The Genesis of **Gendicine**: The Story Behind the First Gene Therapy

In this *BioPharm International* exclusive interview, SiBiono's founder relates the science and manufacture of his company's innovative cancer therapy.

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In October 2003, Shenzhen SiBiono GeneTech made history by becoming the first company approved to market a gene therapy medication. China's State Food and Drug Administration (SFDA) approved Gendicine for treatment of head and neck squamous cell carcinoma (HNSCC). SiBiono believes that continued clinical trials will prove Gendicine to be effective as a wide-spectrum anticancer agent.

In March, *BioPharm International* interviewed Dr. Zhaohui Peng, SiBiono's founder, chairman, and CEO, to learn more about the science behind gene therapy, the clinical experience with Gendicine, and the processes involved in manufacturing this ground-breaking new therapy. In addition to a distinguished academic career, Dr. Peng served as director of a research institute at The First Medical University in Guangzhou and as a visiting professor at both the University of Chiba in Japan and the University of California. He also conducted research at two US biotech companies. Dr. Peng has devoted more than ten years to gene therapy research, development, and commercialization.

GENE THERAPY BASICS

BPI: You have used the term adenoviral vector +p53 tumor suppressor-gene delivery system. Will you briefly describe the science behind this?

Peng: Gendicine is a gene therapy product. To be more specific, it is a replication-incompetent, recombinant, human adenovirus of serotype 5 engineered to contain the human wild-type p53 tumor-suppressor gene. The Ad5-p53 virus particles are approximately 90 nm in diameter.

Gendicine is produced using SBN-Cel, which is a cell line that was subcloned from the human embryonic kidney (HEK) cell line 293. Gendicine is a sterile, slightly-white-to-clear liquid. A Gendicine vial contains 1 x 10^{12} viral particles in 1 mL of WFI (water for injection) buffered with Tris (made by Amresc) and glycerol. We store it frozen in a single-use vial at –20°C.

BPI: What is the role of the p53 gene?

Peng: The p53 gene is one of the most important tumor-suppressor genes existing in normal cells. In normal cells, the expression level of p53 protein is very low. p53 expression is activated upon oncogene activation, growth-factor deprivation, hypoxia, and DNA damage. The upregulation of p53 gene expression occurs at the posttranslational level and is achieved through stabilization of the expressed protein. The activation of p53 gene expression results in either cell cycle arrest or apoptotic cell death.

The p53 gene is mutated or deleted (null) in approximately 50% to 70% of human tumors. Mutant forms of the p53 gene are not necessarily inactive and can gain oncogenic functions that contribute to tumorigenicity. Most importantly, mutant p53 proteins have been associated with the upregulation of the multidrug resistance (MDR) gene, which results in tumor resistance to a variety of chemotherapeutics. Introduction of exogenous wild-type p53 gene and subsequent over-expression of the p53 protein has been shown to control and eliminate tumor cell growth by growth cycle arrest or apoptosis. In addition, over-expresion of wild-type p53 protein has been demonstrated to have a synergistic effect with radiotherapy and chemotherapy.

BPI: What role does the adenoviral vector play?

Peng: Upon intratumor injection, Gendicine binds to the coxsakie adenovirus receptor (CAR) on tumor cells. Subsequently, Gendicine enters tumor cells via receptor-mediated endocytosis and begins to over-express the encoded exogenous p53 gene. The over-expressed p53 protein triggers multiple tumor fighting functions.

First, it induces tumor cell cycle arrest or apoptosis by functioning as a sequence-specific transcriptional-regulator that up-regulates the expression of some anticancer genes and downregulates the

Table 1. The Initial Clinical Stage of Patients with HNSCC

Protocols	N	Sex		Age	Clinical Stage of Cancer			
		M	F		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

GTRT = Gene therapy (Gendicine) in combination with conventional radiation therapy
RT = Radiation therapy alone

MATTERS OF SAFETY

BPI: What are the results of toxicity tests with Gendicine?

Peng: Two groups of rhesus monkeys received intramuscular injections of Gendicine, based on weight, at doses 7.5-fold and 75-fold the proposed clinical dose for 16 successive days. On the 14th day, a neutralizing antibody to Gendicine appeared in all animal sera. A mild pathological effect was observed in kidney tissues in 2 out of the 12 animals at the end of the study. Gendicine can be detected in lung, liver, and kidney tissues by polymerase chain reaction (PCR) analysis. The expressed exogenous p53 protein can be detected by immunohistochemistry analysis in the intestines, lungs, bladders, and kidneys. At three weeks postinjection, the p53 gene and the expressed p53 protein were still detectable in the above-mentioned organs and tissues.

BPI: Did you conduct any genetic toxicology tests?

Peng: In our tests, no genetic toxicity was observed. Results from both a Chinese hamster lung (CHL) cell-chromosome-aberration test and mouse bone-marrow micronucleus assay were negative. Gendicine will not replicate in the infected cells and is incapable of multi-cycle infection and of spreading to the neighboring cells. Therefore, Gendicine will not cause horizontal adenovirus infection or environmental contamination. More importantly, infected adenoviral DNA will not integrate into the human host cell genome. Consequently, Gendicine poses no genetic toxicity.

BPI: What are the distribution and pharmacokinetic parameters?

Peng: *In vivo* animal studies demonstrated that Gendicine entered tumor cells within one hour after injection, whether administrated locally or systemically. A detectable amount of p53 protein is expressed from the transduced p53 gene at three hours postinjection. The level of p53 protein expression increased to 47% at the 12th hour, reached the highest level (100%) at the 72nd hour, and subsequently descended to 30% at the 120th hour postinjection. However, a detectable amount of p53 protein expression was still observed at the 14th day. At three weeks postinjection, recombinant Gendicine DNA began to diminish and eventually became undetectable. When injected locally, Gendicine is distributed mainly in the local tissues with minimal distribution in other organs and tissues. No Gendicine DNA was detected in excrements of urine, stool, or bile.

Table 2. Comparison of Tumor Shrink Rates

Protocols	Mean tumor shrink rate (%)		
	4 wks treatment	8 wks treatment	Follow up for 4 wks
GTRT group	60.77 ± 23.43	81.86 ± 25.12	83.59 ± 30.03
RT group	41.63 ± 26.89	62.51 ± 29.75	66.62 ± 34.37
(GTRT)/(RT)	1.47	1.31	1.25
p value	$p < 0.01$	$p < 0.01$	$p < 0.05$

expression of some oncogenes.

Second, it can directly induce tumor cell apoptosis.

Third, it can function as a tumor-antigen by stimulating human immune cells (cytotoxic T cells) to selectively kill cancer cells that over-express the p53 gene. It can also activate natural killer cells to kill uninfected cancer cells via bystander effects.

Fourth, the expressed p53 protein can downregulate the expression of vascular endothelial growth factor (VEGF) genes and MDR genes, which are involved in tumor progress, metastasis, and chemo-drug resistance. The expression of p53 gene is not activated in normal cells because its DNA is undamaged, thus minimizing the side effects of Gendicine treatment.

CLINICAL TRIALS

BPI: When did you begin researching Gendicine?

Peng: We started in 1989. That is about 14 years ago.

BPI: How have clinical trials proceeded?

Peng: One hundred thirty-five patients with late-stage HNSCC took part in phase 2 and 3 trials during the period from November 2000 to May 2003. The results showed complete regression of tumors in 64% of the patients after eight weekly intratumoral injections of Gendicine in combination with radiation therapy; 29% of the patients

Table 3. Comparison of Protocol Efficiency

Protocol	N	4 Wks Treatment				N	8 Wks Treatment				N	4 Wks Follow-Up			
		CR	PR	SD	PD		CR	PR	SD	PD		CR	PR	SD	PD
GTRT	63	8%	65%	27%	0%	62	45%	47%	8%	0%	56	64%	29%	7%	0%
RT	72	0%	40%	57%	3%	71	8%	62%	28%	0%	63	19%	60%	21%	0%

Comparison of GTRT group with RT group, $p < 0.01$ in each comparison
CR: complete response, PR: partial response, SD: stabilized disease PD: progressive disease

experienced partial regression. Among all the patients, about 75% suffered from advanced nasopharyngeal carcinoma, which is a sub-indication of head-and-neck cancer. Statistically, 80% of the worldwide cases are in China. Tables 1, 2, and 3 show our clinical trial results as of October 2002.

Another 240-plus patients with late-stage HNSCC or terminal-stage non-HNSCC tumors were treated with Gendicine during the period from June 2003 to the present, continuing the phase 2 and 3 clinical trials. Like the previous data, these trials also showed the safety and efficacy of Gendicine.

We find that in combination with chemo- and radiotherapy, Gendicine can improve treatment efficacy by 3.4-fold. Furthermore, this combination not only improves treatment efficacy, but it also appears to alleviate the toxic side effects normally associated with chemotherapy and radiation therapy. We have no test criteria proving reduced side effects, just anecdotal comments from patients or their doctors. Seven scientific papers reporting on the safety and efficacy of the clinical trials have also been published in *National Medicine Journal of China* (December 10, 2003).

BPI: Are there any side effects?

Peng: Clinical results indicate that Gendicine is safe and efficacious. Some patients experienced self-limiting Grade I and II fevers lasting approximately three hours. No other serious side effects were observed.

BPI: Is Gendicine a wide-spectrum anticancer agent?

Peng: In clinical trials, Gendicine has been used to successfully treat cancers of the digestive tract (esophageal, gastric, intestine, liver, pancreas, gallbladder, rectum), lung cancer, sarcoma, thyroid-gland cancer, breast cancer, cervical cancer, and ovarian cancer. Although Gendicine has not been formally approved by SFDA for indications other than HNSCC, terminal patients with no other avenue of treatment have been allowed, on a case-by-case basis, to receive Gendicine with permission from the SFDA, along with a request from the patient, the patient's family, and the agreement of the patient's doctor. We also insist that the patient, family, and the doctor-in-charge carefully read and endorse the informed consent forms.

BPI: What is the incidence of relapse?

Peng: During more than three years of follow-up for the twelve patients with mid-to-late-stage laryngeal cancer who received Gendicine therapy in phase 1 clinical trials, no patient has relapsed. By contrast, among patients who received only surgery, the three-year relapse rate is approximately 30%.

BPI: What is the duration of treatment?

Peng: Patients do not stay on the drug for the rest of their lives. A patient receives one injection per week for four to eight weeks consecutively as a treatment cycle. A standard dose is $1x10^{12}$ viral particles (VP).

GENDICINE AVAILABILITY

BPI: Are you receiving requests for Gendicine from outside of China?

Peng: Yes. Requests are coming from the US, Germany, Denmark, Thailand, the Philippines, Greece, Canada, the UK, Singapore, Russia, Rumania, and Turkey. Patients must come to China to be treated. So far, about 400 patients have been treated.

BPI: How much do you plan to produce in 2004?

Peng: In 2004, 200,000 to 500,000 doses. Gendicine is expected to bring relief to millions of cancer patients over the coming years. We also expect it to accelerate the commercialization of other gene therapy products and to create a significant social and economic impact.

BPI: Is Gendicine currently available?

Peng: Yes. It will be formally launched in late March 2004. Right now, it is just for HNSCC.

MANUFACTURING AND SCALE-UP

BPI: Your manufacturing facility was built in 2000. How is Gendicine produced and how was the process developed?

Peng: Our facility passed GMP approval by SFDA on February 13, 2004. We produced research quantities in roller bottles and parallel-plate reactor systems. However, roller bottles were found unsuitable for production of gene therapeutics using an adenoviral vector.

I used the Cell-Cube (parallel-plate reactor) system in the lab, but yields were too low, and we had cell growth problems. We produced approximately 1×10^{14} VP in a medium sized Cell-Cube (21,250 cm^2 surface area). When we installed a 14-L CelliGen Plus packed-bed bioreactor (from New Brunswick Scientific) in our manufacturing facility, we produced 15 times more than in the Cell-Cube system. Approximately 2×10^{15} VP can be produced in a 14-L bioreactor that is partially loaded with 200-g Fibra-Cel disks, and 1×10^{15} VP adenoviral vector in a 5-L bioreactor loaded with 100-g disks. We observed two periods of peak virus release into the supernatant — on day 3 and day 5 postinfection, respectively. The viral bursts of 40,000 to 50,000 viral particles/cell were attained on day 3 postinfection.

A complete panel of lot-release quality-control criteria for the final product has been established. Those criteria include vector purity, particle concentration, infectivity, gene expression, potency, and product safety criteria such as sterility, adventitious viruses, and level of replication-competent adenovirus (RCA). For example, our released final product has the following quality characteristics: IU/VP ratio of about 4.8%, purity over 97%, and less than 1 RCA in 3×10^{10} VP.

BPI: What is the source of cells?

Peng: Gendicine is based on an E1-deleted adenoviral vector containing the p53 tumor-suppressor gene. Engineered cells expressing adenovirus E1 proteins are required to produce Gendicine. Usually, people use the HEK 293 cell line for this purpose. We have subcloned a cell line called SBN-Cel from the 293 cell line. SBN-Cel is much better than the 293 cells for the production of Gendicine. Compared to standard 293 cells, SBN-Cel has a shorter doubling time and a more uniform, better morphology and attachment to the culture surface in the DMEM [Dulbecco's Modified Eagle Medium] media. Production of Gendicine using the SBN-Cel is carried out in NBS's CelliGen Plus perfusion bioreactor.

A production process under development will use suspension cells instead of attachment-dependent cells. We have been using the NBS CelliGen Plus bioreactor with packed-bed basket for production during the past four years. The suspension process is referred to internally as the second-generation process. We will apply to SFDA for a change to the suspension process if it results in higher productivity (Table 4).

For the production of Gendicine, the most important raw material items are the master and working cell banks and the working virus bank we have prepared. The adenoviral vectors produced from the bioreactors are processed further by clarification, ultrafiltration, and diafiltration, and they are finally purified using an automated bioprocessing system. The overall downstream-processing vector

NBS CelliGen Plus Bioreactor

Table 4. Production Apparatus Comparison

	NBS CelliGen Plus bioreactor with packed-bed basket	NBS CelliGen Plus bioreactor with pitched blade impeller
Culture medium	DMEM 10% fetal bovine serum	CD 293 serum-free medium
Cells	Adherent cells	Suspension cells
Equipment size	14 L (240,000 cm^2/200 g of disks)	14 L
Process Mode	Perfusion	Batch
Number of cells per infection	5×10^{10}	1×10^{10}
Viral yield (VP/mL)	2.1×10^{10}	2.5×10^{10}
Total viral yield (VP)	2×10^{15}	2.5×10^{14}

Note: The 14-L CelliGen Plus can hold up to 500 g of FibraCel disks. For the data presented for the packed-bed basket, only 200 g were used.

recovery rate is approximately 65%. The purified and formulated viral product is filled into sterile glass vials using an automated filling machine and stored frozen for future use.

INSIDE THE COMPANY

BPI: What can you tell us about Shenzhen SiBiono GeneTech?

Peng: Chinese scholars returning to China from the US founded SiBiono in early 1998. We are privately held and currently have more than 50 employees.

SiBiono is a leader and pioneer in gene therapy research, development, and commercialization in China. We have developed two technology platforms for the research, development, and commercialization of gene therapy: viral and nonviral gene-delivery systems. In China, we authored the first comprehensive book covering gene therapy, commissioned the first cGMP facility equipped with validated state-of-the-art equipment for the production of gene therapy products, and established a

SiBiono and New Brunswick Scientific

New Brunswick Scientific (NBS) in Edison, NJ was recently awarded a contract to scale-up the bioreactors used in SiBiono's new gene therapy production facility in Shenzhen. NBS has successfully partnered with the Chinese government and businesses since 1982, when the company was among a handful of biotech and pharmaceutical companies that traveled to China as part of a US-led trade delegation. That first trip landed NBS a major contract with the Institute of Microbiology, a division of the Chinese Academy of Science in Beijing. Today the company does a substantial part of its export business in China, supported by sales and service offices located in Beijing, Shanghai, and Shenzhen. The offices are entirely staffed by native Chinese sales and service personnel to ensure that language, culture, and geographical distance from the US are no obstacle to supporting their China-based customers.

The New Jersey headquarters offers customized training packages, lasting one day or several weeks, to provide on-going support. NBS believes that training in the operation of equipment is essential for their customers to be successful at reaching their fermentor's or bioreactor's full potential. To this end, the company maintains in-house labs, fully equipped with stirred-tank and packed-bed bioreactors from 1 to 150 L and fermentation equipment up to 500 L. The labs are also available for customer assistance in process development, media formulation, computer control, and optimization. It is here that one of SiBiono's head researchers came to train for five weeks during the summer of 1999. According to Dr. Peng, the reasons he works with NBS are simple: "The equipment provides very good yields, and New Brunswick Scientific provides very good technical support."

NBS also supports education by organizing a variety of regional conferences. In 2001, NBS and the World Health Organization brought together 60 cell-culture researchers from across China for a two-day symposium in Nanjing City. That conference presented novel techniques for high-yield, low-cost production of vaccines. The company also backs a variety of US-based technical programs on cell culture and fermentation, including hands-on workshops held throughout the year at Penn State, University of Maryland, and Utah State University.

All Photos courtesy of SiBiono

complete set of quality-control assays and production processes following international regulations and standards.

SiBiono owns five innovative patents issued by the China Intellectual Property Ministry covering production process, products, and a subcloned cell line. SiBiono is the recipient of a number of national high-tech biotechnology grants, Science and Technology Development Foundation grants, and grants from Guangdong Province and Shenzhen municipal projects.

BPI: What other challenges has SiBiono faced?

Peng: China is a huge market, and we need to increase our production capacity in order to meet the market demand. For large-scale production, SiBiono is building a new production facility in Shenzhen. Manufacturing capacity will reach approximately two million doses per year when we commission this new facility in mid-2005.

We also need to make changes in our product formulation to facilitate transition to commercial-scale production. We are consistently improving our quality system to maintain a leading position in the Chinese market. For example, we wrote a technical guide for gene therapy research, development and commercialization, titled "Points to Consider for Human Gene Therapy and Product Control", which the SFDA later adopted. This document can be found in the May issue of *BioPharm*, (p.73)

We are also building our marketing and product distribution network. Currently, there are about 15 companies participating in the distribution of Gendicine in China.

PARTNERING

BPI: What criteria do you use in selecting business partners?

Peng: SiBiono has established collaborations with a few domestic and overseas research organizations and universities during Gendicine's development period.

We are currently looking for strategic partners. Potential partners include well-known organizations, foundations, and biotech corporations. We are seeking a partner that, together with SiBiono, can jointly lead and promote worldwide gene therapy development. The partner is expected to collaborate with SiBiono, however; we are not interested in a merger partner. This partner has to be large enough to deal with China's huge market.

BPI: Did you have any other consulting help?

Peng: Dr. W. French Anderson, director of the Gene Therapy Laboratories at the University of Southern California and widely considered the father of gene therapy, serves as a free advisor to SiBiono. He plans to visit here once a year in an advisory capacity.

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