Evolution and effectiveness of oncolytic virotherapy as a cancer treatment

Abstract
Oncolytic virotherapy behaves as a therapy with increased tumor selective targeting. Enormous amount of natural and genetically engineered anticancer viruses are currently being tested in clinical trials, with two viruses being clinically approved. H101 approved in 2005 showed increased tumor selectivity compared to the viruses studied previously to 2005. Whereas double mutated T-Vec, approved in 2015 showed further increase in tumor selectivity and tumor regression due to the insertion of GM-CSF. However, combinational therapy focusing the immunostimulatory properties of these viruses along with immune-checkpoint inhibitors has exhibited remarkable results over the monotherapy of oncolytic viruses, which shows the potential in becoming a standard treatment modality for cancer patients.

Keywords: virotherapy, oncology, T-Vec, H101, immunotherapy, monotherapy, combination therapy

Hallmarks of cancer
Cancer is a condition characterized as the uncontrolled cell proliferation, which can be induced by a vast number of factors such as chemicals, radiation, hereditary factors etc. Over 100 types of cancers have been recognized as at present, according to its organ or tissue of origin. Cancer cells are specialized in various adaptations to maintain its immortality and rapid proliferation.1-5

Moreover, highlighting the momentous need of a promising therapy in treating cancers, World Health Organization has predicted worldwide cancer deaths to be increased by 70% in another two years’ time.4 Classical regimen of cancer involves surgery, chemotherapy and radiation therapy which are used commonly on cancer patients. Unfortunately, these are tumor non selective and as a result, patients tend to develop side effects. In contrast to these conventional methods, oncolytic virotherapy uses viruses that selectively replicate and lyse cancer cells. Due to its’ high tumor specific targeting, minimal side effects, increased overall survival and its ability to lyse cancer stem cells, it tends to show superiority over the conventional methods.5,6

Oncolytic virotherapy
Oncolytic virotherapy is a therapy that utilizes therapeutically beneficial anticancer viruses as the active drug reagent in treating cancer. There are mainly two groups of oncolytic viruses namely, naturally occurring (Reovirus, newcastle disease virus) and genetically modified (Adenovirus, herpes simplex virus). Furthermore, over 7 types of viruses are being clinically tested to examine their potential in treating different cancers. Whereas two of these oncolytic viruses were clinically approved in 2005 and 2015. These oncolytic viruses may cause tumor destruction through either direct or indirect mechanisms.7

These natural and genetically engineered viruses target and enter tumor cells by targeting extracellular oncogenic receptors, intracellular oncogenic pathways and immune avoidance mechanisms of tumor cells. Hence, directly infect and replicate, inducing the death of host cells (direct virus mediated cell lysis). Thus, healthy cells remain intact. Furthermore, the released virus particles enter the neighboring tumor cells and infect them, causing local inflammation. Moreover released tumor antigens, damage associated molecular patterns (DAMPs) and pathogen associated molecular patterns (PAMPs) are recognized by the antigen presenting cells (APCs) such as dendritic cells located in the tumor microenvironment. The local inflammation caused by the direct tumor lysis, induces the dendritic cell maturation, which then present the tumor antigens to T cells. This induces a T cell response that destroy virus infected and uninfected cells (antitumor immunity). Moreover, oncolytic viruses in the circulation are capable of infecting the tumor associated endothelial cells and expressing viral proteins which inhibit angiogenesis. Thereby lead to necrosis of uninfected tumor cells (tumor vasculature disruption).5,6

Evolution and effectiveness of oncolytic virotherapy
This concept of oncolytic virotherapy existed since 1950’s (Figure 1) where it originated due to a vast number of cases reported on spontaneous tumor regressions following viral infections. A widely cited example of this is, remission of myelogenous leukemia in a patient following an influenza infection.10 These provided the fundamental basis and the curiosity within the scientific community to perform research in finding the exact cause of these spontaneous tumor regressions and its relationship to viruses. As a result, many in vitro and in vivo studies in rodent models were performed as attempts to treat cancers with attenuated, natural oncolytic viruses. Unfortunately these ended as unsuccessful due to the lack of knowledge in controlling the virulence of these viruses while retaining their ability of proliferating in tumor cells. Thus oncolytic virotherapy concept was abandoned during 1970’s due to its’ limited success.11,12

Overcoming all these obstacles, in 1991 the first ever therapeutically beneficial, genetically engineered oncolytic virus was discovered. This substantially changed the outcome of subcutaneous glioblastoma tumor cells in nude mice.13 This was a herpes simplex virus which the thymidine kinase gene (tk gene) was deleted. Thus, it enabled the virus to replicate selectively in rapidly dividing cells. During this study, tumor growth of control tumors which were not treated with the virus was compared with the tumors which were treated with the genetically engineered virus. Accordingly, it was able to demonstrate the reduced tumor growth in the treated tumors when...
Evolution and effectiveness of oncolytic virotherapy as a cancer treatment

During the phase I of this study in 2000, H101 was delivered intratumorally and showed effectiveness in 15 patients with various malignancies. Therefore, proceeded with the phase II and III studies which delivered the virus in combination with chemotherapy. During the phase II study, injected lesions showed overall response rate of 30.4% whereas the control lesions showed overall response of 13%. Thereby it demonstrated the effectiveness of the virus in treating malignant tumors. Furthermore during the phase III study, toxicities and effectiveness of H101 in combination with cisplatin plus 5-fluorouracil (PF) or Adriamycin plus 5-fluorouracil (AF) against the monotherapy of PF or AF were compared. This was able to show the distinct effectiveness of PF + H101 with the overall response rate of 78.8% when compared to the rates of AF + H101, monotherapy of PF and monotherapy of AF which were 50%, 39.6% and 50% respectively. Furthermore, it was noticed that patients who developed fever following the intratumoral injection with H101 showed high tumor regression rate when compared to the patients who did not develop fever. This is mainly due to the induction of heat shock proteins as a response to fever. Due to the capability of heat shock proteins in chaperoning tumor antigens and presenting it to APCs, it tends to activate antitumor immune responses and thereby causing increased tumor regression. Unfortunately, a major drawback of engineered viruses were tested for its potential against various tumor types. With many research been done to produce the ideal oncolytic virus, the year of 2005 gave immense hope to the concept of oncolytic virotherapy with its first ever commercialized virus H101, approved in China (Table 1). This virus was an E1B gene deleted adenovirus, hence selectively replicative in p53 defective cells.

### Table 1 Clinical trials performed with H101 virus

<table>
<thead>
<tr>
<th>Type of study</th>
<th>Method of administration</th>
<th>Tumor type</th>
<th>Study outcome</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phase I</td>
<td>Intratumoral</td>
<td>Various malignancies</td>
<td>Toxicities of intratumoral injection of E1B deleted adenovirus (H101) were low, tolerable and safe.</td>
<td>17</td>
</tr>
<tr>
<td>Phase II</td>
<td>Intratumoral and chemotherapy</td>
<td>Various malignancies</td>
<td>The combination of H101 and chemotherapy possessed some effect for treating the patients with refractory malignant tumors, and the toxicities were low and well tolerated.</td>
<td>18</td>
</tr>
<tr>
<td>Phase III</td>
<td>Intratumoral and cisplatin + 5-FU</td>
<td>HNC and esophageal cancer</td>
<td>Combination intratumoral H101 injection with cisplatin + 5-FU showed a distinct efficacy in patients with squamous cell cancer of head and neck or esophagus and safety compared to chemotherapy alone.</td>
<td>19</td>
</tr>
</tbody>
</table>

Figure 1 Milestones in oncolytic virotherapy evolution.

During the phase I of this study in 2000, H101 was delivered intratumorally and showed effectiveness in 15 patients with various malignancies. Therefore, proceeded with the phase II and III studies which delivered the virus in combination with chemotherapy. During the phase II study, injected lesions showed overall response rate of 30.4% whereas the control lesions showed overall response of 13%. Thereby it demonstrated the effectiveness of the virus in treating malignant tumors. Furthermore during the phase III study, toxicities and effectiveness of H101 in combination with cisplatin plus 5-fluorouracil (PF) or Adriamycin plus 5-fluorouracil (AF) against the monotherapy of PF or AF were compared. This was able to show the distinct effectiveness of PF + H101 with the overall response rate of 78.8% when compared to the rates of AF + H101, monotherapy of PF and monotherapy of AF which were 50%, 39.6% and 50% respectively. Furthermore, it was noticed that patients who developed fever following the intratumoral injection with H101 showed high tumor regression rate when compared to the patients who did not develop fever. This is mainly due to the induction of heat shock proteins as a response to fever. Due to the capability of heat shock proteins in chaperoning tumor antigens and presenting it to APCs, it tends to activate antitumor immune responses and thereby causing increased tumor regression. Unfortunately, a major drawback of these studies related to H101 is considered to be the use and clinical data of this oncolytic virus being strictly limited to China. However in 2015, Talimogene laherparepvec (T-Vec) was approved as the 1st oncolytic virus in USA (Table 2). This virus was a double mutated herpes simplex virus (HSV) with human granulocyte colony stimulating factor gene inserted. The deletion of the ICP34.5 gene enabled the virus to replicate selectively in tumor cells, while attenuating the virus for neurovirulence. Thus it replicated selectively in tumor cells with defective protein kinase R and interferon signaling. Furthermore, ICP47 gene which enables the virus to be dormant in the host cell was also deleted. This promoted tumor antigen presentation and tumor destruction which facilitated the antitumor immune response. Whereas the insertion of human granulocyte colony stimulating factor gene, facilitated the recruitment and maturation of APCs such as macrophages and dendritic cells. This resulted in rapid presentation of tumor antigens to T cells and thereby gave rise to a systemic antitumor immune response.

During the phase I of this study in 2006, the optimal dose, safety and the biological effects of this virus in 30 patients with refractory head and neck cancer, malignant melanoma, breast cancer and gastrointestinal cancer were determined. These patients contained tumors either in cutaneous, nodular or subcutaneous sites that were accessible for intratumoral injection. Following the treatment with the virus, most tumor areas were able to show extensive considerable necrosis. Whereas when all types of cancer patients were stained for HSV proteins, several factors were observed; all necrotic sections showed presence of HSV proteins, no HSV protein staining observed in normal tissues in the tumor microenvironment, HSV staining was rarely observed in non-necrotic tumor areas. This in turn determined the tumor specificity of this virus. Furthermore, the injected lesions and non-injected surrounding lesions were also able to show regression when clinically examined, which was an evidence of antitumor effect. However, common side effects of the virus observed during this study were mild flu like symptoms such as erythema of the injected site and pyrexia. Whereas these were observed to increase in severity at high viral doses in HSV seronegative patients compared to seropositive patients. Based on these results, phase II was conducted in 2009.
with 50 patients in stage III/IV non resectable melanoma. The overall response rate was 26% with mild flu like symptoms in 85% of patients. Furthermore, 92% of the durable responses in all injected, un.injected non visceral and uninjected visceral lesions were observed and were maintained for 7-31 months. This showed tumor flattening in injected tumors, un injected visceral (pancreas, chest wall) and un injected surrounding tumors. Moreover, the phase III randomized clinical trial which involved 436 patients with melanoma was considered to be the first oncolytic virotherapy based study to determine statistically, a significantly beneficial treatment for melanoma. During this study, effectiveness of T-Vec was compared with recombinant granulocyte colony stimulating factor (GM-CSF) in unresectable melanoma patients in stages of IIIB/IIIC/IV. The results showed that the overall survival was significantly high in patients treated with T-Vec when compared to patients treated with GM-CSF.

Table 2 Clinical trials performed with Tvec

<table>
<thead>
<tr>
<th>Study</th>
<th>Number of patients</th>
<th>Clinical outcomes</th>
<th>Immunological outcomes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phase I</td>
<td>30</td>
<td>Well tolerated in patients with cutaneous or subcutaneous deposits of breast, head and neck and gastrointestinal cancers, and malignant melanoma who had failed prior therapy and can be safely administered using the multi dosing protocol described</td>
<td>Evidence of an antitumor effect was seen due to flattening of non-injected surrounding tumors during clinical examination</td>
</tr>
<tr>
<td>Phase II</td>
<td>15</td>
<td>The 26% response rate together with the survival rates are evidence of systemic effectiveness of T-vec</td>
<td>Durable responses in both injected and uninjected lesions including visceral sites.</td>
</tr>
<tr>
<td>Phase III</td>
<td>436</td>
<td>Improved durable response rate (16.3 vs. 2.1%), overall response rate (26.4 vs. 5.7%), and longer median survival (23.3 vs. 18.9 months) in patients with non-surgically resectable melanoma receiving T-VEC vs. GM-CSF</td>
<td>Regression of 22% of uninjected non-visceral and 9% of visceral tumors. Responses in uninjected lesions provide validation of T-VEC-induced systemic immunotherapeutic effects against melanoma</td>
</tr>
</tbody>
</table>

Moreover tumor regression in, 22% of the uninjected non visceral tumors and 9% of the visceral tumors were observed along with the regression in 47% of injected tumors. This demonstrated the induction of systemic antitumor immune response by T-Vec against melanoma. This study also demonstrated the safety of T-Vec in treating melanoma patients. The adverse effects related to the therapy were mild flu like symptoms which were shown in the phase I and II studies as well. Based on the results generated by this study, T-Vec was able to gain clinical approval in USA for treatment of unresectable recurrent melanoma patients in October 2015. Subsequently, it was approved in Europe and Australia as well.

**Combination therapy and future directions**

Along with these positive outcomes of the oncolytic virotherapy, a thriving interest in research with regards to this concept has been observed since 2015 (Figure 2). Across four continents, approximately 40 clinical trials are currently recruiting cancer patients for treatment with various oncolytic viruses, where most being performed in USA. Furthermore, increased clinical focus on the oncolytic viruses and their immune stimulatory properties, have paved the way for the combination therapy with immune check point inhibitors in treating cancer.

Immune checkpoint proteins such as PD-1/PD-L1 and CTLA-4 are usually expressed to maintain immune homeostasis and to avoid autoimmunity, but during tumor condition these are overexpressed which facilitates tumor cell evasion from the host’s immune system. Thus, inhibitors of immune checkpoint proteins, behave as antagonists of immune suppression. In a phase III trial conducted to treat melanoma by inhibiting PD1/PDL1 immune checkpoint protein, showed exceptional results which ultimately led to its clinical approval. Unfortunately, majority of the patients are non-responders to these immune checkpoint inhibitors due to the deficiency in active T cells in the tumor microenvironment. Therefore oncolytic virotherapy helps in overcoming this, by inducing antitumor immunity and thereby increasing the active T cells in the tumor environment. This concept has been able to show impressive synergistic results.

Figure 2 Growth of ongoing clinical trials based on oncolytic virotherapy in the recent years.

A study conducted in an animal model of immune checkpoint inhibitor resistant lung adenocarcinoma, was able to show the reversal of resistance due to the combination treatment with anti PD-1 antibody and a genetically engineered oncolytic adenovirus (hTertAd). When the tumor growth of mice treated with combination treatment compared with the tumor growth in monotherapy received
mice, it was able to reveal that monotherapy of anti-pD1 therapy was not able to effectively reduce the tumor growth. Whereas the combination therapy was able to reduce the tumor burden effectively, even compared to oncolytic virus alone. Therefore it was evident that the local administration of the oncolytic virus was able to facilitate in overcoming the systemic resistance to anti PD-1 antibodies and thereby reducing the tumor and improving survival by synergistically acting along with the PD-1 therapy. Furthermore, prolong survival was observed in the combination treatment received mice compared to the mice treated with either the anti PD-1 antibodies or the virus.

In contrast to the synergistic effect of oncolytic viruses in overcoming immunotherapy resistance, a preclinical study with reovirus and anti PD-1 antibodies was conducted in mice bearing subcutaneous B16 melanoma.36 Reovirus which is a natural oncolytic virus is capable of eliciting direct tumor lysis and antigen immune responses. Unfortunately, regulatory T cells (Treg) tend to suppress these antitumor immune responses and thereby reduce the efficacy of the virus.37 However during this study, mice with B16 melanomas were treated with either intratumoral reovirus (three doses of 7x108pfu/50μl) or PBS during 7th, 10th and 12th days. Whereas 14th day onwards, mice were administered with IgG or intravenous anti PD-1 antibody (0.25mg/mouse) on every other day. This was able to demonstrate the ability of anti PD-1 antibodies in improving T cell and natural killer cell (NK cell) activity, suppressing Treg and enhancing the direct tumor lysis of reovirus. Thus prolonging survival of the mice compared to monotherapy of either of the two agents.37

Furthermore, combination of T-Vec and systemic delivery of different immune checkpoint inhibitors have shown promising results comparatively to T-Vec alone (Table 3).38 Efficacy in combination of T-Vec and ipilimumab was assessed in a phase 1b trial whereas the efficacy of T-Vec and pembrolizumab is currently being assessed in a phase 1b trial.39

<table>
<thead>
<tr>
<th>Tumor response at the lesion level, %</th>
<th>Talimogene laherparepvec+ipilimumab</th>
<th>Talimogene laherparepvec+pembrolizumab</th>
<th>Talimogene laherparepvec+monotherapy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Injected lesions</td>
<td>74</td>
<td>80</td>
<td>64</td>
</tr>
<tr>
<td>Non-injected lesions</td>
<td>52</td>
<td>35</td>
<td>NR</td>
</tr>
<tr>
<td>Non-visceral</td>
<td>54</td>
<td>45</td>
<td>34</td>
</tr>
<tr>
<td>Visceral</td>
<td>50</td>
<td>28</td>
<td>15</td>
</tr>
</tbody>
</table>

A phase 1b study with T-Vec and ipilimumab was conducted in 2016, to demonstrate the safety and efficacy of combinational treatment in stage IIIB-IV melanoma patients.37 Ipilimumab is an immune checkpoint inhibitor which inhibits cytotoxic T lymphocyte associated antigen 4 (CTLA-4). Thus it helps activating T cells in the tumor microenvironment that are inactivated. Thereby facilitates recruitment of lymphocytes and help improving the systemic antitumor effects exerted by T-Vec. The systemic anti immune responses elicited by T-Vec, when combined with increased activation of ipilimumab, was able to demonstrate tumor regression in uninjected surrounding tumors and uninjected visceral tumors along with the tumor regression in injected lesions. Furthermore, it showed 67% overall survival and 50% progression free survival which are higher survival rates compared to rates of T-Vec alone. No severe adverse effects were reported during the study, where only mild flu like symptoms was reported in 26.3% of the patients.40 On the basis of the results generated by this phase 1b trial, a phase II trial is currently ongoing in order to compare the efficacy of T-Vec and ipilimumab combination vs ipilimumab monotherapy.41 The phase Ib/II clinical trial involving the combination treatment of melanoma with pembrolizumab and Tvec is also ongoing.1 Where an analysis of early efficacy have shown, its tolerability along with responses generated in 56% of patients.39 Therefore it can be considered that the combination of T-Vec with immune checkpoint inhibitors shows the potential of treating cancers with a higher efficacy compared to oncolytic virotherapy alone.

**Conclusion**

The potential of oncolytic viruses behaving as therapeutically beneficial anticancer agents is evident from all the studies that have shown promising results. Even though during this review, a main focus was given to the evolution of oncolytic virotherapy based on its milestones and the clinically approved oncolytic viruses, there are many types of oncolytic viruses that are been clinically tested for its potential against various cancer types. This includes both natural and genetically engineered viruses. However concerning the two oncolytic viruses which have gained clinical approval, H101 was able to show improvement in attenuation and tumor selectivity due to its single mutation of E1B gene when compared to the oncolytic viruses which were studied prior to 2005. Nevertheless, Tvec which was produced much later than H101 was able to show further improvement in attenuation and tumor selectivity due its double mutation. Furthermore, elucidating systemic tumor regression was facilitated by the GM-SCF insertion. Hence arming of oncolytic viruses with transgenes was able to direct oncolytic virotherapy concept in a new direction.

Subsequently, various combination therapies were hypothesized in order to enhance the tumor lysis by utilizing attractive immune stimulatory properties of oncolytic viruses. Hence along with the development of immunotherapies and positive outcomes of immune checkpoint inhibitors, several studies showed the synergistic effect of immune checkpoint inhibitors and oncolytic viruses. This combination therapy indeed was able to demonstrate attractive results more than the treatment with oncolytic viruses alone. As a result of these rapid developments in the evolution of oncolytic virotherapy and striking results of oncolytic virotherapy delivered as a combinational treatment, it won’t be long before this concept is established as a standard therapy for treating cancer.
Acknowledgments

None.

Conflicts of interest

The author declares that there is no conflicts of interest.

References

