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Review

Current Status of Gendicine in China: Recombinant Human Ad-p53 Agent for Treatment of Cancers

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INTRODUCTION

OVER THE PAST DECADE, gene therapy has been increasingly applied in clinical trials. According to data published by the *Journal of Gene Medicine* (http://www.wiley.co.uk/genetherapy/clinical/), there were a total of 1020 approved gene therapy clinical trials in the world at the end of January 2005. Among these clinical trials, 66% were for the treatment of cancer. Of these cancer gene therapy trials, 58 used recombinant adenovirus encoding human *p53* tumor suppressor gene (rAd-p53). More than 20 kinds of cancer indications have been treated with rAd-p53 agent, such as head and neck squamous cell carcinoma (HNSCC), lung cancer, breast cancer, liver cancers. Various clinical treatment regimens have been evaluated, including administration of rAd-p53 agent alone or in combination with conventional therapies such as radiotherapy, chemotherapy, and surgery.

Encouraging clinical responses have been reported by a number of study groups. Lang *et al*. (2003) reported the results of a phase I clinical trial study in which rAd-p53 was administered to 15 patients with recurrent glioma. rAd-p53 was injected intratumorally at doses between 3×10^{10} and 3×10^{12} viral particles (VP). Three days after rAd-p53 injection the tumor was resected and more rAd-p53 was injected into the tumor bed. Of the 15 patients treated, 1 survived more than 3 years without evidence of recurrence, 4 patients experienced no recurrence for more than 6 months after treatment, and 2 of these 4 patients survived for more than 1 year. In a multicenter phase II trial study involving 25 patients with non-small cell lung cancer, 7.5×10^{12} VP of rAd-p53 was injected intratumorally in combination with cisplatin and vinorelbine. No significant difference in tumor response was observed from rAd-p53-injected lesions and noninjected lesions (52 versus 48%, respectively); however, the rAd-p53-treated lesions appeared to be smaller than the nontreated controls (Schuler *et al*., 2001). In a separate clinical study of 24 patients with non-small cell lung cancer (Nemunaitis *et al*., 2000), rAd-p53 was injected intratumorally at doses between 1×10^6 and 1×10^{11} plaque-forming units (PFU)/injection in combination with cisplatin. Seventeen patients achieved stable disease, 2 patients achieved partial response, and 4 patients had progressive disease. Intratumoral injection of rAd-p53 in combination with cisplatin was well tolerated and there was evidence of clinical efficacy. rAd-p53 was also used for non-small cell lung cancer treatment in combination with radiation therapy (Swisher *et al*., 2003). The rAd-p53 dose ranged from 3×10^{11} to 3×10^{12} VP/injection. Radiation was given concurrently over 6 weeks to a total of 60 Gy. Of the 19 patients treated, 1 showed complete regression (5%), 11 demonstrated partial regression (58%), 3 showed stable disease (16%), 2 showed progressive disease (11%), and 2 were unevaluable (11%). In a bladder carcinoma trial, 12 patients received either intratumoral or intravesicular injections of rAd-p53 at doses of 7.5×10^{11} to 7.5×10^{13} VP (Kuball *et al*., 2002). Higher transduction efficiency was observed when using the intravesicular delivery method. Seven of the 11 evaluable patients had evidence of p53 expression by reverse transcription-polymerase chain reaction (RT-PCR). Nine of the 12 patients were alive at a median follow-up of 30 months. Pagliaro *et al*. (2003) reported a similar phase I study in which rAd-p53 was instilled intravesicularly at 1×10^{12} VP/dose in 13 patients with locally advanced transitional cell carcinoma of the bladder. A preliminary antitumor effect was observed at a treatment dose of 1×10^{12} VP on days 1 and 4. Clayman *et al*. (1998) reported the results of a clinical study in which rAd-p53 was applied as a single agent to treat advanced recurrent head and neck squamous cell carcinoma. Thirty-three patients received an intratumoral injection of rAd-p53 at doses up to 1×10^{11} PFU/injection. Of the 17 evaluable patients, 2 patients showed objective tumor regression of greater than 50%, 6 patients presented stable disease for up to 3.5 months, and 9 patients showed progressive disease.

In all the reported clinical studies, the most common side effects were pain at the injection site, fatigue, and development of self-limited fever. Overall, rAd-p53 treatment was well tolerated by patients, without serious side effects. On the basis of

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the promising clinical results and benign side effects, Introgen Therapeutics (Austin, TX) is currently pursuing a number of late-phase clinical studies using rAd-p53 in the treatment of cancers, of which treatment for squamous cell carcinoma of the head and neck (SCCHN) is in a phase III clinical study.

There has been an active clinical gene therapy program using rAd-p53 in the treatment of head and neck squamous cell carcinoma in China since 1998. After extensive multiyear and multicenter clinical studies, a recombinant human adenovirus–p53 injection (trademarked as Gendicine) developed by Shenzhen SiBiono GeneTech (SiBiono; Shenzhen, China) was approved by the State Food and Drug Administration of China (SFDA) on October 16, 2003 for the treatment of head and neck squamous cell carcinoma, and was formally launched in April 2004. Gendicine became the world's first gene therapy product approved by a governmental agency for the treatment of cancer indications and thus sets a new milestone in the history of gene therapy and biotechnology.

This review presents a general description of the clinical research and applications of Gendicine and its antitumor mechanisms. An overview concerning the manufacturing process, quality control, and quality assurance of Gendicine is also provided. In addition, the supportive policies and grants that the Chinese Government instituted to encourage the development of gene therapy in China are outlined.

GENDICINE AND ITS ANTITUMOR MECHANISMS

Gendicine is a recombinant human serotype 5 adenovirus in which the E1 region is replaced by a human wild-type p53 expression cassette. The *p53* gene is driven by a Rous sarcoma virus (RSV) promoter with a bovine growth hormone (BGH) poly(A) tail. The recombinant adenovirus is produced in human embryonic kidney (HEK) 293 cells grown in a bioreactor. Virus produced from the bioreactor is further processed and chromatographically purified to produce the recombinant human Ad-p53 injection product.

After more than two decades of study, the *p53* gene is widely regarded as the "genome guardian." It has been estimated that at least half of all human malignancies are related to a mutation of the *p53* gene (Shiraishi *et al*., 2004). Extensive basic research on *p53* gene facilitated its clinical application. In turn, the clinical applications helped us to better understand the functions of the *p53* gene.

Gendicine is composed of the therapeutic *p53* gene and its delivery vehicle, the recombinant adenoviral vector. After Gendicine administration, the adenoviral particle infects tumor target cells and delivers the adenovirus genome carrying the therapeutic *p53* gene to the cytoplasm and the cell nucleus for transcription and translation of the *p53* gene. The expressed *p53* gene appears to exert its antitumor activities by one or more of the following mechanisms:

- Simultaneously triggering apoptotic pathways in tumor cells by a transcription-dependent mechanism in the cell nucleus (Muller *et al*., 1998; Bouvard *et al*., 2000; Matsuda *et al*., 2002; Taha *et al*., 2004) and by a transcription-independent mechanism in the mitochondria (Chipuk *et al*., 2004; Leu *et al*., 2004) and Golgi apparatus (Bennett *et al*., 1998; Ding *et al*., 2000)
- Activation of immune response factors such as natural killer (NK) cells (Yen *et al*., 2000; Cerwenka and Lanier, 2003; Rosenblum *et al*., 2004) to exert "bystander effects"
- Inhibition of DNA repair and antiapoptosis functions in tumor cells (Sah *et al*., 2003)
- Downregulation of the expression of (1) multidrug resistance genes (Krishna and Mayer, 2000) to revert the resistance of tumor cells against radio- and chemotherapies, (2) the vascular endothelial growth factor (VEGF) gene (Dameron *et al*., 1994; Pal *et al*., 2001) to block the blood supply to tumor tissue, and (3) matrix metalloproteinase (MMP) (Toschi *et al*., 2000; Ala-aho *et al*., 2002; Sun *et al*., 2004) to suppress tumor cell adhesion, infiltration, and metastasis
- Blockage of the transcription of survival signals in tumor cells (Singh *et al*., 2002; Yin *et al*., 2003; Rother *et al*., 2004), thus inhibiting the growth of tumor cells in any stage of the cell cycle
- Limitation of the uptake of glucose (Schwartzenberg-Bar-Yoseph *et al*., 2004) and the production of ATP (Brasseur *et al*., 1997; Iizumi *et al*., 2002) in tumor cells

After intratumoral injection of Gendicine, we observed infiltration of many lymphocytes and obvious inhibition of VEGF activity in biopsies of tumor lesions of patients enrolled in clinical trials of Gendicine.

The adenoviral delivery vehicle is known to trigger a strong immune response in patients. It may also induce various nerve factors, hormones, and cytokines to regulate the nerve–endocrine–immune system in the body and thus increase humoral immunity, cellular immunity, and NK cell activities to more effectively kill tumor cells. The increased immune activities are manifested by the development of grade I/II self-limited fevers in approximately 32% of patients. Fever is usually regarded as a side effect in clinical practice; however, it reflects the effectiveness of Gendicine in mobilizing the body's immune systems. A mobilized immune system in advanced or terminal stage cancer patients is expected to be beneficial for tumor containment.

In clinical trials and applications, we also observed that Gendicine reduced the side effects caused by conventional chemo- and radiation therapy. A significant observation was that some patients showed improved appetite and general health status approximately 2 days after receiving Gendicine treatment. This is a positive clinical development for cancer patients who suffer from severe side effects caused by radio- and chemotherapy. The mechanism leading to the improvement is not yet known; we suggest it is related to the infective property of the Gendicine active ingredient, the recombinant adenovirus. Further study is underway to better understand the clinical implications.

SAFETY AND EFFICACY OF GENDICINE IN CLINICAL USE

Safety

Gendicine is an active recombinant adenoviral particle genetically engineered to express the human *p53* gene. The *p53*

gene exists ubiquitously in normal cells and is one of the most widely studied tumor suppressor genes in the human body. Because of its importance in maintaining genome stability, the *p53* gene has been dubbed the "genome guardian." The adenoviral vector is a replication-incompetent serotype 5 adenovirus (Ad5) with a deletion in the E1 region, which limits the infectivity of the virus to only one cycle, and the adenovirus genome does not integrate into host genome DNA. Ad5 has one of the weakest pathogenicity levels in the adenovirus family. Wild-type Ad5 infection generally results in mild upper respiratory disease and fever. Although one fatality has been reported in relation to the use of adenovirus to treat a patient with ornithine transcarbamylase (OTC) deficiency syndrome, the death was later found to be caused by the patient's overwhelming immune reaction against a high systemic dose of adenoviral vector (Han *et al*., 2003; Zhang *et al*., 2003a,b; Chen *et al*., 2003). No fatalities have been reported in other cancer gene therapy trials using adenoviral vectors. Data from our clinical trials and applications demonstrated that Gendicine is safe for clinical use.

In a phase I clinical trial (Han *et al*., 2003) from 1998 to May 2000, 12 patients with advanced laryngeal cancer received various doses of Gendicine before and after surgery. Development of self-limited fever was observed in one patient. The administration of Gendicine did not change the status of wound healing after surgery. No other side effects were found during the subsequent 3 years of follow-up. Extensive multicenter controlled and randomized clinical trials (Zhang *et al*., 2003a) confirmed that the most commonly observed side effects were grade I/II self-limited fever in approximately 32% of Gendicine-treated patients (Table 1). In a few rare cases patient fever reached as high as 40°C. Development of fever was observed as quickly as approximately 3 hr after injection, lasted about 4 hr, and then disappeared spontaneously. On occasion, it lasted more than 10 hr. Gendicine in combination with radiotherapy did not exacerbate any side effects.

Gendicine has been administered via a number of routes, including intratumoral injection, intrapleural and intraperitoneal infusion, intravenous injection, hepatic and lung artery infusion, endotracheal and intravesical instillation, and so on. A few patients receiving an intravenous infusion of 1×10^{12} VP of Gendicine per dose experienced temporary blood pressure decrease (approximately 1.33-kPa drop) when a relatively fast infusion rate was used.

So far, no severe side effects have been found in more than 2500 patients treated with Gendicine. The extensive experience in clinical use showed that Gendicine is safe and well tolerated by patients.

Therapeutic effects

The therapeutic *p53* gene carried by Gendicine exists ubiquitously in normal cells. The recombinant adenovirus can infect, with varying efficiency, almost all human cells including dividing cells and resting cells. Those unique biological properties make Gendicine a wide-spectrum antitumor agent. Its activities have been demonstrated in our clinical applications. Furthermore, the clinical efficacy of Gendicine may be independent of the endogenous *p53* gene status of tumor cells. The published data on Gendicine clinical studies are presented below.

Advanced head and neck squamous cell carcinoma

1. Phase I clinical trial (Han et al., 2003). Gendicine was used for the treatment of 12 patients with advanced laryngeal cancer, with an average clinical course of 41 months. Seven of the 12 patients had not received any treatment before Gendicine administration and 5 of the 12 patients had one or multirecurrent history. One of the patients received six laser surgery treatments because of relapse, with an average interval of 9 months. The patients were divided into three groups receiving escalating doses of Gendicine. Intratumoral injection was administered at a dose of 1×10^{10}, 1×10^{11}, and 1×10^{12} VP every other day for a total of 10 injections. In the subsequent 36- to 42-month follow-up no patient relapsed. In addition, according to data presented by the principal investigator (PI) of this phase I clinical trial, D. Han (Beijing Tongren Hospital, Beijing, China), at The Fourth National Conference of Diagnosis, Therapy and Prevention of Genes held in May 28–30, 2004 in Xian and organized by the Chinese Medical Association (CMA), there was still no patient relapse more than 5 years after Gendicine treatment. In comparison, the 3-year relapse rate for patients with advanced laryngeal cancer receiving surgery alone is generally about 30%.

2. Phase II/III clinical trials (Zhang et al., 2003a,b; Chen et al., 2003). Significant synergistic effects have been demonstrated for the combination of Gendicine with radiotherapy, chemotherapy, surgery, and hyperthermia in the treatment of cancers. A multicenter, concurrently controlled, randomized clinical trial was conducted in which Gendicine was administered to 135 patients with head and neck squamous cell carci-

Table 1. Fever in Patients Undergoing Gendicine Treatment

	Time of administration (weeks)									
	1	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*Total*	*%*
Number of administrations:	103	103	103	101	99	99	99	99	806	—
Number of patients with fever										
Grade I:	31	21	19	17	19	15	7	7	136	16.9
Grade II:	25	29	15	15	14	11	7	6	122	15.1
Total:	56	50	34	32	33	26	14	13	258	32.0
Fever rate (%):	54.4	48.5	33.0	31.7	33.3	26.2	14.1	13.1	32.0	—

Table 2. Comparison of General Data for Two Patient Groups with Head and Neck Squamous Cell Carcinoma

Group	Number of patients	Sex		Age, years ($\bar{X} \pm SD$)	Clinical stage			
		Male	Female		I	II	III	IV
GTRT	63	43	20	48.09 ± 12.59	0	15	19	29
RT	72	47	25	52.46 ± 12.11	0	17	27	28

Abbreviations: GTRT, group receiving both gene therapy and radiotherapy; RT, group receiving only radiotherapy; SD, standard deviation; $\bar{X}$, mean value.

noma. Of the enrolled patients, 77% had late stage III to IV cancer and had failed in either radio- or chemotherapy or were not eligible for surgery. The majority (85%) of the patients had nasopharyngeal cancer. The patients were divided randomly into two groups: one group received gene therapy in combination with radiotherapy (GTRT) and the other group received radiotherapy alone (RT). There were no significant differences ($p > 0.05$) in age, sex, or clinical stage (Table 2) or in size of tumor lesion (Table 3) between the two groups of patients. Conventional or three-dimensional conformal radiotherapy was used at doses of 70 Gy administered in 35 fractions over 7–8 weeks for the RT group. For the GTRT group, Gendicine was given each week at a dose of 1×10^{12} VP 3 days before radiotherapy, for a total of 8 weeks. Radiotherapy in the GTRT group was the same as that used in the RT group. Objective tumor response was evaluated by computed tomography (CT) or magnetic resonance imaging (MRI) according to World Health Organization (WHO) response criteria. The data showed that the response rate in the GTRT group was 93%, with 64% showing complete regression (CR) and 29% partial regression (PR) (Table 4). The response rate in the RT group was 79%, with 19% of the patients showing CR and 60% PR. There is a significant difference ($p < 0.01$) between the two groups in terms of both the CR rate and the PR rate. The CR rate in the GTRT group was 3-fold higher than that in the RT group. In addition, it is encouraging that, 4 weeks after treatment, 65% of patients showing PR were observed in the GTRT group whereas 40% of those with PR and 57% stable disease (SD) were in the RT group. We concluded that Gendicine in combination with radiotherapy showed obvious synergistic effects. Usually, it is somewhat misleading when the total response rate is used to judge clinical efficacy because of the frequently observed high frequency of relapse for patients with partial regression. Instead, we believe that it is more appropriate to use the CR rate to compare real clinical efficacy.

More than 200 new patients with HNSCC were treated with Gendicine in 2004. Furthermore, a new multicenter, concurrently controlled, randomized phase IV clinical trial including 300 patients with HNSCC has been initiated and is in progress.

3. Laboratory studies of patient tumor samples (Zhang et al., 2003b). Laboratory testing of patient blood samples showed an elevated level of serum antibodies against adenovirus 2–3 weeks after Gendicine injection, reflecting the development of a specific immune response against Gendicine. We also analyzed specimens of tumor tissues derived from 11 patients with squamous or adenocarcinoma. The analysis suggests that there is no significant correlation between the effects of Gendicine and the status of the *p53* gene in tumor cells (Table 5). Among six patients with a normal *p53* gene, two had CR, three had PR, and one had SD; of the other five patients, with a mutant *p53* gene, three had CR and two had PR. Other investigators (Inoue *et al*., 2000; Quist *et al*., 2004) also reported the lack of correlation between tumor p53 status and patient response to the rAd-p53 agent.

Advanced liver cancer

Guan *et al*. (2005), at West China Hospital (Sichuan University, Chengdu, China), reported the results of administering Gendicine in combination with hepatic transcatheter arterial chemoembolization (TACE) to patients with advanced hepatic cell carcinoma (HCC). A total of 150 patients were enrolled. Sixty-eight patients were treated with the Gendicine–TACE combination by intratumoral injection of Gendicine under CT guidance or transcatheter perfusion at a dose of $1–4 \times 10^{12}$ VP, once per week for 4 weeks, 2–5 days after TACE was carried out. Eighty-two patients were treated by TACE alone as a control group. TACE in both the treatment group and the control group was performed with 5-fluorouracil (5-FU; 1 g), hydroxy-camptothecin (HCPT; 40 mg), and doxorubicin (Adriamycin [ADM], 40 mg), and arterial embolization with iodized oil (10–30 ml).

The response rates were 67.6% in the GT–TACE group and 51.2% in the TACE-alone group, showing a statistically significant difference ($p < 0.05$) (Table 6). Compared with the control group, patients in the GT–TACE group experienced relief from pain (Table 7) and improvement of Karnofsky performance status (Table 8). It is important to point out that the 6-month survival rate was 76.5% (52 of 68) in the GT–TACE

Table 3. Comparison of Tumor Size in Two Patient Groups with Head and Neck Squamous Cell Carcinoma

Group	Number of tumor lesions	Size (mm^2) of tumor lesion ($\bar{X} \pm SD$)
GTRT	63	1132.49 ± 1107.29
RT	72	804.84 ± 673.59

Abbreviations: See Table 2.

Table 4. Comparison of Real-Time Efficacy between Two Patient Groups with Head and Neck Squamous Cell Carcinoma

	4 weeks					*8 weeks*					*12 weeks (confirmation)*				
Group	*No.*	*CR*	*PR*	*SD*	*PD*	*No.*	*CR*	*PR*	*SD*	*PD*	*No.*	*CR*	*PR*	*SD*	*PD*
GTRT	63	5 (8%)	41 (65%)	17 (27%)	0 (0%)	62	28 (45%)	29 (47%)	5 (8%)	0 (0%)	56	36 (64%)	16 (29%)	4 (7%)	0 (0%)
RT	72	0 (0%)	29 (40%)	41 (57%)	2 (3%)	71	6 (8%)	44 (62%)	20 (28%)	1 (2%)	63	12 (19%)	38 (60%)	13 (21%)	0 (0%)

Abbreviations: CR, complete regression; PR, partial regression; SD, stable disease; PD, progressive disease. For other abbreviations see Table 2.

Table 5. Efficacy of Gendicine and Endogenous p53 Status in Tumor Cells

Case no.	*Age (years)*	*Sex*	*Diagnosis*	*p53 Protein*[a]	*Clinical effect*
9521746	78	F	Cervical squamous carcinoma	–	CR
9522478	27	M	NPC (squamous)	–	PR (73%)
9522313	66	M	Perineum squamous carcinoma	–	CR
9516989	46	M	NPC (squamous)	+	PR (78%)
9522892	39	M	Tongue squamous carcinoma	–	SD (41%)
9523026	64	M	NPC (squamous)	+	CR
9521911	46	M	Lung squamous carcinoma	–	PR (92%)
9522981	47	F	Cervical adenocarcinoma	+	CR
9522122	69	F	Ovary adenocarcinoma	–	PR (57%)
9524614	53	F	NPC (squamous)	+	PR (53%)
9522617	69	F	NPC (squamous)	+	CR

Abbreviations: F, female; M, male; NPC, nasopharyngeal carcinoma. For other abbreviations see Table 14.
[a]–, normal *p53* gene; +, mutant *p53* gene.

group and only 23.2% (19 of 82) in the control group. The difference is significant ($p < 0.01$) (Table 9). The preliminary results showed that Gendicine in combination with TACE is effective for the treatment of HCC to enhance patient survival rate and to improve patient quality of life.

Zhu *et al*. (2004), at the Second People's Hospital of Shenzhen (Shenzhen, China), reported a clinical study of 38 patients with refractory, inoperable advanced hepatic cell carcinoma. Thirty of 38 patients received Gendicine treatment alone and the other 8 patients were treated with Gendicine in combination with hyperthermia. All patients received the same Gendicine dosage, $1–2 \times 10^{12}$ VP/week (injection) for a total of 4 weeks, by intralesional injection via percutaneous hepatic paracentesis or hepatic arterial infusion. The preliminary clinical data for the 30 patients receiving Gendicine alone showed the following: 2 of 30 had PR, 24 of 30 had SD, and 4 of 30 had PD. Twenty-six of the 30 patients showed an improvement in Karnofsky performance status, from 55.79 ± 11.30 to 61.05 ± 21.64. All the patients showed good tolerance for Gendicine administration.

Another 300 patients are enrolled in a concurrently controlled, randomized trial of Gendicine in combination with chemotherapy via TACE for the treatment of advanced hepatic cell carcinoma; this trial is in progress in 11 clinical centers across China.

Advanced lung cancer

Fifteen patients with stage IIIb–IV lung cancer were treated with Gendicine by intratumoral injection at a dose of 1×10^{12} VP each time (Weng *et al*., 2004). The Gendicine was injected by percutaneous lung paracentesis under CT guidance once a week for 4 consecutive weeks as a treatment course. Clinical response was evaluated by CT imaging, tumor biopsy, and a 2-month follow-up. It showed PR in 5 of 15 patients (33.3%), SD in 7 of 15 patients (46.7%), and PD in 3 of 15 patients (20%). Tumor biopsy for 6 of the 12 patients with PR or SD revealed obvious tumor tissue necrosis and reduction in tumor cell number. Except for the development of self-limited fever, no other side effect was observed.

In another, independent study 13 patients with advanced lung cancer were treated with Gendicine in combination with conventional chemotherapy at Sichuan University (Guan *et al*., 2005). Gendicine was injected at a dose of $1–4 \times 10^{12}$ VP once per week for 4 weeks. Chemoagents (cisplatin [DDP], 100 mg; 5-fluorouracil [5-FU], 1.0 g; and etoposide [VP-16], 100 mg) were infused through the bronchial artery 4 days after Gendicine administration. Eight of the 13 patients showed a positive response at 1- and 4-month follow-up: 1 had CR, 6 had PR, and 1 patient showed thoracic liquid reduction. Eleven of the 13 patients showed alleviation of clinical symptoms such as cough, thoracodynia, hemoptysis, and dyspnea.

Other advanced solid tumors

Zhang *et al*. (2004), at the Cancer Center of Sun Yat-Sen University (Guangzhou, China), reported the use of Gendicine for the treatment of 24 patients with advanced solid tumors (13 kinds of tumor). All the patients had failed conventional therapies such as chemotherapy and radiation therapy. The treat-

Table 6. Effect of Gendicine in Combination with Transcatheter Arterial Chemoembolization in Patients with Hepatic Cell Carcinoma

Group	*No. of patients*	*CR*	*PR*	*NC*	*PD*	*CR + PR (%)*
Gendicine–TACE	68	0	45	15	7	67.6
TACE alone	82	0	42	27	13	51.2

Abbreviations: TACE, transcatheter arterial chemoembolization; CR, complete response; PR, partial response; NC, no change; PD, progressive disease.

Table 7. One-Month Follow-up for Clinical Symptoms of Patients with Hepatic Cell Carcinoma

Group	No. of patients	Fever [no. (%)][a]	GI reaction [no. (%)][a]	Pain relief [no. (%)][a]	Pain in muscles and joints [no. (%)][a]
Gendicine–TACE	68	35 (51.5)	20 (29.4)	30 (44.1)	9 (13.2)
TACE alone	82	24 (29.3)	28 (34.1)	21 (25.6)	1 (1.2)

Abbreviation: GI, gastrointestinal; see also Table 6.
[a]$p < 0.05$.

ment regimen was 1×10^{12} VP/week for a total of 4 weeks. Administration routes included intratumoral injection, intrabronchial spray, intraperitoneal injection, arterial infusion, and intravenous injection. Gendicine was administered in combination with a variety of therapies: in the case of 18 patients, with chemotherapy; for 2 patients, with radiotherapy; for 1 patient, with radio- and chemotherapy; for 1 patient, with abdominal thermotherapy and gefitinib; and for 1 patient, with immunotherapy; 1 patient was treated solely with Gendicine. One patient withdrew from the study early because of rapid disease progression. Twenty patients received 4 injections of Gendicine treatment, 2 patients received 8 injections of Gendicine, and 1 patient received 20 injections of Gendicine. Results from 21 evaluable cases showed PR in 5 of 21 patients (24%), SD in 5 of 21 patients (24%), and PD in 11 patients. Clinical responses were observed in 10 of 21 patients (48%). The most common side effect was grade I–II self-limited fever, with two patients experiencing grade III fever. Other, rarer side effects were pain at the injection site, shivering, and muscle soreness.

Zhang *et al*. (2003a), at the Department of Radiation Therapy of Beijing Cancer Hospital (Beijing, China), reported a clinical study of Gendicine in combination with thermotherapy administered to seven patients with advanced malignant tumors. Gendicine was injected intratumorally at 1×10^{12} VP/week for a total of 4 weeks. On day 3 after Gendicine administration hyperthermia was applied, using either a microwave at 915 MHz (warming to 43-44°C) or radioheating at 40 MHz (warming to 42–43°C). The data showed one patient with CR, two with PR, and four with SD after eight doses of Gendicine treatment. Biopsy for the CR and PR cases revealed the occurrence of tumor necrosis.

Cancerous ascites

A significant effect was reported when Gendicine was used to treat cancerous ascites (Zhu *et al*., 2005). Thirteen patients with advanced cancers (8 patients with gastric carcinoma, 4 with colon carcinoma, and 1 with carcinoma of the gallbladder) and a large amount of ascites were treated with Gendicine via peritoneal paracentesis and intraperitoneal infusion at a dose of $1–2 \times 10^{12}$ VP/week for a total of 4 weeks. Six of 13 patients also had jaundice. Clinical response was evaluated by measurement of abdominal girth, CT, or MRI and 1 month of follow-up. After 3 weeks of treatment, 7 of the 13 patients showed significant reduction in ascites buildup, alleviation of disease symptoms (such as abdominal distention and shortness of breath), and improvement in Karnofsky performance status ($p < 0.05$). Except for the development of self-limited fever, no other side effects were observed. The results suggest that Gendicine can be used to treat patients with cancerous ascites to relieve disease symptoms and improve quality of life in a short time period.

Although Gendicine is approved as a new class of drug for cancer treatment and has demonstrated clinical efficacy in a large number of patients, more clinical experience and application data are needed to optimize its use and to maximize clinical benefit to better serve the cancer patient population. We believe the available data on Gendicine has proven the concept of gene therapy as a new modality for cancer treatment at the gene level. We hope our experience with Gendicine will help to speed up the development of other gene therapy products.

MANUFACTURE OF GENDICINE

The following is a general description of the large-scale production technologies that are used for the manufacture of Gendicine.

Large-scale cell culture technology

Large-scale culture of HEK293 cells is a critical step in the production of Gendicine. The technology has undergone significant development, from the first-generation adherent cell culture using roller bottles, the CellCube bioreactor (Corning Life Sciences, Acton, MA), and the packed-bed CelliGen Plus

Table 8. One-Month Follow-up for Karnofsky Score for Patients with Hepatic Cell Carcinoma

Group	No. of patients	Karnofsky score				Total no. (%) of patients with increased Karnofsky score
		≥20-point change	≥10-point change	No change	Decrease	
Gendicine–TACE	68	14	28	18	8	42 (61.8)[a]
TACE alone	82	12	24	18	28	36 (43.9)[a]

Abbreviation: See Table 6.
[a]$p < 0.05$.

Table 9. Three- and Six-Month Survival Rate After Treatment of Patients with Hepatic Cell Carcinoma

Group	*No. of patients*	*Three months [no. (%)]*	*Six months [no. (%)]*
Gendicine–TACE	68	61 (89.7)	52 (76.5)
TACE alone	82	49 (60.0)	19 (23.2)

Abbreviation: See Table 6.

bioreactor (New Brunswick Scientific, Edison, NJ) to the second-generation suspension, serum-free culture in large-scale bioreactors. Gendicine is produced with a patented large-scale bioreactor system. The patented producer cell line, SBN-Cel, is a subclone derived from the HEK293 cell line. The subclone was established at SiBiono through genetic engineering and molecular cloning for the commercialization of Gendicine. The subclone showed stronger attachment to culture surfaces, a faster growth rate (doubling time, approximately 18 hr) than the parental HEK293 cells, and good virus productivity.

Chromatography purification technology

SiBiono has developed and optimized a complete downstream processing and automated chromatography purification process for the production of Gendicine. Downstream processing includes tangential flow filtration for harvest clarification and tangential flow ultrafiltration for concentration and diafiltration. The concentrate is further treated with Benzonase (Merck, Darmstadt, Germany) to break down large cellular DNA. The material is further clarified and chromatographically purified in an automated chromatography system (fast protein liquid chromatography [FPLC]). After a single-step purification, the purity of the Gendicine final product can be greater than 98%. Approximately 4×10^{15} VP of purified final product can be produced from a single-batch 14-L bioreactor run. SiBiono has established and qualified both master/working cell banks and master/working virus banks, which are critical raw materials for the commercial production of Gendicine.

Table 10. Quality Control Methods and Specifications for Gendicine

No.	*Assay*	*Method*	*Specification*
1	Physical characteristics		
	Appearance	Examination under light	Opalescence
	Recoverable volume	Capacity assay	≥1.5 ml/dose
2	Chemical characteristics	pH	8.0–8.5
3	Identity		
	RE mapping	Restriction mapping	Given segment
	p53 gene	PCR analysis	396 bp
4	Purity	HPLC	≥95.0%
	$A_{260/280}$ ratio	$A_{260/280}$ assay	1.2–1.3
5	Viral titer		
	VP	A_{260} assay	$\geq 6.7 \times 10^{11}$ VP/ml
	Infectious units	$TCID_{50}$	$2–4 \times 10^{10}$ IU/ml
	IU:VP ratio		≥3.3%
6	Gene efficacy		
	Gene expression	Western blot	Positive
	Bioactivity	Saos-2 cell bioassay	Positive
7	RCA	A549 cell bioassay	≤ 1 RCA/3×10^{10} VP
8	Detection of AAV	PCR	Negative
9	Residuals		
	BSA	Cell agglutination	≤33 ng/ml
	HCP	ELISA	≤66 ng/ml
	Cell DNA	Southern blot	≤6.6 ng/ml
	Benzonase	ELISA	≤0.6 ng/ml
10	Microbes		
	Sterility test	Membrane filtration	Negative
	Mycoplasma test	DNA fluorescence suckling mice	Negative
11	General safety	Guinea pig and	Safe
12	Endotoxin	LAL	≤6.6 EU/ml

Abbreviations: A_{260}, absorbance at 260 nm; AAV, adenovirus; BSA, bovine serum albumin; ELISA, enzyme-linked immunosorbent assay; EU, endotoxin unit; HCP, host cell protein; HPLC, high-performance liquid chromatography; IU:VP ratio, infectious unit:viral particle ratio; LAL, *Limulus* amebocyte lysate; PCR, polymerase chain reaction; RCA, replication-competent adenovirus; RE, restriction endonuclease; $TCID_{50}$, median tissue culture infective dose; VP, viral particles.

QUALITY CONTROL AND QUALITY ASSURANCE OF GENDICINE

In March 2003, the SFDA officially issued a document, *Guidance for Human Gene Therapy Research and Its Products* (State Food and Drug Administration of China, 2003), that provides national guidance for research on and commercialization of gene therapy products in China. This document was translated and published in *BioPharm International* in May 2004 (Shenzhen SiBiono GeneTech and National Institute for the Control of Pharmaceutical and Biological Products, 2004). This guideline is one of the most comprehensive documents published by a government agency for gene therapy research and product quality control. It outlines 12 quality control tests and 21 test methods. Table 10 outlines the quality control tests and specifications performed on in-process and final product samples during the manufacture of Gendicine.

The tests can be divided into the following three groups:

- *General tests required for biopharmaceuticals*: General tests include verification of physical characteristics and chemical characteristics, determination of purity and detection of residual impurities, and performance of sterility test, *Mycoplasma* test, general safety test, and bacterial endotoxin test. These tests are generally applied to all biopharmaceuticals.
- *Basic tests*: Basic tests include identity and efficacy tests. The purpose of these tests is to demonstrate the presence of the correct therapeutic gene, gene expression, and biological activity of the expressed gene product. Restriction mapping and PCR analysis are used to identify the presence of the correct therapeutic gene. The efficacy test comprises measurement of gene expression and biological activities of the expressed gene product. Gene expression is measured by Western blot or ELISA of cells infected with the recombinant adenovirus product (e.g., expression of p53 protein in H1299 cells). Bioactivity of the expressed gene product is assayed on a specific cell line infected with the recombinant adenovirus product (e.g., induction of Saos-2 cell apoptosis by p53 protein).
- *Unique tests*: Unique tests include viral particle titer determination (viral particles, infectious units [IU], and IU:VP ratio) and detection of replication-competent adenovirus (RCA).

Viral particle titer determination

Viral particle titer determination is generally done by the A_{260} ultraviolet absorption method. In the presence of sodium dodecyl sulfate (SDS), 1 absorption unit at 260 nm equals 1.1×10^{12} VP/ml.

Infectivity

Infectivity is measured by a median tissue culture infective dose ($TCID_{50}$) method, using serial dilution. The infectivity titer is calculated according to the formula $T = 10^{1+d(S-0.5)}$ (IU/100 μl), where d is dilution and log S is the sum of the infection rate from the highest dilution.

IU:VP ratio

According to the SFDA guideline, the specific activity (IU:VP ratio) of clinical-grade recombinant adenovirus needs to be at least 3.3%. Gendicine generally has an IU:VP ratio of about 4.0% with an infectious titer of $4–5 \times 10^{10}$ IU/ml, exceeding the guideline requirement.

Purity test

Although purity determination is not unique to recombinant adenovirus product, the use of the $A_{260/280}$ absorbance ratio as a purity indication for rAd is unique and should be in the range of 1.2 to 1.3. HPLC is also used for purity determination. According to HPLC analysis, Gendicine generally has a purity of greater than 98%, exceeding the SFDA guideline specification.

Detection of replication-competent adenovirus

Determination of the RCA level is an important safety criterion for recombinant adenovirus product. RCA can arise by homologous recombination between adenoviral vector and the host cell genome during the adenovirus production process. Amplification in A549 cells is generally used to detect the presence of RCA. The RCA level for Gendicine meets the less than 1 RCA/3×10^{10} VP specified in the SFDA guideline.

In addition to performing all the necessary quality control tests, Gendicine is produced in accordance with strict GMP regulations as outlined in document ICH Q7A (International Conference on Harmonization, Topic Q7A: GMP for Active Pharmaceutical Ingredients). SiBiono has instituted an independent quality assurance function to ensure the consistent production of high-quality Gendicine product.

SFDA REGULATIONS AND REQUIREMENTS FOR HUMAN GENE THERAPY STUDY

In May 1993, the Chinese Ministry of Public Health released *An Outline of Quality Controls for Clinical Studies of Human Somatic and Gene Therapy*. It was further revised in June 1999 and reissued as *Guiding Principles for Human Gene Therapy Clinical Trials*. In realization of the rapid development in gene therapy and the potential gene therapy promised for scientific and medical advancement, and to promote gene therapy research and eventual commercialization in China, the SFDA published *Guidance for Human Gene Therapy Research and Its Products* (State Food and Drug Administration of China, 2003; the quality control section of this document was translated and published as *Points to Consider for Human Gene Therapy and Product Quality Control* [Shenzhen SiBiono GeneTech and National Institute for the Control of Pharmaceutical and Biological Products, 2004]). This guidance document outlined requirements for application of gene therapy clinical study, study protocol format, and requirements for construction of a recombinant DNA and gene delivery system. The document also outlined requirements for the establishment and testing of cell bank and engineered strains, manufacturing process, quality controls, and product efficacy and safety tests.

In the 7 years from the submission of a phase I clinical trial application in March 1998 to March 2004, when its GMP certificate was issued, Gendicine underwent five stringent review and approval steps. The steps included application for initiation of a phase I clinical trial, application for initiation of phase II/III clinical trials, New Drug License application, Production Li-

cense application, and GMP certification application. For each step numerous submission materials are required by the SFDA. A strict review by an expert advisory team is conducted for each phase of a clinical trial application. A clinical trial can begin only after the sponsor satisfactorily addresses any questions the reviewers have, and after approval of the application. For example, the material submitted for the Gendicine phase I clinical trial study comprised more than 17 volumes with about 1000 pages (more than 1200 pages when translated into English). The SFDA expert team consisted of 39 experts (10 of whom were members of the Chinese Academy of Sciences). Such a large expert team is rare in China's New Drug Review history.

After the issuance of the New Drug License, the Guangdong Food and Drug Administration inspected SiBiono's facility and submitted three batches of sealed samples to the National Institute for the Control of Pharmaceutical and Biological Products (NICPBP) for lot release testing. The SFDA granted the production license only after the NICPBP successfully passed all three lots. China's drug law specifies that a sponsor must first obtain a production license before applying for a GMP certificate for its facility. And before market launch, the sponsor must apply for pricing approval from the State Price Bureau. There are two stages of GMP certificate approval. The first GMP certificate is valid for 1 year. The second GMP certificate application needs to be submitted in the second year. After review and approval, the second GMP certificate is valid for 5 years. The Gendicine production facility and the 1100 SOPs used for the production of Gendicine have passed the second GMP certification.

SUPPORTIVE POLICY OF CHINESE GOVERNMENT TOWARD HUMAN GENE THERAPY

Since the reform and open market policy instituted in the late 1970s and early 1980s, China has emphasized science and technology development as the key to the country's modernization and economic development. The government has placed particular emphasis on the development of biotechnology. The birth of the world's first gene therapy product, Gendicine, is the result of the productive interaction between Chinese scientists, entrepreneurs, and the government, which has consistently encouraged the development of innovative, high-tech business in China (Li *et al*., 2004; Sabine, 2004; Sue *et al*., 2004). Since the establishment of SiBiono in 1997, the company has received generous support from various branches of the government such as the Shenzhen Municipality, Guangdong Province, and the Ministry of Science and Technology and the National Development and Reform Commission of China. SiBiono's gene therapy program received grant support from the National 973 Program (2005), the National Model Engineering Bases (2004), the National 863 Program (1999 and 2004), the National Core Scientific and Technological Project (2002–2005), the National Key Research Program (2001), the National Foundation for Innovation (2000), the Key Technology Platform Program of Guangdong Province (2003), and Industrialization Projects of Shenzhen. With this support, SiBiono has made significant progress in the education of the general public on gene therapy and eventual commercialization of a gene therapy product in China:

- Significant progress in disseminating gene therapy knowledge to the general public and to government agencies in China. As a result, gene therapy is accepted as a new medical intervention for a variety of diseases.
- Successful commercialization of first gene therapy product, Gendicine, in China. The product has been shown to improve the health and qualify of life of cancer patients. The launch of Gendicine is expected to promote further development of gene therapy as a whole.
- Establishment of a production facility that is a quality control and production technology platform for the commercialization of gene therapy products.
- Emphasis on intellectual property rights and establishment of a large intellectual property portfolio to protect key products and technologies; a number of domestic and international patents have been acquired. Gendicine is an internationally recognized trademark.

SiBiono is confident that it has the know-how and intellectual properties for future exploration of other international markets. With long-term government support and the return of outstanding scholars from overseas study, a number of notable successes have been achieved in biotechnology in China. The Chinese government is pursuing a similar supportive policy toward the development of gene therapy. We are encouraged and optimistic about the future of gene therapy development in China.

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