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RESEARCH ARTICLE

First Study of the Safety and Tolerability of Allvec-1, a Gene Therapy Vector, in Patients With Advanced Stage IV Malignant Solid Tumors

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Abstract

Background: Gene therapy relies on the delivery of foreign DNA into cells. More than 50% of all reported clinical trials for gene therapy are for cancer.

Objectives: To test the tolerability, safety, and recommended phase II dose of Allvec-1, a highly selective gene therapy vector, after systemic administration in patients with advanced stage IV solid tumor malignancies.

Patients and Methods: A phase I trial evaluated escalating doses of Allvec-1, administered 3 times weekly for 8 weeks in 6 patients with gastric, breast, esophageal, non-small cell lung cancer (NSCLC), and leiomyosarcoma. Clinical lab parameters, blood pressure, pulse, and patients' own-reported adverse events were used for evaluation of safety. The maximum dose was set based on the first sign of any minor side effect to be likely related to Allvec-1. Tumor imaging techniques were applied before and after Allvec-1 treatment for any tumor response. No further concomitant anti-tumor treatment was admitted during the study period.

Results: Six patients [median age, 50.5 years (range 23-66), they were heavily pretreated; received Allvec-1 starting at a dose of 1.25×10^{10} and increasing to the final dose of 2×10^{11} thrice weekly. During the study period 3 patients have received 24, one patient 21, one patient 14, and one patient 13 intravenous (I.V) injections, respectively. Treatment-related adverse events were nausea (1 out of 6) and increase of body temperature (38°C, 2 out of 6). These side effects were minor and lasted only up to 30 minutes, and disappeared after repeated dosing. The increase of body temperature occurred 24 to 48 hours after the treatments and was observed only during the second week. No other side effects were reported. All clinical lab and vital functions remained unaffected. An increase of body weight and an improvement of general condition could be observed in 4 out of 6 patients. One of these patients showed stable disease until the end of 4 weeks surveillance period. A partial response was seen in 1 out of 6 patients. Four patients died within one month after termination of the treatment due to the progressive dieses.

Conclusions: Allvec-1, as the first gene therapy vector for systemic administration, was tolerated without any side effects. Dose-limiting toxicities were not observed in this study. Therefore, higher doses can be recommended in phase II trials. Despite extensive prior treatment and final stage of all patients a partial response and stable disease could be reached during the treatment period. It could be expected that a treatment beyond 8 weeks, even in those terminally ill patients, might increase the life expectancy without any side-effects. Therefore, additional clinical trials are well warranted in defining the role of Allvec-1 in treatment of cancer.

Keywords: *Gene Therapy; Solid Tumors; Genetic Vectors*

1. Background

Gene therapy relies on the delivery of foreign DNA into cells. More than 50% of all reported clinical trials for gene therapy are for cancer. Successful systemic gene therapy has been hindered by vector-related limitations, including toxicity and inefficient gene delivery to tumor cells after intravenous administration. For detailed information one can refer to many review articles (1-5) presenting the pros and cons of those gene transporters. Gene transfer vectors can be broadly categorized into two groups: mammalian-viral and non-viral vectors. In general, viral vectors tend to provide for longer-term gene expression but often come with additional safety concerns, ranging from fears of generating replication of a competent virus during vector production, random insertion of the transgene into the genome following treatment, or development of a harmful immune response (4). Non-viral vectors are less efficient in transferring the genetic cargo through all cellular barriers; they lead often only to a transient gene expression, and after systemic application they are quickly inactivated (6-10). Understandably, due to the need for extensive modifications of non-mammalian viruses such as bacteriophages or insect viruses (baculovirus) (11-14) for systemic gene transfer, they have not generated great interest among many researchers in

this field. Therefore, there are merely a few researchers advocating the use of bacteriophages for gene therapy, and even for systemic administration, over a long period of more than a decade. Their work, however, has not progressed beyond in vitro and preclinical studies (15-18). All clinical trials so far, including all different types of vectors, have remained limited to a local administration of the vector; for example, local injection of the vector into the tumor tissue. In order to achieve any significant clinical benefit, however, it is obvious that a vector with the capability for systemic administration would be very desirable. Systemic administration of the wild type bacteriophages, also known as bacteriophage therapy for infectious diseases, has been reviewed (19, 20), and has shown very good safety records in clinical trials.

Allvec-1 is a new type of gene transporter based on a modified recombinant bacteriophage, and it is especially designed for the targeted treatment of cancer. It is the first vector of its type to have ever been used in animals and humans for systemic drug administrations. Allvec-1 can target and destroy tumor cells through apoptosis. Apoptosis is induced selectively in tumor cells by caspase-dependent and independent pathways. Allvec-1 disrupts mitochondrial functions, degrades cellular DNA, and inhibits protein degradation. The apoptotic effects of Allvec-1 can be confirmed in vitro by FACS (Fluorescence Activated Cell Sorting), DNA laddering and cell histology. In addition, Allvec-1 significantly decreases intracellular ATP. This effect supersedes those induced by cisplatin (data are not presented yet). The pharmocokinetics of Allvec-1 shows profound differences to the unmodified bacteriophages. It does not accumulate in any organ, and in contrast to a normal bacteriophage, it may not be degraded by the reticuloendothelium system. The plasma concentration of Allvec-1 after repeated dosing remains almost unchanged after 6 weeks of treatment in rats. This also represents a major difference from unmodified bacteriophages. Acute toxicological studies were performed in rats (intraperitoneal injection) and rabbits (intravenous injection). The doses in rats were 5×10^{12} /kg and 4×10^{12} /kg in rabbits. No organ changes or side effects were observed. The repeated dose toxicity in rats up to 7 weeks at a dose of 2×10^{12} /kg did not show any organ damage or any side effects. Therefore, we can claim that Allvec-1 is inert in healthy animals.

Allvec-1 has also been tested in 16 animals with spontaneous tumors, including 7 cats and dogs with breast cancer, 3 dogs with mast cell tumors, 2 dogs with lymphoblastic leukemia, 2 dogs and one cat with fibrosarcoma, 1 dog with osteosarcoma, 1 horse with equine sarcoid and 1 cat with lymphosarcoma. Some animals in this study suffered from two different types of tumor, amounting to 19 tumor cases in total. The response rates were: 5 out of 14 tumor cases (28%) were cured, 9 out of 14 showed a partial response (64%) and 1 out of 14 was likely non-responsive (8%). Allvec-1 doses ranged between 1.3×10^9 /kg and 1.6×10^{11} /kg. These doses were administered either intravenously or subcutaneously. Both routes of administration were tolerated very well. The intravenous route of administration shows a better response rate. The side effects were vomiting and shivering. These effects were related to the anti-tumor activities of Allvec-1 caused by tumor cell destruction. Therefore, the therapeutic doses should be determined based on tumor size and types and not according to body weight alone. The longest treatment period was 15 weeks and the longest observation time 12 months. These results were collected from several different general veterinary practitioners. In addition, Allvec-1 was tested in mice with transplanted human ductal adenocarcinoma of the breast. In 30% of animals the tumor growth was stopped and tumor growth was delayed in the rest of the animals.

2. Objectives

According to these preclinical safety and efficacy data it was decided to enter the first clinical trial in end-stage cancer patients which did not have any chances of clinical benefit with standard treatments.

3. Patients and Methods

3.1. Eligibility

Patients with advanced solid tumors and multiple metastases with no chances of survival with other standard treatments but with a minimum life expectancy of two months were admitted to this study. Patients with severe cardiac malfunctions were excluded from the study. All patients and a first-degree relative had to sign a consent form. This study was approved by an ethics committee and all patients were insured for potential Allvec-1 related adverse effects.

3.2. Study Treatment

In a phase I, open-label, single-center dose escalation trial, the safety, tolerability, and tumor response to Allvec-1 were evaluated. Patients received intravenous thrice weekly doses of Allvec-1 for 8 weeks starting at 1.25×10^{10} vectors and increasing to 2×10^{11} vectors per patient per dose. The maximum tolerated dose (MTD) was defined as the highest dose level at which less than 33% of subjects experienced dose-limiting toxicities (DLT) after an injection. Subjects were expected to receive treatment for at least three weeks, at which time the first evaluation for efficacy occurred.

3.3. Treatment Assessment

Safety and toxicity evaluation at baseline included a physical exam, review of systems, vital signs (Eastern Cooperative Oncology Group performance status, blood pressure, pulse rate, body temperature, and weight), 12-lead electrocardiogram, complete blood cell count with differential, hepatic (transaminases [alanine amino-

transferase (ALT) and aspartate aminotransferase (AST)]), and renal function assessment. Efficacy was evaluated by the measurements of all visible and palpable tumors by chest radiographs, computed tomography, sonography, or bone scans. The cardiac function was assessed at the beginning and if necessary for suspected side effects.

3.4. Analytic Methods

Patients were evaluable for safety if at least three low doses of study medication were received. Adverse events and laboratory tests were summarized by normal range. Cumulative dose, dose intensity, and overall dose were summarized descriptively (n, median and range). Tumor response included rates of objective response (complete and partial response) and stable disease. Objective response and stable disease rate were defined as the percentage of subjects based on the total number of response-evaluable subjects. Evaluation of efficacy was a secondary objective of this clinical phase I study. Complete response was defined as disappearance of all clinical evidence of the tumor. Partial response was defined as more than 30% decrease in the sum of the tumors diameter without an increase in any lesions or the appearance of new lesions at the end of the study period. Progressive disease was defined as an increase in lesion by more than 25% or the appearance of new lesions. The patient could achieve stable disease status if criteria for complete or partial response were not met, and progressive disease did not occur within the first 8 weeks of the study.

4. Results

4.1. Patients' Baseline Characteristics

Eight patients were screened, and 6 patients participated in this study. All patients were from the Chemotherapy and Radiotherapy Day Clinic Center of Shahid Rajahi in the city of Babolsar in Iran. Their data is summarized in Table 1. One patient with gastric cancer and post-operative metastatic invasion of the liver had received 12 cycles of epirubicin (100 mg) plus carboplatin (450 mg), and 2 cycles of paclitaxel (120 mg) prior to the treatment with Allvec-1. One patient with post-operative ovarian leiomyosarcoma had developed metastasis in the abdominal cavity, lungs and bones. The bone metastasis was severe and infiltrating the spinal canal from T2-T12, leading to paralysis. She had received 10 courses of radiation prior to the treatment with Allvec-1. One patient had dual breast (with metastasis in lymph nodes) and gastric cancer which were diagnosed simultaneously. Breast tumors were identified in both breasts and were removed a year earlier, but before the treatment with Allvec-1 a clear recurrence developed in both breasts, spreading into ipsilateral infra- and supraclavicular lymph nodes. These lymph nodes were clearly visible. No further surgical procedure was undertaken on advanced gastric cancer in this patient. She was treated with 6 cycles of paclitaxel (120 mg) prior to the treatment with Allvec-1. Another patient had NSCLC with extensive metastasis into the brain. The patient had received 2 cycles of carboplatin (450 mg) in combination with vinblastin (10 mg), and 10 sessions of radiotherapy prior to the Allvec-1 treatment. One patient with a post-operative condition of invasive esophageal cancer was admitted to this study. The patient had 10 sessions of radiotherapy prior to the Allvec-1 treatment. This patient had concomitant heart failure and diabetes mellitus. One patient had breast cancer with widespread metastasis in the lung leading to severe apnea. The patient has been under chemotherapy and radiotherapy for more than four years with progressive disease. The patient also suffered severe hypoxia prior to the treatment with Allvec-1. None of the patients received any other treatment during the Allvec-1 study period. All other treatments were stopped at least two weeks prior to the Allvec-1 treatments.

Table 1. Phase I Study of Allvec-1 in Patients With Solid Tumors: Baseline Characteristics

Variable	Value
Number of Patients	
Male	2
Female	4
Age, y [a]	50.5 (23 - 66)
Tumor Types, n	
NSCLC, metastasis in brain	1
Gastric cancer (adenocarcinoma), metastasis in liver	1
Breast cancer (squamous cell), metastasis in lung	1
Esophageal cancer	1
Breast (adenocarcinoma) + advanced gastric cancer [b]	1
Leiomyosarcoma, metastasis in bone, lung and abdominal cavity	1
Previous Treatments, n	
Cytotoxic therapy	4
Radiotherapy	4
Surgery	6

[a] Age is presented as median (range).
[b] Combined beast and gastric cancer likely independent from each other.

4.2. Drug Delivery and DLTs

The starting dose of Allvec-1 was 1.25×10^{10} and it was increased to the final dose of 2×10^{11} thrice weekly within 3-4 consecutive injections. Allvec-1 was administered intravenously in 250 mL saline as a short infusion (10 minutes). The patients were observed for 2 hours after each infusion and discharged from the clinic. There was no sign of toxicity in any patient. The lab parameters were

all unaffected by Allvec-1 treatment. Therefore, a clear DLT as shown by chemotherapy could not be observed in this study. Since this study was the first study with Allvec-1 in humans, in order to avoid any risk to the patients it was decided to stop the dose escalation at the occurrence of the first potential side effect.

4.3. Patient Disposition

All 6 patients received at least 13 injections within a time period of 28 days and were assessable for safety and toxicity. None of the patients experienced side effects related to Allvec-1, so none stopped the treatment because of side effects. One patient (NSCLC) stopped treatment due to the progression of his disease, and another patient (esophageal cancer) stopped treatment by her own choice.

4.4. Toxicity Assessment

Treatment-related adverse events were nausea (1 out of 6 patients) and increase of body temperature (38°C, 2 out of 6 patients). These side effects were minor and lasted only up to 30 minutes, and disappeared after repeated dosing. The increase of body temperature occurred 24 to 48 hours after the treatments and was observed only during the second week. We could not exactly explain these effects; however, they could be related to the antitumor effects of Allvec-1 rather than a toxic effect. Hence, the highest dose was set at 2×10^{11} thrice weekly. The 6 patients received a total of 118 injections of Allvec-1. The median duration of treatment was 51 days. A total of 45 adverse events were observed with 15 events (30%) being study drug-related. None of the patients experienced any severe side effects (Table 2). Four deaths occurred at least one month after the cessation of the treatment with Allvec-1. All four deaths were attributed to progression of the underlying disease. For all subjects, regardless of dose and treatment cycle, the side effects with the highest frequencies were increased body temperature (24%), nausea (9%), and asthenia (9%). The majority of treatment-related adverse events (64%) were reported during cycles 1 - 4 of this trial. Laboratory abnormalities were reported much less frequently and none of them were treatment-related.

Table 2. Adverse Events [a]

Dose, 1/kg	Cycle	Patient	Adverse Event
1.25×10^{10}	1	GC	Asthenia, pain in right upper abdomen, nausea
2.5×10^{10}	2	GC	Nausea, shivering
5×10^{10}	3	GC	Nausea
1×10^{11}	4	GC	Pain in right upper abdomen, depression, nausea
2×10^{11}	7	GC	Pain in right upper abdomen, increased: SGPT, SGOT, ALP
5×10^{10}	3	LMS	Asthenia, headache
2×10^{11}	8	LMS	Return of pain in legs
2×10^{11}	10	LMS	Back pain
2×10^{11}	16	LMS	Pressure in abdomen
2×10^{11}	22	LMS	Pain in lower abdomen, back pain
2.5×10^{10}	2	BC + GC	Shivering, increased body temperature, apnea
5×10^{10}	3	BC + GC	Increased body temperature, asthenia
1×10^{11}	4	BC + GC	Increased body temperature
2×10^{11}	5	BC + GC	Stomach pain, increased body temperature
2×10^{11}	7	BC + GC	Increased body temperature
2×10^{11}	10	BC + GC	Increased body temperature
1.25×10^{10}	1	NSCLC	Apnea
1×10^{11}	4	NSCLC	Haemapnea
2×10^{11}	14	NSCLC	Seizures
5×10^{10}	3	EC	Increased fasting blood glucose, asthenia, difficulty swallowing
1×10^{11}	4	EC	Stomach pain, depression
1.25×10^{10}	1	BC	Increased body temperature, apnea
2.5×10^{10}	2	BC	Increased body temperature, apnea
1×10^{11}	4	BC	Increased body temperature
2×10^{11}	5	BC	Increased body temperature
2×10^{11}	8	BC	Increased body temperature

[a] Abbreviations: GC, gastric cancer; LMS, leiomyosarcoma; BC, breast cancer; NSCLC, non-small cell lung cancer; EC, esophageal cancer.

Table 3. Patients General Information

Total Treatment Cycles	Patient	Response	Body Weight, kg		Survival in Weeks
			Before	After	
21	GC	PD	62.5	57	11
24	LMS	PD	86	86	11
24	BC+GC	PR	46	47	52
22	BC	SD	64	62.5	12
14	NSCLC	PD	43	44.5	7.5
13	OC	PD	63.5	64.5	6

[a] Abbreviations: PD, progressive disease; SD, stable disease; PR, partial response.

4.5. Efficacy Results

All 6 patients were evaluable for tumor response and no patient was withdrawn from the study due to toxicity (Table 3). Among these patients one partial response and one stable disease were observed after 24 and 22 cycles respectively. Four other patients had progressive disease. Although these patients showed towards the end of their treatment progressive disease, there was some improvement in their general clinical conditions after the first few cycles, such as increase in body weight. This improvement, however, did not last. The patient with leiomyosarcoma was paralyzed prior to the treatment with Allvec-1 due to the tumor invasion into T2-T12. After 8 cycles the patient could again move her legs and sensation returned in her lower abdomen down to her toes. Furthermore, she regained control of her bladder. At the end of the 24 cycles (2 months), however, an increase of tumor mass in the abdomen could be observed by CT. The patient died four weeks after the end of the treatment. A patient with progressive gastric cancer and liver metastasis showed improvement in his general clinical conditions with an increase of body weight after the first 7 cycles. His condition, however, deteriorated after 15 cycles. He died four weeks after the end of the treatment. A patient with esophageal cancer was treated with 13 cycles of Allvec-1. During this period an increase in body weight was recorded. This patient stopped the treatment by her own choice and died four weeks later. A patient in the final stage of NSCLC with massive brain metastasis was treated with 14 cycles of Allvec-1. During this period an improvement in general clinical condition and an increase in body weight were recorded. Due to the complication of brain metastasis, however, the treatment was interrupted. The patient succumbed to the underlying disease and suffered a brain hemorrhage three weeks after the end of the treatment with Allvec-1. One patient with advanced breast cancer and metastasis in the lungs showed stable disease during the course of 22 cycles of treatment with Allvec-1. The patient's body weight was stable and the original apnea caused by the underlying tumor infiltration in the lungs seemed to improve. During the four weeks post-treatment there was no change in her condition. Another patient with combined gastric and breast cancer received 24 cycles of Allvec-1. At the beginning of the treatment the patient was suffering from apnea, and there was visible ipsilateral infra- and supraclavicular infiltration of the lymph nodes. After about 10 cycles the lymph nodes reduced in size and they became normal towards the end of the treatment. The patient has survived the four weeks surveillance period without reappearance of clavicular lymph nodes. Her general condition improved, including the apnea, and she even experienced a slight weight gain (partial response). This patient survived for a year after the last treatment with Allvec-1. During this period she received no other treatment.

5. Discussion

Gene therapy has received several setbacks during recent years due to negative publicity caused by adverse events. Some of these adverse events have culminated to death of the patients. These tragic events can be attributed to the use of mammalian-viral vectors (21-23). To our knowledge this is the first study using recombinant bacteriophage for the treatment of cancer after intravenous injections in humans. The fact that the only "stage" past "end stage" is usually death, and the fact that all subjects admitted to this study were absolutely end stage patients with no other viable therapeutic measure available for them, had set the standard for proof of safety at a very high level right from the beginning. Nonetheless, this study has shown that Allvec-1 is safe and well tolerated at doses up to 2×10^{11} thrice weekly in 24 cycles. No sign of toxicity was observed at any time during the treatment period up to 8 weeks, or during the post-treatment surveillance period of 4 weeks. Although higher doses would have been tolerated due to the terminal stage of the diseases, no such risk was taken. Therefore, the maximum dose was set at the appearance of the first adverse event related to the treatment. These adverse events were mild increases in body temperature or nausea. Those events occurred 24 to 48 hours after the treatment and could be related to apoptosis process induced by the expression of pro-apoptotic genes of Allvec-1 in the tumor tissue. The safety profile of Allvec-1 in this study after systemic application, compared to reported

safety and tolerability of other vectors after local or systemic administrations puts Allvec-1 in a leading position. Based on these preliminary studies with highly variable patient populations, there seems to be some limited indication of efficacy in a phase I setting. Although clinical antitumor effects were not a primary endpoint, one patient had stable disease and one partial response was observed. This patient could survive a year longer after the Allvec-1 treatment without any other therapeutic measures. Even those patients who ultimately succumbed to their underlying disease showed some improvement in their general condition during the Allvec-1 treatment. This limited response rate observed in the current study is very comparable with that seen for several other anti-cancer drugs in phase I setting, but without having any sign of toxicity. This would make Allvec-1 an ideal candidate for many combination treatments with other chemotherapeutic agents. It remains to be elucidated whether certain tumor types are more susceptible.

In general, we can expect that the clinical benefit of any antitumor activities will be more pronounced in the early stages of disease rather than in the terminal stage. In addition, immune stimulation through cell killing can also enhance local tumor killing (24) and help to generate systemic immunity to other tumor deposits (25). Rigorous testing of the hypothesis, however, requires adequate selection of patients and design of future studies. Therefore, additional clinical trials are warranted in further defining the role of Allvec-1 in the treatment of cancer.

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