

# Phase II Trial of Intravenous CI-1042 in Patients With Metastatic Colorectal Cancer

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**Purpose:** To evaluate the antitumor activity, safety, immune response, and replication of CI-1042 (ONYX-015), an E1B 55-kd gene-deleted replication-selective adenovirus, administered intravenously to patients with metastatic colorectal cancer

**Patients and Methods:** Eighteen patients with metastatic colorectal cancer for whom prior chemotherapy failed were enrolled onto an open-label, multicenter, phase II study. CI-1042 was administered intravenously at a dose of  $2 \times 10^{12}$  viral particles every 2 weeks. Patients were evaluated for tumor response and toxicity; in addition, blood samples were taken for adenovirus DNA and neutralizing antibody analysis.

**Results:** Common toxicities included flu-like symptoms, nausea, and emesis. All 18 patients eventually were

removed from study because of progressive disease. Seven patients were assessed as having stable disease after 2 months of treatment, whereas two patients were considered to have stable disease after 4 months. Detectable circulating CI-1042 DNA was identified in 36% of patients 72 hours after last infusion, which is suggestive of ongoing viral replication.

**Conclusion:** In this phase II study, intravenous CI-1042 was administered safely to patients with advanced colorectal cancer. Toxicity was manageable, consisting primarily of flu-like symptoms. Stable disease was experienced by seven patients for 11 to 18 weeks.

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DESPITE RECENT advances made in the treatment of colorectal cancer, more than half of colorectal cancer patients die of metastatic disease, including the approximately 25% of patients with evidence of metastasis at diagnosis.<sup>1</sup> The discovery and development of anticancer therapies with novel mechanisms of action and their subsequent incorporation into combination regimens should improve outcome in this malignancy. CI-1042 (ONYX-015), a genetically modified adenovirus, represents one such novel agent.

Adenoviruses are DNA viruses that are able to infect dividing or nondividing human cells and cause direct cytotoxicity during replication. The replication of adenoviruses occurs in part by dysregulation of the cell cycle control machinery through the action of the E1A and E1B region gene products of the virus. The E1B region of adenovirus expresses early gene products that bind to and inhibit the function of the tumor suppressor *p53*.<sup>2</sup> Mutation of the *p53* gene occurs in approximately 50% of all human cancers, and *p53* is mutated or lost in approximately 65% of colorectal carcinomas.<sup>3</sup>

CI-1042 is an attenuated chimeric human group C adenovirus (serotypes 2 and 5) that efficiently replicates in and lyses tumor cells deficient in *p53* tumor suppressor activity (*p53*-), such as malignant cells, while theoretically sparing cells with normal *p53* function (*p53*+).<sup>4-6</sup> The replication selectivity of CI-1042 is conferred through deletion of the part of the E1B region of adenovirus that encodes for a 55-kd protein, the functions of which include binding to and inactivating *p53* in infected cells.<sup>7</sup> Administration of CI-1042 is predicted to result in a localized active infection leading to lysis of the infected tumor cells.<sup>2</sup> Although this infection is expected to spread within the *p53*- tumor cell population, its effects on *p53*+ normal cells should be limited by the poor replication potential of the virus in these cells.<sup>8,9</sup>

Phase I and II studies have investigated the toxicity of intravenous (IV) and hepatic artery administration of CI-1042 in advanced solid tumor and liver-predominant gastrointestinal carcinoma. Mild transient fever and dose-related elevations in serum AST and ALT (grade 1/2) were the most common adverse events.<sup>10-12</sup> Viral doses up to  $10^{12}$  plaque-forming units (pfu) were given in various dosing schedules and combinations. No dose-limiting toxicity or maximum-tolerated dose was identified. There was no clinical evidence of treatment-emergent hepatotoxicity or clotting abnormalities. Neutralizing antibodies increased in all patients. Viral replication was observed in patients receiving a dose of at least  $10^{11}$  pfu ( $2 \times 10^{12}$  viral particles). Circulating viral genome was noted after day 7 in one of 10 samples at a dose of  $\leq 3 \times 10^9$  pfu. Biopsy results obtained from metastatic lung tumors showed selective replication in tumor tissue. In addition, radiographic and histologic evidence of acute necrosis was seen in some patients who received hepatic arterial administration at high dose ( $2 \times 10^{12}$ ) after ONYX-015 alone.

We selected to initiate a phase II, multicenter, open-label, noncomparative study in the United States investigating the antitumor activity and safety of IV CI-1042 as a single agent in

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patients with metastatic colorectal cancer for whom at least one prior chemotherapy regimen had failed.

## PATIENTS AND METHODS

### Study Population

Patients were required to have histologically or cytologically metastatic colorectal cancer, to have experienced treatment failure from at least one chemotherapy regimen, and to have measurable lesions (by computed tomography [CT] scan) of at least 2 cm. Further eligibility requirements were age  $\geq 18$  years; Karnofsky performance status of  $\geq 60$  and estimated life expectancy of  $\geq 3$  months; adequate bone marrow, renal, and hepatic function; and a time lapse of 4 weeks from prior therapy. Patients were excluded if they had any of the following: metastases involving more than 25% of the lung volume or a pulmonary lesion greater than 10 cm;  $\geq 50\%$  of the liver replaced by tumor; ascites requiring paracentesis; positive serology for hepatitis B or C; previous chemotherapy, radiation, or surgery to the metastatic site within 4 weeks of baseline; prior participation in a research protocol involving an adenovirus vector; active systemic infection or history of human immunodeficiency virus; or a diagnosis of another malignancy within 5 years of study enrollment (except adequately treated carcinoma-in-situ of the cervix or nonmelanoma skin cancer). Pregnant or lactating women were also excluded. The protocol was approved by the institutional review boards at Wayne State University, Detroit, MI; Montefiore Medical Center, Bronx, NY; University of California at Los Angeles, Los Angeles, CA; Northwestern University, Chicago, IL; and The Mayo Clinic, Rochester, MN. Informed consent was obtained from all patients.

Pretreatment evaluation included a history and physical examination, complete blood cell count, differential and platelet count, serum chemistries and coagulation profile, carcinoembryonic antigen (CEA) analysis, abdominal CT scan, upper-body positron emission tomography (PET) scan, ECG, and chest x-ray. Follow-up studies included physical examinations, complete blood cell counts, differential and platelet counts, serum chemistries, CEA analyses, and imaging procedures (every 8 weeks). In addition, viral titers, cytokines, and neutralizing antibody titers to CI-1042 were evaluated at baseline and at specified time points throughout the study.

CT scan was the primary parameter used to guide patient therapy and progress. PET imaging using 18-fluoro-deoxyglucose was used as a secondary method of lesion assessment in patients in which response may have been visible on the basis of metabolic changes but could not be seen with CT.

### Treatment Schedule

CI-1042 ( $2 \times 10^{12}$  viral particles) was administered IV on days 1 and 15 of each 28-day cycle for a planned six cycles. The drug was supplied frozen ( $-20^{\circ}\text{C}$ ) as  $1 \times 10^{12}$  viral particles/mL in single-use vials. After the drug was thawed, 2 mL of CI-1042 was mixed in 50 mL of normal saline (0.9%) and administered as an IV infusion over 5 to 10 minutes. Preparation was done in biologic safety cabinets, and all wastes were disposed in biohazard containers as defined in each institution's policies.

### Response and Toxicity Criteria

Response was evaluated by CT imaging after every two cycles. A complete response was defined as complete disappearance of all measurable and nonmeasurable but assessable disease, no new lesions, no disease-related symptoms, and no evidence of nonassessable disease maintained for at least 4 weeks. A partial response was defined as a decrease of  $\geq 50\%$  in the product of the perpendicular diameters of all measurable lesions maintained for at least 4 weeks, with no new lesions and no progression of assessable disease. Progression of disease was defined as an increase of  $\geq 25\%$  in the sum of the products of the perpendicular diameters of all measured lesions, appearance of new lesions, or worsening of any assessable disease. Patients with stable disease are those not meeting the criteria for complete response, partial response, or progression of disease. Toxicity was graded on a scale of 0 to 4 by the National Cancer Institute common toxicity criteria (version 2.0).

### Statistical Methods

A patient was considered assessable for response if he or she completed at least two full cycles of CI-1042 and had a response assessment. A Simon

two-stage design<sup>13</sup> was used such that 21 assessable patients would be treated in the first stage of enrollment and advancement to the second stage (an additional 20 assessable patients) would not proceed unless two or more responses were documented in the initial stage.

Because difficulties in manufacturing of this complex biologic agent led to drug shortage and no clear-cut evidence of efficacy was observed in the initial patients, enrollment was prematurely halted at 18 patients. All patients who had received at least one dose of treatment were evaluated for toxicity.

### Methods for Correlative Studies

*Detection of CI-1042 adenovirus in plasma and autopsy tissue by polymerase chain reaction (PCR) assay.* A TaqMan PCR assay (Applied Biosciences, Inc [ABI], Foster City, CA) was used to detect and quantify CI-1042 DNA in plasma and tissues samples. All samples were analyzed independently by Althea Technologies (San Diego, CA). The assay detected a 120-nucleotide sequence unique to the CI-1042 virus. Total DNA was extracted from plasma by adding 3  $\mu\text{g}$  of carrier DNA to 500  $\mu\text{L}$  of plasma and isolating total DNA using the QiaAmp Blood Kit (Qiagen, Alameda, CA).

PCR amplification was performed using the following primers and probe: GGATAATTGCGCTAATGAGCTT 5' primer, GGTGCGGGTCTCATCGTAC 3' primer; FAM-TCGACGGATCTGGAAGGTGCTGA-TAMRA probe. DNAs were amplified using 10  $\mu\text{mol/L}$  of each primer and the probe for 45 cycles of  $60^{\circ}\text{C}$  for 1 minute and  $95^{\circ}\text{C}$  for 15 seconds in an ABI7700 DNA sequencer (Perkin-Elmer, ABI, Foster City, CA). Three replicate PCR reactions were performed on each test article DNA using the oligonucleotide primers and fluorescent probe, reaction conditions, and cycling conditions. One of the three replicate reactions was spiked with 100 viral particles of CI-1042 to check for the presence of PCR inhibitors. In addition to the test article reactions, each PCR run contained one set of standards, the extraction controls of which corresponded to the test articles on the plate, and PCR reagent control. The limit of detection for the assay was 500 virus particles/mL as determined by use of a control virus that had been quantitated by electron microscopy. The limit of quantitation was 1,000 virus particles/mL.

*Detection of CI-1042 adenovirus in autopsy tissue by immunohistology (IH).* CI-1042 was analyzed in one patient by IH on 11 autopsy tissue samples, including two from the tumor. Sections of frozen tissue were fixed in acetone and analyzed by indirect immunofluorescence with murine ascites monoclonal antibodies to the adenovirus hexon protein (Chemicon International, Temecula, CA) diluted 1:400 in Tris-buffered saline with 1% bovine serum albumin and 0.03% Tween 20 (Dako, Carpinteria, CA). This was followed with fluorescein isothiocyanate-conjugated goat antibody to mouse immunoglobulin (Biosource/Tago, Camarillo, CA).

*Detection of neutralizing antibody titers.* Neutralizing antibodies to CI-1042 were detected by their ability to inhibit the cytopathic effect of adenovirus on cells. Analysis was done independently by ViroMed Laboratories (Minnetonka, MN). Human 293 cells, which are permissive for replication of CI-1042, were grown at  $37^{\circ}\text{C}$  in Eagle minimal essential medium. Five to 7 days before determining antibody titers, 293 cells were seeded in 96-well microculture plates. Frozen sera from patients were quickly thawed and an aliquot was transferred in a sterile tube and heat inactivated. Serial four-fold dilutions of the heat-inactivated serum were prepared in culture medium, and CI-1042 was added to the serum dilutions and incubated at room temperature for 1 hour. Medium was removed from the cells in the microtiter plates and immediately replaced with the virus and serum dilutions. Plates were quantitated using a spectrophotometric plate reader at 450 nm. The optical density of the culture media control wells (no cells) was subtracted from each well, and the percentage of viral neutralization was calculated. The neutralizing titer was determined by identifying the serum dilution that corresponded to a 60% reduction in cell viability caused by virus infection.

## RESULTS

### Patients

Eighteen patients were enrolled onto this clinical trial (and received at least one dose of study drug) from October 2000 to March 2001. Patients were primarily male ( $n = 13$ ; 72%), with median age of 67 years (range, 41 to 74 years). Additional

**Table 1. Patient Demographics**

Patient Characteristic	No. of Patients	%
No. of patients enrolled	18	
Age, years		
Median	67	
Range	41 to 74	
Sex		
Male	13	72
Female	5	28
KPS		
90 to 100	12	67
70 to 80	6	33
Previous therapy		
Surgery, metastatic site	2	11
Chemotherapy		
One to two regimens	4	22
Three to five regimens	14	78
Radiotherapy	4	22
No. of organs involved		
One	5	28
Two	9	50
Three	4	22
Organs involved		
Liver	16	89
Lung	9	50
Multiple	13	72
CEA level		
< 50 ng/mL	4	22
> 50 ng/mL	13	72
Unknown	1	

Abbreviation: KPS, karnofsky performance status; CEA, carcinoembryonic antigen.

demographic and disease characteristics at baseline are listed in Table 1. All 18 patients had been treated with a fluorouracil (FU)-based chemotherapy regimen and more than three fourths had been treated with at least three prior chemotherapy regimens (14 of 18 patients; 78%). Other prior chemotherapy regimens used include irinotecan (16 [89%] of 18 patients) and oxaliplatin (12 [67%] of 18 patients; not listed in table).

### Response

A total of 93 doses of infusions were administered (Table 2) to the 18 patients. The median number of cycles per patient was 2.0, and the range was 1.5 to 4.5 cycles. Treatment was interrupted in one patient before the second dose because of a 2-day history of flu-like symptoms. The patient initially had some flu-like symptoms after the first dose of CI-1042, but like other patients, these resolved within 48 hours. Flu-like symptoms then returned just before the second dose, but this was thought by the treating physician to be unrelated to drug but

**Table 2. Treatment Parameters**

	No. of Cycles per Patient
Median	2
Range	1.5 to 4.5
Total no. of infusions	93
No. of days on study per patient	
Median	42.5
Range	27 to 118

**Table 3. Toxicity Occurring in More Than 10% of Patients and Possibly Related to Study Treatment**

Associated Toxicity (n = 18)	Grade			Total		No. of Events Requiring Hospitalization
	1	2	3	No.	%	
Chills	9	6		15	83	2
Fatigue/lethargy	4	10	1	15	83	
Fever	4	9	1	14	78	3
Diarrhea	12		1	13	72	
Nausea	8	2		10	56	
Emesis	3	4		7	39	1
Rigors	1	5		6	33	
Loss of appetite	5	1		6	33	
Dyspnea	3			3	17	
Headache	2			2	11	
Constipation	2			2	11	

attributed to the common cold. One patient was not assessed because of death before scheduled response assessment.

All 18 patients who received CI-1042 on this study eventually experienced disease progression. Seven patients were assessed as having stable disease after 2 months. Two patients received more than four cycles of CI-1042 before disease progression. The median time of stable disease was 16 weeks (range, 11 to 18 weeks). Three patients did have minor reduction of CEA (7.0%, 7.5%, and 15%, respectively) after two cycles, whereas one patient experienced a decrease in CEA from 59.2 to 37.7 (36%) after four cycles. Of the 18 patients who received drug, 17 developed progressive disease on the basis of either radiologic or clinical finding, and one patient died from tumor progression while receiving treatment. PET imaging confirmed CT findings on all patients; no unexpected findings were noted. In addition, there was no obvious PET response in the stable disease patients.

### Toxicity

Toxicities that occurred in more than 10% of patients are presented in Table 3. Among the 18 treated patients, none withdrew from the study as a result of toxicity, and common toxicity criteria grade 4 was not reported. One patient died of sepsis secondary to ischemic bowel disease and gastrointestinal bleeding after receiving three doses of virus; this was most likely related to the patient's cancer under study.

As expected with adenovirus, flu-like symptoms were the most frequent adverse event. Chills and fatigue or lethargy occurred in 83% of patients, whereas fever occurred in 78% of patients. Although most of these events were considered grade 1 or 2, one patient did develop a grade 3 fever that required hospitalization. Symptoms generally started within the first hours of dose and patients subsequently recovered within 48 hours. Patients who received antihistamines, IV corticosteroids, antipyretics, or any combination of these drugs before dosing were less likely to develop flu-like reactions. There were no reports of patients who developed pneumonia during the study.

Two serious adverse events occurred during treatment that were considered to be related to the study medication. One patient was hospitalized for severe lethargy and recovered without interruption in dosing. Another patient with normal coagulation levels at baseline and no significant past medical

**Table 4. Kinetics of Virus Plasma Levels and Neutralizing Antibody Titers in CI-1042-Treated Patients**

Patient No.	Dose	Neutralizing Antibody Titer	Virus Particles/mL Hours Postdose						
			0	1	3	6	24	48	72
1	1	1:241	BLD	4.20E + 05	NA	2.08E + 03	BLD	BLD	BLD
	2	1:99,496	4.24E + 04	3.28E + 05	9.47E + 03	1.06E + 03	BLD	BLD	BLD
	3	1:73,516	5.43E + 04	4.90E + 05	9.59E + 03	3.22E + 03	BLD	BLD	BLD
2	1	1:557	BLD	7.70E + 05	5.50E + 03	3.30E + 03	BLD	BLD	BLD
	2	1:275,531	BLD	2.93E + 05	4.30E + 03	1.29E + 03	BLD	BLD	NA
3	3	>1:1,310,720	1.88E + 03	1.36E + 05	4.64E + 03	BLD	BLD	BLD	BLD
	1	1:6	BLD	1.37E + 06	2.70E + 04	7.87E + 03	BLD	2.27E + 04	4.48E + 03
4	2	1:874	1.06E + 03	2.12E + 06	1.88E + 04	5.13E + 03	BLD	BLD	NA
	1	1:1,323	BLD	6.85E + 05	1.24E + 04	2.35E + 03	BLD	BLD	BLD
5	2	1:613,319	BLD	5.38E + 05	4.29E + 04	5.76E + 03	BLD	BLD	BLD
	3	1:188,299	BLD	4.83E + 05	8.15E + 03	1.72E + 03	BLD	BLD	BLD
	1	1:60	BLD	1.66E + 06	2.22E + 04	1.77E + 03	BLD	BLD	BLD
6	2	1:43,175	BLD	8.92E + 05	4.88E + 03	2.98E + 03	BLD	BLD	BLD
	3	1:12,457	BLD	9.84E + 05	3.94E + 03	1.64E + 03	BLD	BLD	BLD
	1	<1:5	BLD	1.87E + 05	1.17E + 04	9.07E + 03	658*	4.01E + 04	6.39E + 03
7	2	1:5,997	287*	9.73E + 04	1.12E + 04	4.07E + 03	NA	606*	BLD
	3	1:21,574	2.26E + 05	7.34E + 04	1.29E + 04	6.71E + 03	BLD	BLD	BLD
	1	1:8,500	BLD	2.98E + 05	7.87E + 03	2.45E + 03	BLD	BLD	BLD
8	2	>1:1,310,720	9.44E + 05	1.86E + 05	1.61E + 04	2.43E + 03	BLD	BLD	BLD
	1	1:9	BLD	1.34E + 06	2.93E + 03	2.56E + 03	9.84E + 03	7.63E + 04	3.53E + 04
9	2	1:1,902	BLD	1.33E + 05	2.08E + 04	4.34E + 03	BLD	BLD	BLD
	1	1:5,0	BLD	NA	3.54E + 07	2.80E + 05	6.01E + 05	9.74E + 05	3.27E + 05
	2	1:773	BLD	7.16E + 07	4.80E + 05	5.24E + 06	1.07E + 06	NA	BLD
10	3	1:14,322	BLD	2.05E + 07	1.93E + 06	4.13E + 03	BLD	BLD	BLD
	1	1:1,458	BLD	1.22E + 09	2.19E + 08	1.92E + 06	1.03E + 06	BLD	BLD
	2	1:267,600	BLD	1.13E + 08	1.90E + 05	3.78E + 05	BLD	3.07E + 03	BLD
11	3	1:778,117	BLD	NS	2.01E + 04	7.23E + 03	BLD	8.72E + 03	BLD
	1	1:1,384	BLD	2.99E + 07	6.90E + 05	3.94E + 05	3.15E + 06	3.77E + 05	3.18E + 05
12	2	1:975,304	4.37E + 05	5.28E + 08	2.36E + 06	5.98E + 05	1.23E + 06	NA	NA
	1	1:9	NA	NA	NA	NA	NA	NA	NA
	2	1:51,821	NA	NA	NA	NA	NA	NA	NA
13	3	1:9,482	NA	NA	NA	NA	NA	NA	NA
	1	1:4,134	BLD	9.52E + 06	3.59E + 04	1.82E + 04	BLD	BLD	BLD
	2	1:115,133	BLD	9.21E + 06	9.00E + 04	1.03E + 04	BLD	BLD	BLD
14	3	1:260,719	BLD	7.39E + 05	1.45E + 05	3.44E + 03	BLD	BLD	BLD
	1	1:531	BLD	1.44E + 06	4.22E + 03	BLD	BLD	BLD	BLD
	2	1:1,254,120	BLD	1.89E + 06	1.61E + 04	950*	899*	BLD*	BLD
15	1	1:19	BLD	1.43E + 05	1.28E + 04	BLD	BLD	BLD	BLD
	2	1:18,228	NA	NA	NA	NA	NA	NA	NA
	3	1:18,248	NA	NA	NA	NA	NA	NA	NA
	4	1:11,573	NA	NA	NA	NA	NA	NA	NA

NOTE. Serum samples obtained from each patient immediately before dosing with virus were quantitated for neutralizing antibodies to CI-1042. Plasma samples were obtained at the indicated times postdose and the virus particles/mL (viral DNA molecules/mL) were determined by quantitative polymerase chain reaction.

Abbreviations: BLD, below limit of detection (500/mL); NA, not available; E, exponent.

\*Below limit of quantitation (1,000/mL).

history other than the cancer under study developed asymptomatic increase in partial thromboplastin time (125.8 seconds) during cycle 2, which required dose interruption. The patient was hospitalized for observation and complete work-up. The patient was diagnosed as having lupus inhibitor, and subsequent partial thromboplastin time tests normalized during dose interruption; however, the patient never resumed treatment and was eventually withdrawn from the study because of disease progression.

Other grade 1 or 2 associated adverse events include diarrhea (72%), nausea (56%), emesis (39%), and rigors (33%). Other grade 3 toxicities include one case of diarrhea in a patient who responded well to antidiarrhea medication and was able to continue receiving treatment.

There were no reports of drug-related increase in serum creatinine or liver ALT or AST during treatment; however, transient, mild to moderate increases in alkaline phosphatase and lactic dehydrogenase levels occasionally were noted.

#### *Circulating CI-1042 (Viral Kinetics)*

Fourteen patients were tested for circulating CI-1042. PCR analysis was used as previously described. Detectable levels of CI-1042 were noted in all patients 6 hours posttreatment, and seven patients (50%) had detectable levels at  $\geq 24$  hours (Table 4). Five patients (36%) had detectable levels at 72 hours. Two patients who had detectable virus at 72 hours achieved stable disease through cycle 4. Otherwise, no correlation was noted between detectable virus and response.

Table 5. CI-1042 Viral Distribution in Various Tissue Samples From an Autopsy Patient

Organ	Fluorescence Staining Results			Viral DNA Molecules of CI-1042/ $\mu$ g DNA
	Result (+/-)	Positive Cell Numbers	Fluorescence Intensity (1 to 4)	
Brain	-	—	—	LLD
Heart	-	—	—	138
Lung	-	—	—	352
Liver	+	Small	2	8994
Spleen	+	Moderate	4 (some less)	16878
Pancreas	+	Many (approximately 70% of acinar)	2	LLD
Kidney	+	Small	3	LLD
Small intestine, nonnecrotic	+	Small	3	47
Small intestine, necrotic	+	Small	3	248
Tumor, mesentery	+	Rare	3	91
Liver tumor	+	Rare	2	412

Abbreviation: LLD, lower limit of detection.

### Neutralizing Antibody Titers

Of the 15 patients who were analyzed for neutralizing antibodies, six were considered as having low ( $\leq 1:20$ ) neutralizing antibody titers at baseline (Table 4). In general, the rate and magnitude of changes in titer varied considerably among patients. Titers of specimens collected at approximately 2 weeks after the initial specimens ranged from 28- to 5,758-fold higher than the titers of the initial specimens. Patients with low initial titers ( $\leq 1:20$ ) seemed to generate lower subsequent titers (peak titers of  $< 1:45,000$ ). In addition, those patients with lower initial titers also had higher circulating adenovirus at 24 and 48 hours, indicating greater virus bioavailability.

The time to peak titer ranged from 2 weeks to 2 months, and titers remained substantially elevated through the last sampling point in each patient. There was no correlation of baseline titer levels to time to progression or tumor response or the severity of flu-like symptoms.

### Autopsy Results

Autopsy results were obtained from a female patient (patient 3 in Table 4) who died 56 hours after receiving her third dose of virus. The patient had developed increased abdominal pain and intermittent vomiting over the course of the previous cycle. Two days after her third dose of treatment, she presented with bloody emesis, hypotension, and polymicrobial sepsis and died. At autopsy she was found to have tumor at the root of the mesentery that obstructed the small bowel and produced areas of bowel infarction. The patient's death was attributed to disease progression, but one cannot entirely rule out a contribution from the therapy. PCR and fluorescence immunohistology assays were conducted on 11 tissue samples from this autopsy (Table 5), two of which were tumor specimens. Both analyses clearly indicate that the highest amount of virus was found in the spleen and normal liver cells. Viral distribution in liver tumor cells was clearly modest compared with that of normal liver cells. Even less viral antigen was noted in the tumor mesentery and tumor collected from the small intestine.

### DISCUSSION

Chemotherapy is the mainstay of treatment in metastatic colorectal cancer but is limited by the eventual resistance of

tumor cells and the relative toxicity of these agents. Viral agents may be an alternative agent to target tumor-specific cells while sparing normal cells. In this phase II study, we demonstrated that IV delivery of an E1B 55-kd gene-deleted adenovirus (CI-1042) can be administered to patients with metastatic colorectal cancer safely and feasibly. Mild to moderate fever, rigors, chills, and emesis were the most common toxicities.

Proof of viral replication was demonstrated after IV administration. Half of the plasma samples showed persistence of viral genome in blood more than 24 hours after administration, and five (36%) of 14 patients showed persistence of viral genome in blood at 72 hours. Of note, the levels of viral genome in plasma samples in three patients 48 hours after infusion were higher than levels at 6 hours, suggesting that ongoing replication is occurring.

The organ distribution of virus found in the patient who died is clearly of interest, because we are unaware that such data have been previously reported after human administration of genetically modified, replicating adenovirus. Although death was apparently caused by tumor production of bowel ischemia, we were able to measure the presence of the virus in this patient who died 56 hours after treatment with CI-1042. Tissue virus was assayed by PCR measurement of the viral DNA levels and by the immunohistologic demonstration of adenovirus antigen. CI-1042 contains portions of adenovirus serotypes 2 and 5, both of which are part of subgroup C. In children, these serotypes have been associated with respiratory infections and intussusception. Serotypes 2 and 5 are also frequently found in the stool, as well as the tonsils. Despite the pattern seen in patients infected with unmodified virus, we found little virus in the lung or small intestine in this single patient treated with CI-1042. Both PCR and immunostaining showed the highest levels of virus in the spleen. Splenic involvement, associated with widespread dissemination to other organs including the liver, has been reported for serotype 5 infection in marrow transplant recipients.<sup>14</sup> In immunocompromised patients, adenovirus 2 and 5 have been associated with hepatitis. The next highest level of viral DNA was found in the liver. A small number of positive liver cells were also demonstrated by immunofluorescence histology. The rapid postmortem autolysis that is characteristic of liver tissue may explain the presence of only small numbers of antigen-positive cells. Low

levels of virus were noted with both techniques in tumors obtained from both the liver and mesentery. The lack of efficacy of CI-1042 may be related to the limited tumor retention and replication as demonstrated in this patient.

Although evidence of antitumor activity could not be demonstrated in this trial, experience in previous clinical trials in various tumor types using various dose delivery routes have shown encouraging results. In the metastatic colorectal cancer trial by Reid et al,<sup>10</sup> hepatic arterial infusion of the virus in combination with IV FU and leucovorin (LV) demonstrated antitumor activity. One patient with FU-refractory carcinoma had a partial response after combination therapy with ONYX-015, FU, and LV. In addition, two patients who received  $2 \times 10^{12}$  viral particles had stable disease, with the combination lasting from 7 to 17 months.

The largest experience with the virus involved trials in patients with squamous carcinomas of the head and neck (HNSCC). In a phase II trial reported by Khuri et al,<sup>15</sup> the virus was administered by intratumoral injection at a dose of  $1 \times 10^{10}$  pfu in combination with cisplatin and FU in recurrent HNSCC patients. Tumor shrinkage was reported in 25 of 30 patients, with 19 patients having a decrease of  $\geq 50\%$  and eight patients having a complete response. In a subsequent phase II study by Nemunaitis et al,<sup>16</sup> the virus was administered at a dose of  $2 \times 10^{11}$  particles by intratumoral injection in patients with recurrent HNSCC. Patients were either given virus daily for 5 days (standard regimen) or twice daily for 2 consecutive weeks (hyperfractionated regimen). Objective responses were observed in 14% (three patients) of patients who received standard therapy and 41% (12 patients) of patients who received the hyperfractionated regimen. Interestingly, antitumor activity was significantly correlated with presence of *p53* gene mutations.

IV administration of ONYX-015 was conducted in 10 patients with advanced lung cancer.<sup>11</sup> Doses of  $2 \times 10^{10}$  to  $2 \times 10^{13}$  particles were administered weekly in a 21-day cycle. No dose-limiting toxicity was reported. Eight patients were considered to have stable disease at 2 months, and there was one mixed response. No objective response was reported. Other reported trials with the virus include a phase I trial of intraperitoneal administration of ONYX-015 in patients with platinum-resistant ovarian carcinoma<sup>17</sup> and a phase I trial of local administration of ONYX-015 into unresectable pancreatic tumors.<sup>12</sup> Although no objective response was reported in these trials, stable disease was reported in four ovarian cancer patients at 2 months, and minor responses were demonstrated in six patients in the pancreatic tumor trial.

Given the safety and toxicity profile of IV CI-1042 in metastatic colorectal cancer, the goal of future trials clearly is to optimize efficacy. Several strategies should be considered that could improve efficacy. One is to increase the IV dose beyond the  $2 \times 10^{12}$  viral particles administered in this trial. A true maximum-tolerated dose in the phase I trials was never reached. The maximum reported dose of virus administered systemically was  $2 \times 10^{13}$ . At doses greater than  $2 \times 10^{12}$ , mild to moderate fever, rigors, and mild transient transaminitis were the most common toxicities. Clearly, increasing the dose beyond  $2 \times 10^{13}$  particles may be warranted in future trials. In addition, the

HNSCC studies clearly demonstrated efficacy when the virus was administered at doses of  $2 \times 10^{11}$  particles intratumorally and  $1 \times 10^{10}$  particles directly into a single tumor in head and neck cancer.<sup>15,16</sup> Given the systemic administration of the virus in an IV setting and its distribution throughout the body, a much greater and more concentrated amount of virus would be needed to match the viral load in head and neck tumors. Moreover, the higher tumor density, potentially greater number of tumors, and multiple organ involvement in this patient population warrant a higher IV viral dose.

Increasing the frequency of administration beyond twice monthly is another approach that may be considered to improve efficacy. Nemunaitis et al<sup>11</sup> demonstrated no dose-limiting toxicity when the virus was administered weekly for 3 weeks in 10 patients with advanced lung cancer. Again, flu-like symptoms and a dose-dependent transient ALT/AST were the most common adverse events.

Another strategy to optimize the efficacy of CI-1042 is to combine standard chemotherapy regimens such as FU, LV, and irinotecan (CPT-11) with the virus. In preclinical animal studies, the combination of CPT-11 and ONYX-015 induced significant increase of apoptosis compared with CPT-11 alone in human colon cancer cell lines.<sup>18</sup> Other preclinical combination studies have demonstrated similar synergistic results with cisplatin and FU.<sup>19,20</sup> In the clinic, Reid et al<sup>10</sup> reported minor responses when the virus was given in combination with FU and LV via the hepatic artery; however, to date no trial has been conducted with the virus in combination with FU, LV, and CPT-11.

When delivering the virus to those patients with a low baseline neutralizing antibody, removing the antibody perhaps by immunophoresis or administering immunosuppressive agents may significantly prolong the virus bioavailability and increase infection.<sup>21</sup> In this trial, two patients who were able to maintain stable disease for 5 months also had detectable virus 72 hours after infusion.

Finally, tumors are also known to vary in their expression of the coxsackievirus-adenovirus receptor (CAR), a cell surface adenovirus receptor. The ability of the virus to enter a tissue cell and cause infection may depend on the affinity of the receptor for the virus. CAR expression is frequently lost or reduced in certain malignant tumors. One way to improve therapy with adenoviruses might be to select tumors that clearly express CAR or use modified viruses that bind to other cellular receptors.<sup>22,23</sup>

In summary, this study demonstrated that IV delivery of CI-1042 can be accomplished with limited toxicity in refractory colorectal cancer patients. Although no objective responses were demonstrated, evidence of biologic activity was observed. Preclinical data indicate that the virus may be most effective if given in combination with cytotoxic agents, such as a CPT-11 and FU combination. Additional trials identifying a true maximum-tolerated dose and investigating the virus as part of a combination regimen are warranted.

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