (8 patients of CG0070 [53%] vs 4 of control group [57%]), CG0070 treatment demonstrated a better durable response in a subset of high-risk patients. In a single arm phase III trial that is underway, patients with BCG-refractory non-muscle-invasive bladder cancer are given CG0070 intravesically at a dose of 10^12 vp weekly for 6 weeks. Patients who achieved a partial or complete response at 6 months after the first intervention are maintained with the same induction cycle every 6 months (BOND2, NCT02365818).

**Naturally occurring oncolytic viruses**

The idea of using naturally occurring viruses for the treatment of cancer was almost abandoned after vigorous attempts during the 1960s and 1970s because of the lack of means to control viral pathogenicity at the time. However, the idea was revived with the emerging development of genetically engineered viruses, and newly developed naturally occurring viruses are typically those that are not pathogenic in humans.

**Reolysin.** Reoviruses are double-stranded RNA viruses that replicate preferentially in transformed cell lines but not in normal cells. In theory, oncolytic properties of reovirus depend on activated Ras signaling. Reolysin is the T3D strain of reovirus, which has been most extensively studied among several serotypes as an anticancer agent, and is currently the only therapeutic wild-type reovirus in clinical development.

The first phase I trial involved intravesional administration of Reolysin in patients with advanced solid tumors. The most common treatment-related adverse events were nausea (79%), vomiting (58%), erythema at the injection site (42%), fevers/chills (37%) and transient flu-like symptoms (32%). Further phase I studies demonstrated the safety and broad anticancer activity of Reolysin in prostate cancer, malignant glioma, metastatic colorectal cancer, multiple myeloma, and solid cancers. Multiple phase II studies have investigated intravesional injection of Reolysin together with local irradiation for the treatment of refractory or metastatic solid tumors, intravesical administration of Reolysin for metastatic melanoma and intravesical administration of Reolysin in combination with chemotherapy for head and neck cancer or lung squamous cell carcinoma.

A randomized double-blinded phase III trial has been performed, comparing intravesical Reolysin in combination with paclitaxel and carboplatin versus chemotherapy alone, in patients with metastatic and/or recurrent head and neck cancer (NCT01166542). Patients were treated with intravesical administration of 3 × 10^10 tissue culture infectious dose-50 (TCID50) of Reolysin on days 1–5 with standard doses of intravesical paclitaxel and carboplatin on day 1 only every 21 days, versus standard doses of intravesical paclitaxel and carboplatin alone. According to a report by the company developing Reolysin, of 165 patients analyzed, 118 patients had regional head and neck cancer with/without distant metastases and 47 patients had distant metastases only. In patients with regional cancer, a significant improvement in OS was...
observed for the Reolysin group versus the control group \((P = 0.0146)\).\(^{57}\) The FDA in the USA granted Reolysin an orphan drug designation for malignant glioma, ovarian cancer and pancreatic cancer in 2015.

**Limitations of oncolytic virus therapy**

A wide variety of oncolytic viruses are currently under clinical development worldwide, and, as described in this review, each oncolytic virus carries the characteristics of the parental wild-type virus, not only the advantages but also the disadvantages. For example, in regards to oncolytic HSV-1, such as T-Vec and G47Δ, because HSV-1 spreads from cell to cell and does not naturally cause viremia, oncolytic HSV-1 is best administered intrathecally and may not be well suited for intravenous delivery. However, as proven by the phase III study of T-Vec in melanoma patients at advanced stages,\(^{13}\) local intrathecally injections with oncolytic HSV-1 can act on remote lesions via induction of systemic antitumor immunity.\(^{30}\) It has been shown that expression of GM-CSF does not augment the efficacy of oncolytic HSV-1, while IL-12 expression does, in immunocompetent mouse tumor models.\(^{31}\) Therefore, it is likely that the systemic effect via antitumor immunity was due to the characteristics of HSV-1 itself rather than the effect by GM-CSF.

One major concern of oncolytic virus therapy has been that the efficacy may be diminished by the presence of circulating antibodies.\(^{57}\) Viruses that naturally cause viremia are likely vulnerable to neutralizing antibodies; therefore, for such viruses, the antitumor effect of intravenous administration may be limited in patients who have had previous treatment or vaccination. An unfavorable effect of circulating antibodies was well documented in a clinical trial using oncolytic measles virus (MV-NIS) in patients with multiple myeloma.\(^{70}\) In this dose escalation study, it was only after the dosing level reached a very high dose of 10\(^{11}\) TCID50 that intravenous infusion with MV-NIS showed efficacy. In a preclinical study using tumor-bearing immunocompetent mice, intravenous treatment with reovirus resulted in regrowth of tumors 3 weeks after initial tumor growth inhibition, which coincided with the rise in serum anti-reovirus antibody titers.\(^{71}\) Phase I data showed that the maximum neutralizing anti-reovirus antibody titers were reached by day 7 in 12 (36%) of 33 patients and at day 14 in 20 patients (61%).\(^{72}\) It was, therefore, recommended that, for systemic treatment, reovirus should be administered in rapid, repeated, high doses within the first week of treatment before the rise of serum neutralizing antibodies, and that it should be used in combination with other anticancer therapies.\(^{57}\)

**Oncolytic virus as immunotherapy**

All genetically engineered oncolytic viruses described in this review were designed to enhance the induction of antitumor immunity that accompanies the oncolytic activity. Both T-Vec and G47Δ have a deletion in the \(z\)-7 gene, the product of which inhibits the transporter associated with antigen presentation; therefore, cancer cells subjected to the oncolytic activities of these viruses are vulnerable to immune surveillance, and the processing by antigen presenting cells is likely facilitated.\(^{21,22}\) A combination with systemic administration of immune checkpoint inhibitor is a reasonable strategy to enhance the efficacy of oncolytic viruses. In a preclinical study, intrathecally Reolysin treatment in combination with intravenous anti-PD-1 antibody administration was significantly more efficacious than Reolysin or anti-PD-1 alone in mice with subcutaneous melanoma.\(^{73}\) A phase Ib/II clinical trial of T-Vec in combination with ipilimumab (anti-CTLA4) is currently ongoing in patients with stage IIb-IV melanoma (NCT01740297). Preliminary results from the first 18 patients showed that the median time to response was 5.3 months, and the 18-month PFS and OS rates were 50% and 67%, respectively, with a median follow-up of 17 months.\(^{74}\) An open-label Phase Ib/III study in patients with previously untreated, unresected stage IIb-IV melanoma will further evaluate the safety and efficacy of the combination of T-Vec and pembrolizumab (anti-PD-1) compared with pembrolizumab alone (NCT02263508).\(^{75}\) A phase I study of T-Vec in combination with pembrolizumab has also started for head and neck cancer in late 2015 (Masterkey232, NCT02626000). For all oncolytic virus therapy, long-term side effects from the induction of systemic antitumor immunity, including development of autoimmune diseases, should be closely investigated.

Like T-Vec, JX-594 and CG0070 that have the GM-CSF gene inserted in the viral genome, “arming” oncolytic viruses with transgene(s) is a useful strategy to add certain antitumor functions to oncolytic viruses. According to preclinical studies with oncolytic HSV-1, however, GM-CSF is not exactly an ideal transgene for “arming”; rather, interleukin 12, interleukin 18 or soluble B7-1 would significantly enhance the antitumor efficacy via augmenting the antitumor immunity induction.\(^{13,26,76}\) Besides immunostimulatory genes, various transgenes of other antitumor functions, including antiangiogenesis, have been utilized to arm oncolytic viruses.\(^{77–79}\)

**Conclusion**

It would not be too early to say that oncolytic virus therapy is now established as an approach to treat cancer. Because an induction of specific antitumor immunity in the course of oncolytic activities is the common feature that plays an important role in presenting antitumor effects, the efficacy of oncolytic virus therapy is expected to improve further when combined with immunotherapy. By arming oncolytic viruses with functional transgenes, a whole panel of oncolytic viruses with a variety of antitumor functions would be available in the future, from which a combination of appropriate viruses can be chosen according to the type and stage of cancer. A new era of cancer treatment seems at dawn, where cancer patients can freely choose oncolytic virus therapy as a treatment option.

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