Clinical Trials with Oncolytic Adenovirus in China

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Abstract: Since the 1990s, oncolytic viruses were utilized to treat cancer patients from phase I to phase III. Oncolytic virus development in China has been keeping in step with that in other countries and even accelerated the process in some fields, especially in conducting clinical trials. H101 is one kind of oncolytic adenovirus with E1B-55KD and partial E3 deleted developed by Shanghai Sunwaybio. From 2000-2004, phase I to phase III clinical trials for treating head and neck cancer were conducted in China. Clinical data show that H101 is well tolerable and has good efficacy when combined with chemotherapy in some cancer treatment modalities. We review the clinical results and relative issues of H101 in treating cancer and discuss approaches and possible improvements for the future. Information on other oncolytic viruses developing in China is also provided.

Keywords: Oncolytic virus, clinical trial, China; H101, adenovirus.

INTRODUCTION

More than one thousand clinical trials of gene therapy were approved as of January 2005 around the world. Cancer gene therapies have now become the foremost field in which gene therapy is being applied. Oncolytic virotherapy has accounted for almost half of the cancer gene therapy trials. Utilizing viruses to treat cancer is not an altogether innovative concept. Over the past century, the approach was temporarily abandoned due to safety and toxicity issues, genetic engineering technology developed now, more and more applicable oncolytic viruses are emerging.

An oncolytic vector is a virus which has intrinsic or engineered tumor selectivity and especially targets tumor cells, whereas non-oncolytic vectors mostly express transgenes that correct gene mutation (like p53 tumor suppression gene) or generate an anticancer product (such as IL-2, IFN-β, or GM-CSF). Thus, the oncolytic virus especially provides the potential for targeted tumor cell destruction without destroying normal cells. The oncolytic viruses which have been developed and reported include adenovirus, HSV, vaccinia, Newcastle disease virus and reovirus. The first oncolytic virus with recombinant technology HSV-1 demonstrated enhanced safety and selectivity for tumor cells in a malignant glioma model [1]. In 1996, ONYX-015 became the first engineered oncolytic adenovirus to undergo a clinical trial [2]. But in 1999, gene therapy suffered a major setback with the tragic death of an 18-year-old man Jesse Gelsinger, at the University of Pennsylvania. His death led to the discontinuation of all gene therapy trials in the United States for a time and eventually to more stringent safety precautions for gene therapy.

Among oncolytic viruses used in cancer gene therapy clinical trials, adenovirus and vaccinia virus are double-strand DNA viruses that are easy to manipulate but have high immunogenicity. HSV, are more readily used in brain tumors due to their affinity to neuron cells. Newcastle disease virus and reovirus are RNA viruses which have intrinsic tumor selectivity. Adenovirus-based vectors have been used extensively in cancer gene therapy. Same basic strategies as above for oncolytic virus have been pursued in treating cancer: Direct tumor cell killing through delivery of replication-competent oncolytic viruses or non-replicating vectors encoding tumor suppressor genes, suicide genes or anti-angiogenic genes, while aiming to stimulate body anti-tumor immune responses that can destroy tumor cells at both primary and distant locations, even preventing recurrence through inserted genes of cytokines. Extensive pre-clinical and clinical studies have been explored and conducted based on these strategies. Although encouraging results have been obtained, robust clinical efficacy must still be confirmed [3].

Basic research on adenovirus biology has been quite thorough. Adenoviruses are non-enveloped DNA viruses commonly causing upper respiratory tract infections with other viruses such as Para influenza virus. Type C adenovirus has been used for gene therapy. The adenoviral genome contains double-stranded DNA, with a total length of about 36 kb. Up to 10 kb of foreign DNA can be inserted after some region is deleted. E1A, E1B, E2, E3 and E4 regions compose the early region of the adenovirus genome. To generate an E1-defective virus, plasmids were constructed to have the adenovirus packaging signal, an E1 deletion, and some adenovirus sequences downstream from the E1 region by using recombination technology. E1-expressing 293 cells were also used for plasmid recombination generating an E1-defective virus that can replicate in those 293 cells. As a gene therapy vector adenovirus has some advantages including: 1) high titer availability (up to 10^{12} viral particles/ml) by manufacturing; 2) replication in dividing or non-dividing cells, so that some tumor cells can be targeted in G0; and 3) non permanent inserted gene expression, which is good especially for some therapeutic gene products. Disadvantages include: 1) Entry to tumor cells depends on Coxsackievirus and adenovirus receptors (CARs), which may limit infection efficacy and spread; and 2) systemic administration is limited due to its immunogenicity. High

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immunogenicity remains difficult to classify as either an advantage or disadvantage, because an immune response may prevent over shedding to other organs and warrant more safety. On the other hand, a high immune response may also induce an anti-tumor imune response. An association was reported between the cellular p53 and the adenovirus 5 E1B-55kd proteins, thereby reducing the oncogenicity of Ad-transformed cells [4]. A typical oncolytic adenovirus virus is ONYX-015 in which E1B 55kd is deleted. It is known that replication of ONYX-015 is severely impeded compared to wild-type adenovirus, probably as a result of loss of E1B-55 kDa protein function for the late virus mRNA transcription [5]. Meanwhile tumor cells can fortunately supplement E1B function in late viral mRNA transported from nucleus [6], so that the virus can replicate selectively in tumor cells without normal p53 protein or with a deficient p53 pathway. This is why ONYX015 is one of the so-called conditionally replicative adenoviruses (CRAds). The promising preclinical data of (CRAds) make them readily available for human clinical trials.

CLINICAL EXPERIENCE WITH ONYX-015

Onyx-015 is the first oncolytic virus used in clinical trials since 1996, and a wealth of experience and clinical data have been accumulated and reported. Almost 300 cancer patients have now been treated in approximately ten clinical trials (from phase I to III). Onyx-015 has also been tested in different administration routines from intratumoral, intravascular, intraperitoneal, mouthwash to intraesophageal instillation. Many lessons and experiences can be drawn from the clinical trials. And ONYX-015 has now been tried in a variety of tumors, including recurrent or advanced head and neck [2,7], pancreatic [8], sarcoma [9], glioma [10], colorectal metastatic to liver [11], ovarian [12], and hepatobiliary [13] cancers. Viral replication was tumor-selective and samples were tested and documented after administration by all routes during trial; however, no evidence showed that the viral replication and response depended on tumor histology. Head and neck squamous cell carcinoma (HNSCC) is the most applicable indication. Bladder cancer and non small cell lung cancer (NSCLC) were also planned as further indications. The applied dose for HNSCC is $10x10^{11}$pfu. The highest dose in vein can be escalated to $2x10^{13}$vp without obvious toxicity. In general, the virus was well tolerated at doses of up to 2×10^{12} virus particles by different administration routines. Following intra-vascular administration, maximally-tolerated doses were not identified. The most frequent side events have been flu-like symptoms, fever, nausea and leucopenia among others, and can be solved with or without medication. Variable clinical responses were observed in these trials. Single agent efficacy has been relatively limited to about 14% local tumor regression rates. The combination of this agent with chemotherapy (5-FU+CDDP) has demonstrated clear benefits for recurrent head and neck cancer in phase II clinical trial [14,15]. A promising phase III clinical trial of HNSCC in combination with chemotherapy was suspended because of funding problems in 2003. These clinical research results show the potential of this novel platform for cancer therapy, as well as the difficulties that must be faced. The call is for greater potency of the replication-selective agents.

SUMMARY OF RECOMBINANT ADENOVIRUS H101

Shanghai Sunwaybio initiated oncolytic virus cancer gene therapy clinical trial in 2000. After almost 4 years, in Nov 2005, the State Food and Drug Administration of China approved the replication-competent adenovirus (E1B-55KD and E3 regions segments deleted) injection (H101) for treating advanced nasopharyngeal carcinoma in combination with chemotherapy (regimen of 5-FU+CDDP). H101 thus became the first approved oncolyitc virus product on the china market in the cancer gene therapy field. This review summarizes the detailed clinical trial of oncolytic virus (H101) in head and neck cancer, provides information of other ongoing oncolytic virus clinical trials in China, and addresses issues from the standpoint clinical experience for future reference and development.

CLINICAL EXPERIENCE WITH H101 (ONCOLYTIC VIRAL THERAPIES) IN CHINA

H101 is a recombinant human type-5 adenovirus (Ad5) with E1B-55 kDs gene totally deleted, making it a CRAd similar to ONYX-015. The virus is produced by Shanghai Sunway Biotech, with an additional deletion of 78.3-85.8 μm gene segment in the E3 region which includes the adenovirus death protein (see Fig. **1**). The same above mentioned works for replication in tumor cell when the protein of E1B-55kD gene is deleted: Activated p53 of cells would prevent virus efficient replication in normal cells. Thus, cancer cells lacking functional p53 would be sensitive to viral replication and subsequent cytopathic effects. Dysfunctional tumor suppressor genes like p53 are the most common genetic lesions identified in human cancers cells,

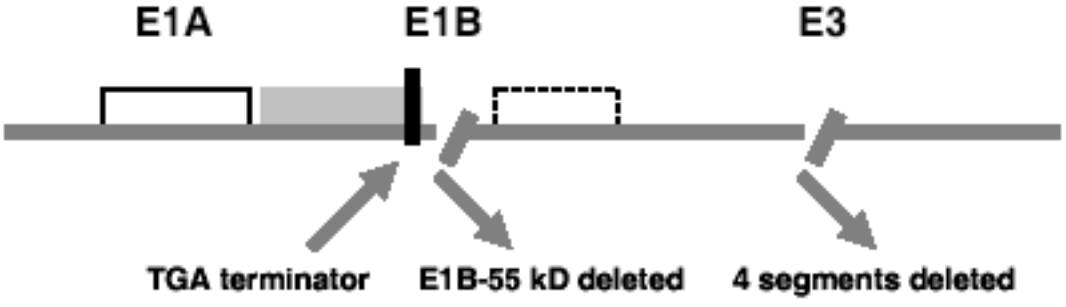

Fig. (1). Schematic diagram of constructed adenovirus (H101). This figure shows the deletion of adenoviral early region genes to form oncolytic virus (H101). E: early region of adenovirus genome.

which is why H101 can be utilized for cancer gene therapy. Recent research has also hinted at a new and complicated mechanism of E1-B55 KD deleted adenovirus lysing tumor cells. Although the exact mechanism is not clear, the oncolytic effect has been confirmed by data from clinical trials and basic research. Deleted segments of E3 region may enhance the safety of the product.

PHASE I CLINICAL TRIAL

From September 2000 to March 2001, 15 patients were accrued in the trial including: HNSCC (5 cases), melanoma (3 cases), breast cancer (2 cases), ovarian cancer (1 case), carcinoma of penis (1 case), sarcoma of soft tissue (2 cases), and one case of neuroblastoma. Baseline characteristics of patients were summarized in Table **1**. After systemic examination was performed, patients were treated with H101 intratumoral injection in a dose–escalation manner (dose from $5.0x10^7$ to $1.5x10^{12}$vp per day for 5 consecutive days). Intratumoral injection is the same as that of ONYX-015. There were five different dosage groups ($5.0x10^7$vp, $5.0x10^9$vp, $5.0x10^{10}$vp, $5.0x10^{11}$vp and $1.5x10^{12}$vp), and each group enrolled 3 patients. The first patient received only one-time injection (at the dose of $5.0x10^7$vp), and the rest were given total a five injections (once daily). All patients were monitored, and physical signs and toxicity were observed and recorded carefully according to the protocol during treatment. Samples of plasma, urine and swabs from oral pharyngeal wall were collected and then tested by analysis using PCR at different time points (D1, 6, 12, 19, 26 after first injection) to detect DNA of the replicated adenovirus.

Table 1. Baseline Patient Characteristics in Phase I Clinical Trial

Age (years)	Median: 54 Range: 28-72
Gender	Male: 7 Female: 8
Performance status	1 grade 10 2 grade 5
Prior therapy	No prior therapy 1 (6.7%) Surgery 11 (73.3%) Radiotherapy 4 (26.7%) Chemotherapy 7 (46.7%) ›2 modalities 6 (40%)
Tumor size[1]	Median: 12.7 Range: 2.5-78
P53 gene status	Normal 4 (36.4%) Mutated 7 (63.6%)
Baseline neutralizing antibody levels	(+) 14 (93.3%) (-) 1 (6.7%)
CD4 cell count[2]	Median: $0.50x10^9$ Range: $0.14\text{-}0.97x10^9$
CD8 cell count[2]	Median: $0.42x10^9$ Range: $0.12\text{-}0.64x10^9$

[1]Max. cm^2. [2]Cells/l

All 15 patients were included in the 'intent–to-treat' (ITT) analysis. No patient withdrawal from the study. The treatment caused the tumor to shrink remarkably in 3 out of 15 patients (1 PR, 2 MR). The 15 evaluable patients well tolerated H101. Dose limited toxicity (DLT) and serious adverse events were not seen in the course of treatment. The most frequent complications were fever, flu-like symptoms and pain at the injection site (see Table **2**). Good safety was shown when treating patients with H101 based on this data. The recommended H101 dosage for phase II trial was $5.0x10^{11}$vp/day for five consecutive days [16].

PHASE II CLINICAL TRIAL

A multi-center and open-label phase II clinical trial was conducted from October 2001 to June 2002 in China. A total 53 patients suffering from advanced malignant tumor were recruited. All patients were refractory to conventional therapy or had no standard therapy available. The baseline characteristics of patients were summarized in Table **3**. The treatment regimen consisted of intratumoral injection at the dose of H101 $5x10^{11}$vp for 5 consecutive days, with an interval of 16 days, a 21 days as one cycle. Evaluable patients should have received at least two cycles. 46 patients were evaluable and 4 patients dropped out of the study (3 patients of HNSCC, 1 patient of lung cancer). The objective response rate (ORR) was evaluated according to WHO criteria. Efficacy for different tumor types was summarized in Table **4**. Among these 46 evaluable patients, CR was obtained in 3, PR in 11, and ORR was 30.4% (14/46). ITT analysis showed a total response rate of 28.0%. Among 14 patients who obtained a good response, 10 patients received at least 2 courses of treatment and 4 patients one course of treatment. The response rate in the treatment group was 28%, against 12.0% 9(6/50) in the control group. Enhanced efficacy was observed when combining H101 and chemotherapy for treatment of tumors refractory to conventional therapeutics (see Table **5**). ORR of this group was less than that reported for ONYX-015 [14,15]. Three main reasons may contribute to the result: 1) patients in this trial (H101) were refractory to conventional therapy including chemotherapy, in contrast to patients (ONYX-015) naïve to chemotherapy though radiotherapy or only surgery had been applied; 2) all patients in H101 trial were in the advanced stage including metastasis to other sites; and 3) not enough treatment cycles were applied (most patients underwent 3 cycles of treatment). The response rate with single-agent cancer treatment is similar to that of ONYX-015. And different tumor type or pathological type can also affect the efficacy because different levels of CAR expression on tumor cell surface which play a key role in adenoviral cell entry on cell, and also deficiency of p53 pathway must be different too. Screening patients through p53 mutation or CAR expression on tumor cells may provide a means to improve the response rate in future trials. Similar efficacy of a single agent was confirmed compared to ONYX-015. This phase II trial further shows the safety of CRAds as an

Table 2. Adverse Events During Phase I Clinical Trial of H101

Adverse events	Grade according to NCI criteria					Total (%)
	I	II	III	IV	V	
Fever	4	1	0	0	0	5 (33.3)
Injection site pain	9	1	0	0	0	9 (60.0)
Nausea	3	0	0	0	0	3 (20.0)
Leucopenia	1	0	0	0	0	1 (6.7)
Hepatic dysfunction	1	0	1	0	0	2 (13.3)
Flu-like symptoms	7	1	0	0	0	8 (53.3)

oncolytic agent. Patients well-tolerated to the oncolytic virus treatment regimen. The most frequent adverse events were fever, flu-like symptoms and local site pain, which were well tolerated, similar to ONYX-015 (see Table **6**). Although HNSCC was selected as an indication for phase III trial due to limiting the administration route, other indications were still worth being explored in the future [17].

Table 3. Baseline Patient Characteristics in Phase II Clinical Trial

Age (years)	Median: 52 Range: 18-76
Gender	Male: 35 Female: 18
KPS	80-100
Prior therapy	No prior therapy 14 (26.4%) Surgery 24 (45.3%) Radiotherapy 20 (37.7%) Chemotherapy 37 (69.8%) Biotherapy 8 (15.1%) ›2 modalities 31 (58.5%)
Tumor size[1]	Median: 12.5 Range: 1.43-360

[1]Max. cm^2.

Table 4. Efficacy in Patients Treated with H101 and Chemotherapy in Phase II Clinical Trial

Type of tumor	Cases	Response (CR+PR)
HNSCC	15	4
Breast cancer	3	1
Non-small cell lung cancer	4	1
Malignant melanoma	2	1
Carcinoma of esophagus	8	3
Ovarian cancer	1	0
Gastric carcinoma	5	0
Rectal cancer	3	1
Lymphoma	1	1
Chordoma	1	1
Soft tissue sarcoma	3	1

PHASE III CLINICAL TRIAL

A multi-center, randomized and controlled phase III trial combining H101 with chemotherapy was conducted from October 2002 to March 2004 in China [18]. 13 hospitals participated in the trial and a total 170 patients were recruited. 160 patients finished the trial. Baseline characteristics of patients were summarized in Table **7**. Evaluable patients should receive at least two cycles. 18 patients (11.3%) were excluded for failing to meet the enrollment criteria and 19 others withdrew from the trial or were disqualified. A total 123 patients completed the trial and were evaluable, accounting for 76.9% of the total patients (123/160). All recruited patients received chemotherapy. Those without prior chemotherapy or whose prior chemotherapy was PF and showed a good response underwent the PF regimen (DDP 20 mg/m^2 iv., Day1-5;5-FU 500 mg/m^2 iv., Day1-5). Patients refractory to the PF regimen received the AF regimen (ADR 50 mg/m^2 iv at Day1, 5-FU 500 mg/m^2 iv., Day1-5). Patients were randomized to the treatment group (H101 combined with chemo) or control group (chemo alone). H101 was injected into the tumor at the dose of $5x10^{11}$vp for 5 consecutive days, and 21 days was one course of treatment. All patients received at least 2 courses of treatment and no more than five. During the trial period, no other anti-tumor drugs were allowed. The results from 105 evaluable patients (PF regimen)were summarized in Table 8. The most common side effects were fever (45.7%), local site pain (28.3%), flu-like symptoms (9.8%), leucopenia, decreasing platelets, malfunctions of liver, alopecia and nausea, all of which were well-tolerated. Objective response rate (ORR) was evaluated according to WHO criteria. Statistical analysis showed that the response rate from combining H101 with chemotherapy to treat patients naïve to chemotherapy was higher than by chemotherapy alone (79% to 39.6%, p=0.000). The response rate of combination therapy for patients (including naïve and refractory to chemotherapy) was 72.7%, in contrast to 40.4% of control (including naïve and refractory to chemotherapy). In China, nasopharyngeal carcinoma (NPC) accounts for the

Table 5. Efficacy of H101 on Injected Lesion and Control Lesion in Phase II

Lesion	n	Median size (cm^2)	CR	PR	SD	PD	Response rate (%)
H101	46	12.5	3	11	24	8	30.4
Control	46	11.3	1	5	28	12	13.0

Table 6. Toxicities Experienced During Trial in Phase II Clinical Trial

Toxicity	Grade				Total (%)
	I	II	III	IV	
Fever	10	5	1	0	16 (30.2)
Injection site pain	12	2	0	0	14 (26.4)
Nausea	13	5	0	0	18 (34.0)
Leucopenia	12	7	3	4	26 (49.1)
Hepatic dysfunction	2	0	0	1	3 (5.7)
Flu-like symptom	13	2	0	0	15 (28.3)
Alopecia	3	3	1	0	7 (13.2)

majority of head and neck cancer, so patients suffering from (NPC) were stratified and analyzed. The response rate of NPC patients (including naïve and refractory to chemotherapy) treated with H101 and chemotherapy was 75.6%, against 57.1% in the control group (p=0.081). The response rate of NPC patients without prior chemotherapy was significantly higher that that of control (86.5% to 59.4, $p<0.05$, data unpublished). Among 66 patients who received H101, 34 developed a fever. Based on analysis of patients who received H101 without prior chemotherapy, 81.5% (22/27) of patients developed a fever and showed an objective response compared to 76.0% (19/25) who had no fever and showed an objective response (see Table **8**-**9**). It seems that fever may even enhance the efficacy of H101 and chemotherapy and such patients may be more sensitive to the virus. Although there is still no clear relationship between the efficacy and fever, new experiments have proved that high temperatures can enhance the replication of virus, which may be through heat shock protein to help late RNA export [19].

In November 2005, Chinese government regulators (SFDA) approved H101 especially for advanced nasopharyngeal carcinoma in combination with cisplatin and 5-FU chemotherapy based on clinical trial data. At that time, the objective response rate was still the primary endpoint for SFDA new drug approval in treating cancer, although over survival (OS) is recognized as the best endpoint for an oncology trial.

OTHER ONGOING CLINICAL TRIALS OF H101 FOR EXTENDED INDICATIONS

Advanced non small cell lung cancer (NSCLC Stage IIIB-IV) poses a great challenge for oncologists worldwide. All treatment regimens including chemotherapy or radiotherapy yield about a 30% response rate, median TTP of 5-6 months and survival time of 8-12 months according to the data from large clinical trials [20-22]. Shanghai Sunwaybio is conducting a clinical trial with H101 through CT-guided puncture to treat NSCLC by combination with chemotherapy in order to prolong patient survival. The trial is still open and recruiting patients. Other indications considered include hepatocellular carcinoma and colon cancer metastatic to liver.

OTHER PRODUCTS DEVELOPING BY SHANGHAI SUNWAYBIO

H102

An α-fetoprotein **(**AFP) promoter gene was inserted with a CMV enhancer into E1-B55KD deleted adenovirus, aiming specifically to replicate in hepatocellular carcinoma (HCC), which has a very high morbidity and mortality in China. H102 is still in the preclinical stage.

H103

Another product based on CRAds has been developed by Shanghai Sunwaybio, by insertion with a gene of HSPs (heat shock proteins) using a CMV enhancer. HSPs have been discovered to perform critical functions in maintaining cell homeostasis as molecular chaperones. HSPs are over-expressed in a wide range of human cancers and are implicated in tumor cell proliferation, differentiation, invasion, metastasis, death, and recognition by the immune system [23,24]. HSPs in an extracellular milieu can modulate innate and adaptive immunity due to their ability to chaperone polypeptides and to interact with the host's immune system, particularly professional antigen presenting cells (APCs), which can be potentially applied in cancer immunotherapy [25]. Different vaccines based on HSPs have been tested in cancer immunotherapy. Normally, the vaccine

Table 7. Baseline Patient Characteristics in Phase III Clinical Trial

		Treatment	Control
Median age		51.1 yrs	50.9 yrs
Gender	Male	76	50
	Female	16	18
Height (Median)		165.2 m	165.8 m
Weight (median)		58.8 kg	61.0 kg
Diagnosis*	Nasopharyngeal carcinoma	50	41
	Esophageal carcinoma	11	5
	Laryngeal carcinoma and glottic carcinoma	6	6
	Metastatic Lung cancer	2	0
	Metastatic squamous cell carcinoma	5	1
	Carcinoma of sinus	1	1
	Squamous cell carcinoma of neck	1	1
	Oral cavity carcinoma	16	12
	Squamous cell carcinoma of thyroid gland	0	1
Clinical stage	Stage I	6	3
	Stage II	12	13
	Stage III	34	22
	Stage IV	35	29
	Unknown stage	5	1
History	No prior therapy	50	39
	Radiotherapy	28	13
	Chemotherapy	25	11
	Surgery	21	16
	Biotherapy	3	0
Performance states	0	29	23
	1	52	34
	2	11	11
Lesion numbers	1	54	37
	2	22	20
	3	9	6
	4	3	2
	5	4	2
Median superficial tumor size (cm^2)		19.4 (24.9)	15.9 (27.6)

*: lip, gum, tongue, base of oral cavity, salivary carcinoma were classified as oral cavity carcinoma.

Table 8. Comparison of Response Rate between Two Groups of First-Line Cases

Group	n	Injected lesion, n (%)	Total lesion, n (%)
H101+PF	52	41 (78.8)	40 (76.9)
PF alone	53	21 (39.6)	19 (35.8)
P value		= 0.000	= 0.000

PF regimen (DDP 20 mg/m^2 iv, Day1-5; 5-FU 500 mg/m^2iv, Day1-5).
Statically mean: P value < 0.05 between H101 + PF and PF alone on either Injected lesion or Total lesion.

Table 9. Comparison of Response Rate between Two Groups

Group	n	Injected lesion, n (%)					Total Lesion, n (%)				
		CR	PR	MR	SD	PD	CR	PR	MR	SD	PD
H101+PF or AF	66	6 (9.1)	42 (63.6)	3 (4.5)	12 (18.2)	3 (4.5)	3 (4.5)	44 (66.7)	3 (4.5)	11 (16.7)	5 (7.6)
PF or AF alone	57	2 (3.5)	21 (36.8)	6 (10.5)	24 (42.1)	4 (7.0)	1 (1.8)	19 (33.3)	6 (10.5)	22 (38.6)	9 (15.8)
P value				< 0.001					= 0.000		

PF regimen (DDP 20 mg/m^2 iv, Day1-5;5-FU 500 mg/m^{2}iv., Day1-5). AF regimen (ADR 50 mg/m^2 iv at Day1, 5-FU 500 mg/m^{2}iv, Day1-5).
Statically mean: P value < 0.05 between H101 + PF or AF and PF or AF alone on either Injected lesion or Total lesion.
CR: Complete response, PR: Partial response, MR: Minor response, SD: Stable disease, PD: Progressive disease.

is made from individual patients' tumors and heat shock proteins from the patients' own white blood cells or tumor tissue. The process is conducted in the lab to purify heat shock proteins from the white blood cells and combine purified antigens from the tumor tissue or the heat shock protein. Its associated peptides are directly isolated from the tumor. The process *in vitro* could not simulate the natural folding and chaperoning the antigen and tumor cells selectively survive under press may contribute to the barely satisfactory efficacy of the vaccine. Animal tests had demonstrated that H103 can not only lyse tumor cell primary sites but stimulate immune response, treat tumors at distant sites and enhance oncolytic efficacy, somehow working as a vaccine *in vivo* [26]. H103 is under investigation in Phase I clinical trial for safety in treating cancer which was just completes (data unpublished yet).

OTHER ONCOLYTIC VIRUSES UNDER INVESTIGATION

A few researchers have focused on oncolytic viruses as weapons against tumors in new and different ways in China. Liu et al., for example, reported a normal anti-tumor strategy called “targeting gene virotherapy”, based on E1B-55KD-deleted adenovirus vector (ZD55). Two therapeutic genes such as mda-7/IL-24 were inserted into ZD55 with a specific promoter for targeting tumor cells. Possible candidate therapeutic genes had been applied according to published studies [27-31]. Data from *in vivo* experiments showed good efficacy in treating xenograft tumors. Combining targeting gene-virotherapy with 5-FU enhanced the antitumor efficacy [32]. Clinical trials are to be conducted with ZD55-based dual gene virotherapy after SFDA approval [33].

CONCLUSION AND FUTURE DIRECTIONS

Clinical data on H01 have attracted the attention of western medicine journals. There are certain issues can now be addressed, thanks to the clinical results of H101 [34-37]. Although some issues remain and must be faced when conducting human clinical trials, the cumulative clinical experience oncolytic viruses clinical in China has at least laid the foundation for further improvement of oncolytic virus cancer therapy. Data from H101 clinical trials indeed show promising results for oncolytic virus as a platform for cancer gene therapy.

In the field of anti-tumor therapy, more interest and grants are being devoted to targeting cancer therapy with small molecules and antibody-based therapy. EGFR and VEGFR are the most popular targets. There are problems with viral vectors, toxicity, immune and inflammatory responses, along with gene control and targeting issues. In particular, there is always the genuine concern that the viral vector may recover its ability to interact with wild-type virus to cause disease inside the patient, so oncolytic viral therapy is not the mainstream platform under investigation in oncology. Given the clinical trial data from ONYX-015 and H101, there are still hurdles for extensive application. First of all efficient spread and replication within solid tumor masses poses a problem. One study estimates that the replicating adenoviral infection travels 5-10 times slower than the tumor wave front velocity [38,39]. Expression of CAR on the tumor cell surface has also affected the efficacy of treatment [40]. Second, neutralizing antibodies prevent systemic administration from human adaptive immune response. The role of immune response in the efficacy of oncolytic therapy is not yet clear. Although the immune system may restrict the distribution of virus and be more safe, the active immune response is beneficial for the anti-tumor effect too. Third, no obvious systemic efficacy has been described from updated clinical data of ONYX-015. Thus, knowledge of the detailed replication cycle of Ads should make it possible to develop advanced CRAd systems that achieve a better oncolyitc effect than that achieved with this first-generation CRAd. New approaches have been tried in several ways. Preventing neutralizing antibodies or redirecting virus particles away from liver in one of them [41]. But such improvements are still quite far from clinical application and may cause some problems. From the standpoint of clinical application, more emphasis should be placed on the virus kinetic, interactive effect between oncolytic virus and chemotherapy agents. Although E1A of adenovirus has been recognized to enhance the sensitivity of tumor cells to chemotherapy, little attention is put on its detailed mechanism when applied. For instance, adenovirus replication depends on microtubule system transportation. When a certain cytostatic agent like Taxanes are used at the same time or before CRAds, it probably decreases the oncolytic effect by damaging microtubules and causing G2-M arrest, potentially affecting adenovirus entry into the nucleus. This is an area calling for much more investigation when combining virus therapy. Different agents or different time order may also cause apparently different efficacy, another area worthy of exploration. Combining heating or other therapy should be considered seriously in order to enhance efficacy, not only for the innate quality of HSP as

an antigen chaperone, but high temperature is helpful for oncolytic viral replication through HSPs [19]. Hyperthermia reportedly enhances CTL cross-priming [42] except as a chaperone interacting with APC. We mentioned earlier the phenomena in the H101 clinical trial by which fever induced by virus administration without antipyretic drugs showed a better response.

And is it true that the indication of oncolytic virus cancer gene therapy is only for refractory or recurrent cancer? Cancer in the advanced stages is difficult to cure. Some types of tumor which can be detected in the early stage but tend to recur, like bladder cancer, may be a better candidate. Malignant ascites must also be considered. Appropriate selected indications may speed up the stage of clinical application for oncolytic virus.

This writer agrees that the approval of oncolytic virus in China would mean "the end of the beginning, not the beginning of the end" [37]. Based on the available clinical data on viruses, optimizing the combination with conventional therapy in different way and creating new approaches for this platform is a good way to encourage the development of oncolytic virotherapy in the future.

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