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Oncolytic Adenovirus ICOVIR-7 in Patients with Advanced and Refractory Solid Tumors

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Abstract

Purpose: Twenty-one patients with cancer were treated with a single round of oncolytic adenovirus ICOVIR-7.

Experimental Design: ICOVIR-7 features an RGD-4C modification of the fiber HI-loop of serotype 5 adenovirus for enhanced entry into tumor cells. Tumor selectivity is mediated by an insulator, a modified E2F promoter, and a Rb-binding site deletion of *E1A*, whereas replication is optimized with E2F binding hairpins and a Kozak sequence. ICOVIR-7 doses ranged from 2×10^{10} to 1×10^{12} viral particles. All patients had advanced and metastatic solid tumors refractory to standard therapies.

Results: ICOVIR-7 treatment was well tolerated with mild to moderate fever, fatigue, elevated liver transaminases, chills, and hyponatremia. One patient had grade 3 anemia but no other serious side effects were seen. At baseline, 9 of 21 of patients had neutralizing antibody titers against the ICOVIR-7 capsid. Treatment resulted in neutralizing antibody titer induction within 4 weeks in 16 of 18 patients. No elevations of serum proinflammatory cytokine levels were detected. Viral genomes were detected in the circulation in 18 of 21 of patients after injection and 7 of 15 of the samples were positive 2 to 4 weeks later suggesting viral replication.

Conclusions: Overall, objective evidence of antitumor activity was seen in 9 of 17 evaluable patients. In radiological analyses, 5 of 12 evaluable patients had stabilization or reduction in tumor size. These consisted of one partial response, two minor responses and two cases of stable disease, all occurring in patients who had progressive disease before treatment. In summary, ICOVIR-7 treatment is apparently safe, resulting in anticancer activity, and is therefore promising for further clinical testing. *Clin Cancer Res; 16(11); 3035–43. ©2010 AACR.*

Oncolytic adenovirus-based therapy represents a novel approach for cancer refractory to conventional therapies (1–3), and can be combined with other modalities for synergistic effects (4–7). Oncolytic adenoviruses have been safe in dozens of clinical trials and typical adverse events include flu-like symptoms, fever, and pain at the injection site (5, 8, 9). Infection of tumor cells results in replication, oncolysis, and subsequent release of the virus progeny. The replicating virus kills infected cancer cells, amplifies and spreads locally, and can also disseminate through the vasculature into metastases (10, 11). Moreover, oncolytic replication is an immunogenic phenomenon and antitumor immune responses can be body-wide (2).

ICOVIR-7 is based on a serotype 5 adenovirus and features a 24-bp deletion in *E1A*, which has been placed under the control of a tumor-specific E2F-modified promoter (12). Both features convey selectivity for cells defective in the Rb-p16 pathway (12–14), which includes most if not all advanced solid tumors (15). Additionally, a DM insulator has been placed 5′ of the promoter for increased specificity and a Kozak sequence leads E1A for optimal expression. The capsid of ICOVIR-7 has been modified with an RGD-4C motif in the HI-loop of the fiber knob for enhanced infectivity of various cancer types (16–19). In this study, we report the safety and efficacy of oncolytic adenovirus ICOVIR-7 in 21 patients with cancer.

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Translational Relevance

Despite recent developments in cancer therapy, there are still no curative treatments for most advanced metastatic solid tumors. Oncolytic adenoviruses provide a novel approach against cancer and they can be successfully combined with conventional therapies for increased efficacy without increased side effects. Oncolytic adenoviruses have shown good safety in clinical trials, which suggests their suitability for treating advanced refractory tumors. However, improvements in efficacy are needed to make the approach more attractive for routine clinical use. In this study, we report the first clinical use of ICOVIR-7, an oncolytic RGD capsid–modified adenovirus with enhanced tumor specificity via a modified E2F-promoter in 21 cancer patients with advanced metastatic tumors. ICOVIR-7 treatment resulted in good safety, immunologic activity, and objective evidence of anticancer effects in more than half of the patients. Thus, improvements in viral technology led to promising clinical results and encourage further clinical evaluation of ICOVIR-7.

Materials and Methods

Patients

Twenty-one patients with solid tumors refractory to available anticancer modalities were treated with a single round of ICOVIR-7 (Table 1). All patients had progressive metastatic tumors and signed written informed consent. Symptoms were collected by interviewing the patient at each visit and also by collecting their medical records in case they visited other health care providers. Hematologic side effects and cytokines (possibly predictive of immunologic toxicity; refs. 20–23) were assessed by laboratory analysis. Side effects were graded according to CTCAE v3.0. Treatments were done according to Good Clinical Practice and the Helsinki Declaration of the World Medical Association. This Advanced Therapy Access Program is in compliance with EU and Finnish regulations and has been evaluated by the Medicolegal Department of the Finnish Ministry of Social Affairs and Health and by The Gene Technology Board.

Treatment protocol

All patients received a single round of ICOVIR-7 intratumorally in ultrasound guidance. The largest safely accessible tumors were selected for injection and the typical number of needle tracts was 10 to 15 per patient. The starting dose of 2×10^{10} viral particles (VP) was chosen based on published safety results (1–9, 11, 12, 24–29). Subsequently, the dose was escalated to 7×10^{10}, 1×10^{11}, 2×10^{11}, 3×10^{11}, 4×10^{11}, 5×10^{11}, 6×10^{11}, 7×10^{11}, and 1×10^{12}. "Time-lapse" dose escalation was used to maximize patient safety but minimize delays in enrolling new patients at potentially more effective higher doses. A dose could be escalated when sufficient time (typically 2 weeks) had lapsed (and relevant safety information collected) from the treatment of the first patient at that dose. If intratumoral injection was not possible, intravenous injection was done. In case of intraperitoneal or intrapleural disease, intratumoral injection was done intracavitary. Patients were monitored for 24 hours in the hospital and for 4 weeks as outpatients. If there were no contraindications (e.g., gastrointestinal, hematologic, or neurologic), low-dose oral metronomic cyclophosphamide (50 mg/d) was administered to selected patients to reduce regulatory T-cells (refs. 30, 31; Table 4). Cyclophosphamide was started 1 week prior to virus and continued until disease progression or withdrawal of consent. One patient (U157) received cyclophosphamide intravenously before treatment and then orally starting 2 weeks later.

Analysis of efficacy

Tumor assessment by computer tomography or magnetic resonance imaging was done before treatment and again ~6 weeks later. Response Evaluation Criteria in Solid Tumors (RECIST) guidelines (32, 33) were applied

Table 1. Characteristics of patients treated with oncolytic adenovirus ICOVIR-7

Sex	No. of patients
Male	10
Female	11
Age (y)	
Median	56
Range	9-68
WHO performance status (0-5)	No. of patients
0	1
1	12
2	5
3	3
Tumor type	No. of patients
Head and neck cancer	4
Breast cancer	3
Pancreatic cancer	3
Ovarian cancer	3
Colon cancer	2
Bladder cancer	1
Cholangiocarcinoma	1
Gastric cancer	1
Prostate cancer	1
Leiomyosarcoma	1
Wilms tumor	1
Previous treatments	No. of patients
Surgery	17
Chemotherapy	21
(mean, 5 regimens; range, 2-13)	
Radiotherapy	14
Autologous stem cell transplantation	1

Table 2. Treatment-related side effects according to CTCAE v3.0 criteria

	Grade 1 (no. of patients)	Grade 2 (no. of patients)	Grade 3 (no. of patients)	Grade 4 (no. of patients)
Hematologic				
Anemia	6	6	1	
Elevated liver transaminases	9	4		
Hypokalemia	7			
Hyponatremia	12			
Pain				
Injection site		1		
Chest	2			
Abdominal	5	6		
Muscular/extremity		5		
Back	1	1		
Head	1	1		
Gastrointestinal system				
Constipation		4		
Diarrhea	1			
Heartburn	1			
Loss of appetite	3			
Nausea, vomiting	6	2		
Abdominal swelling	3	1		
Respiratory system				
Dyspnea	3	1		
Cough	2	1		
Sore throat	2			
Speech difficulties	1			
Constitutional symptoms				
Fever	11	7		
Chills	7	3		
Fatigue	3	13		
Sweating	2			
Edema (lower extremity)		1		
Itching	2			
Thirstiness		1		

to overall disease status including injected and noninjected tumors. In addition to the standard criteria, we used minor response (MR, 10-30% reduction in the size of lesions) as an indicator of cases in which biological activity might be present. Tumor density was evaluated according to the "Choi criteria" (34), which has been proposed to be useful in the context of oncolytic viruses (11).

Virus

ICOVIR-7 is based on serotype 5 adenovirus (12). The virus capsid has been modified with the RGD-4C motif in the HI loop of the fiber. ICOVIR-7 features the 24-bp deletion in the E1 region conferring cancer cell specificity (35). The tumor-specific E2F-1 promoter was placed to control *E1A*, and the promoter was further modified by additional E2F-binding hairpins for enhanced activity. The myotonic dystrophy locus (DM-1) insulator sequence reduces transcriptional leakage from the left inverted terminal repeat and a Kozak sequence ensures optimized transcription. ICOVIR-7 was produced on A549 cells by Oncos Therapeutics, Inc. (Helsinki, Finland) to avoid the risk of recombination with transcomplementing sequences. The viral particle titer of ICOVIR-7 was 1.2×10^{12} VP/mL and the functional titer was 2.6×10^{10} plaque-forming units/mL, for a genome to plaque-forming unit ratio of 46.5. Virus stock buffer formulation was 10 mmol/L Trizma base, 75 mmol/L of NaCl, 5% (w/v) sucrose, 1 mmol/L of MgCl, 10 mmol/L of L(+) histidine, 0.5% (v/v) ethanol, 0.02% Tween, 100 μmol/L of EDTA. A 0.9% (w/v) NaCl solution (B. Braun Melsungen AG) was used as a diluent.

Cytokine analysis

Cytokine analysis was done with BD Cytometric Bead Array Human Soluble Protein Flex Set (Becton Dickinson) according to the instructions of the manufacturer. FCAP Array v1.0.1 software was used for data analysis.

Neutralizing antibody titer determination

293 cells were seeded at a density of 1×10^4 cells/well on 96-well plates and cultured overnight. Serum samples were incubated at 56°C for 90 minutes to inactivate complement, and a 4-fold dilution series (1:1 to 1:16,384) was prepared in serum-free DMEM (36). Ad5lucRGD (ref. 16; identical capsid with ICOVIR-7), was mixed with serum dilutions and incubated at room temperature for 30 minutes. Cells in triplicates were infected with 100 VP/cell, and growth medium with 10% FCS was added 1 hour later. Twenty-four hours postinfection, cells were lysed and luciferase activity was measured (Luciferase Assay System, Promega; TopCount Luminometer, Perkin-Elmer). Luciferase readings were plotted relative to gene transfer achieved with Ad5lucRGD alone. The neutralizing antibody (NAb) titer was determined as the lowest degree of dilution that blocked gene transfer by more than 80% (24).

Quantitative real-time PCR for presence of ICOVIR-7 in serum

The presence of ICOVIR-7 virus in PCR-positive samples was confirmed by real-time PCR using LightCycler480 SYBR Green I Master mix (Roche) and specific primers (forward primer 5′-GCGGGAAAACTGAATAAGAGG-3′ and reverse primer 5′-CGGAGCGGTTGTGAACTG-3′). For detailed methods, see ref. (37).

Results

Treatment of cancer patients with ICOVIR-7 is well tolerated and safe

Treatments were well tolerated up to the highest dose used (1×10^{12} VP). All patients experienced mild to moderate grade 1 or 2 side effects (Table 2). Most common side effects were fever (18 of 21 patients), fatigue (16 of 21 patients), elevated liver transaminases (13 of 21 patients), anemia (13 of 21 patients), hyponatremia (12 of 21 patients), abdominal pain (11 of 21 patients), and chills (10 of 21 patients). There was no apparent relationship between viral dose or administration route and the severity of side effects. Grade 3 posttreatment anemia was detected in one patient (H111), and no grade 4 to 5 side effects were observed.

Effect of treatment on proinflammatory cytokines

Interleukin (IL)-6, IL-8, IL-10, and tumor necrosis factor-α levels were measured in all patients. The highest absolute cytokine concentration was measured in patient H41 with pancreatic cancer whose IL-8 value was 1,159 pg/mL 4 days after treatment. However, the patient's pretreatment IL-8 value was already 626 pg/mL, indicating only a moderate increase (Supplementary Table S1). There was no apparent relationship between the measured cytokine levels, patients' clinical status, administration route,

Table 3. NAb titers for patients treated with ICOVIR-7

Type of cancer, patient ID code	Treatment dose × 10^{11} (VP)	Metastatic sites	NAb, reciprocal of titer			
			Baseline pretreatment	1st week	2nd week	3-4 weeks
Breast, R39	0.2	Bone, liver, nodes	1		16,384	16,384
Pancreatic, H41	0.7	Bone, lungs	1			
Wilms tumor, U157	1	Abdominal cavity	4	4		4
Bladder, V45	2	Lungs, liver	0	1,024		4,096
Prostate, P44	2	Bone, liver	1	16,384		
Breast, R55	3	Bone, liver	0		256	
Breast, R85	3	Liver, nodes	4,096	16,384		16,384
Head and neck, N56	3	Bone, liver	1			1,024
Ovarian, 048	3	Lungs, mediastinum	1,024			16,384
Colon, C93	4	Liver	0	16		
Colon, C104	4	Bone, liver, lungs, nodes	16	64		
Gastric, G59	4	Abdominal cavity	64			
Ovarian, O53	4	Abdominal cavity	4		16,384	
Ovarian, O92	4	Abdominal cavity	64	64		
Head and neck, N90	5	Lungs, mediastinum, nodes	0	1		64
Head and neck, N106	6	Lungs, mediastinum	4	16,384		
Pancreatic, H107	6	Abdominal cavity, liver	4	4,096		
Leiomyosarcoma, S102	6	Lungs, mediastinum	16			16,384
Cholangiocarcinoma, Y120	7	Liver	16	64		16,384
Pancreatic, H111	7	Abdominal nodes	1	4,096		
Head and neck, N127	10	Bone, lungs	256			

Table 4. Summary of all ICOVIR-7–treated patients in this study according to viral dose

Type of cancer, patient ID code	Treatment dose × 10^{11} (VP)	Virus in blood (VP/mL)		
		Day 0 pretreatment	Day 1	Days 2-7
Breast, R39	0.2	neg.	neg.	63,424
Pancreatic, H41	0.7	neg.	neg.	6,791
Wilms tumor, U157*	1	neg.	neg.	2,450
Bladder, V45†	2	neg.	<500	814
Prostate, P44	2	neg.	1,144	4,038,049
Breast, R55†	3	neg.	neg.	17,465
Breast, R85†	3	neg.	neg.	neg.
Head and neck, N56	3	neg.		
Ovarian, 048†	3	neg.		
Colon, C93	4	neg.		68,831
Colon, C104	4	neg.	<500	96,689
Gastric, G59	4§	neg.	<500	neg.
Ovarian, O53†	4	neg.	<500	
Ovarian, O92	4	neg.	neg.	neg.
Head and neck, N90†	5	neg.	<500	<500
Head and neck, N106†	6§	neg.	<500	<500
Pancreatic, H107	6	neg.	neg.	<500
Leiomyosarcoma, S102†	6	neg.	<500	
Cholangiocarcinoma, Y120	7	neg.	neg.	1,413
Pancreatic, H111†	7	neg.	<500	2,528
Head and neck, N127	10§	neg.	<500	

NOTE: ↓, tumor marker decreased; ↑, tumor marker increased; blanks, data not available.
*Cyclophosphamide i.v. 500 mg on day of virus injection and 25 mg/d p.o. starting 2 wk later.
†Cyclophosphamide 50 mg/d p.o. starting 1 wk before treatment until 4 wk after treatment.
‡Alive at the end of follow-up.
§Intravenous treatment only.

or treatment response. Generally, only minor posttreatment elevations in proinflammatory cytokine levels could be detected.

Induction of neutralizing antibodies

NAb titers against the capsid of ICOVIR-7 were analyzed for all patients (Table 3). Eight patients had titers higher than 1:4, which was considered the cutoff for neutralizing activity. In addition, five patients had a titer of 1:1 whereas four patients had a titer of 1:4, possibly reflecting past infection with adenovirus. Treatment resulted in NAb induction within 4 weeks in 16 of 18 patients.

ICOVIR-7 replication

Viral genomes were detected in the circulation in 18 of 21 patients (Table 4). Three patients (R85, O48, and O92) remained negative at all analyzed time points. The highest viral titer (>4 × 10^6 VP/mL) was seen at day 4 in a patient with prostate cancer (P44). In 10 of 15 patients, there was more virus detected between days 2 and 7 than on day 1, suggesting viral replication. Virus could be detected in 7 of 15 patients for 2 to 4 weeks (Table 4), which also suggests viral replication because injected virus is typically cleared rapidly (5, 9, 25, 26, 38). Three patients (G59, N106, and N127) received virus only intravenously. Interestingly, all three had virus present in the blood on day 1. G59 was negative on two subsequent measurements and N127 had no other data points. However, N106 had circulating virus on day 7, which suggests viral replication.

Antitumor efficacy of ICOVIR-7

Overall, objective evidence of antitumor activity was seen in 9 of 17 evaluable patients. In 5 out of 12 radiologically evaluable patients (Y120, N127, H107, O48, and U157), tumor size measurements suggested benefit from the treatment (Table 4). These consisted of one partial response, two minor responses, and two cases of stable disease which lasted until the end of follow-up: 93 and 316 days (disease was progressing in all patients before treatment). Nine patients did not fulfill RECIST criteria and could therefore not be evaluated for radiological response. With regard to tumor density ("Choi" criteria), all three evaluable patients had a decrease, which has been suggested to indicate antitumor activity of oncolytic viruses (11). Three patients (R39, P44, and O48) had a decrease or stabilization in tumor markers.

Table 4. Summary of all ICOVIR-7–treated patients in this study according to viral dose (Cont'd)

Virus in blood (VP/mL)			Tumor markers	RECIST	Tumor density (HU)	Survival (d)
Days 8-14	Days 15-21	Days 21-28				
<500		<500	MR: CEA↓ PD: CA15-3 ↑			58
<500		<500	PD: CA19-9 ↑ CEA ↑	PD		92
		neg.		PR (–37%)		192
<500		neg.		PD (+29%)	73 → 60 (–18%)	320
2,580			SD: PSA↓			51
neg.		neg.	PD: CA15-3 ↑ CEA ↑	PD (+24%)	79 → 57 (–28%)	142
		neg.	PD: CA15-3 ↑			79
804	<500		PD: CEA ↑	PD		113
	neg.		MR: CA12-5 ↓	MR (–17%)		268‡
				PD		62
						34
	neg.		PD: CA12-5 ↑			109
neg.	neg.		PD: CA12-5 ↑			79
			PD: CA12-5 ↑	PD		73
		<500				371‡
						49
		<500		SD (+9%)		93
		neg.		PD (+30%)		56
		neg.		SD (+13%)	93 → 78 (–16%)	316‡
						60
				MR (–10%)		167

Patient U157 was a 9-year-old boy with Wilms tumor, a pediatric kidney malignancy. He had a partial response with a 37% overall reduction in the sum of tumor diameters. Also, he had complete eradication of some of his tumor lesions (Fig. 1). Patients N127 and O48 also showed minor responses (–10% and –17%, respectively). The RECIST measurements of the tumors of Y120 and H107 resulted in the classification of stable disease. Y120 also had a decrease in tumor density.

Two of three patients injected only i.v. could be evaluated for antitumor efficacy. One had an increase in CA12-5 (G59) whereas the other (N127) had a 10% minor reduction in the overall sum of tumor diameters. Both patients remained free of documented progression until the end of follow-up. After a minimum of 9 months of follow-up (Fig. 2), 4 of 21 patients were still alive (longest follow-up, 371 days).

Discussion

Treatment of metastatic cancer refractory to available treatments requires novel approaches such as oncolytic adenoviruses. The first generation of such viruses has completed clinical testing with good safety data, and although there are some examples of efficacy in nearly all trials, the overall single agent efficacy has been less than satisfying (1–3, 39). However, it is promising that even such prototype viruses seem quite effective when combined with chemotherapy or radiation (4, 6, 7). ICOVIR-7 embodies several improvements over viruses tested previously in patients including RGD modification of the fiber, and a deletion of constant region 2 of E1 combined with a tumor-specific promoter enhanced by E2F binding hairpins. These modifications may represent an important improvement in the technology and the use of RGD-modified viruses in humans has not been reported previously.

No serious side effects were detected in this study, except for one patient with pancreatic cancer who experienced grade 3 anemia (patient H111). His hemoglobin value decreased from 97 to 68 g/L on the first posttreatment day. This may have been treatment-related, as such a rapid decrease is rare despite anemia being very common in patients with pancreatic cancer (40). At the seventh posttreatment day, his hemoglobin value was 94 g/L and it stabilized between 80 and 94 g/L during the following 4 weeks.

Although adenoviruses have been quite safe in the treatment of cancer, with thousands of patients reported in the literature (1–7, 39), some safety concerns remain in the context of newer generation viruses. It has been proposed that cytokines could predict harmful inflammation (20, 22). IL-6, IL-8, and tumor necrosis factor-α were selected for analysis due to their proinflammatory role (21, 23), which could be helpful for analyzing systemic inflammatory response. High levels are associated with systemic inflammatory response syndrome, which can result in multiple organ failure in the worst case scenario (20, 22). All patients had elevated cytokine levels prior to

treatment, which is in line with previous reports on patients with advanced cancer (41–43). Taking into account the detected side effects and cytokine data, it seems reasonable to propose that ICOVIR-7 treatment was well tolerated at the dose range used. The highest dose used here was 6-fold lower than the highest dose of oncolytic adenovirus safely used in humans previously (9).

As suggested by others (7, 26–29), no apparent relationship was seen between NAb titers and the injected viral dose, as NAbs increased in nearly all evaluable patients, except U157 and O92, although in N90, there was only a slight increase. No viral replication was seen in O92, perhaps correlating with lack of NAb induction. In the case of U157 and O92, we speculate that antibody induction might have been thwarted by the cyclophosphamide used in conjunction with virus administration because viral replication seemed likely based on quantitative PCR. Alternatively, or in addition, these patients' tumors might have been particularly immunosuppressive. Further studies are needed to resolve these issues. In the two patients with the highest baseline NAb titers (R85 and O48), there was no virus detected in the blood after treatment. There seemed to be no correlation between baseline NAbs and efficacy. For example, O48 had a minor response despite a baseline NAb titer of 1,024 and N127 had a minor response after i.v. injection, despite a baseline NAb titer of 256. These data seem to support the hypothesis that antiviral antibodies could increase the efficacy of treatment, as they can help in the clearance of (tumor) cells containing virus.

Although most patients received the virus intratumorally, there were three patients whose tumors could not be injected and therefore received virus intravenously. It is tantalizing that there was a minor response in one of these patients. Although there is a substantial body of preclinical evidence suggesting that efficacy is possible through the i.v. route (12, 44), initial trials did not seem to support this notion (6, 26, 27, 29). However, in one trial, there were prostate-specific antibody responses in three of eight patients treated at the highest dose level (9), possibly indicating antitumor activity. These findings might be explained in part by the low activity of ONYX-015 in general (7, 26–29), whereas the virus used by Small et al. may have been more active (9). Preclinical data suggests that ICOVIR-7 might be even more active (12).

All patients in this study were heavily pretreated with a mean of five previous chemotherapy regimens, in addition to other modalities such as radiotherapy and/or surgery. Despite this, 9 of 17 of patients had objective evidence of antitumor activity of the virus (Table 4). The preliminary activity seen here might justify further evaluation of the virus in larger studies, which might ultimately lead to randomized trials, needed to reliably evaluate the safety and efficacy of any cancer therapeutic. Nevertheless, it is interesting to note that the patients who experienced some clinical effect had different tumor types, including cholangiocarcinoma, head and neck, pancreatic, prostate, ovarian cancer, and Wilms tumor. This is a promising indication that ICOVIR-7 could be used successfully for various tumor types. One issue that complicates the analysis

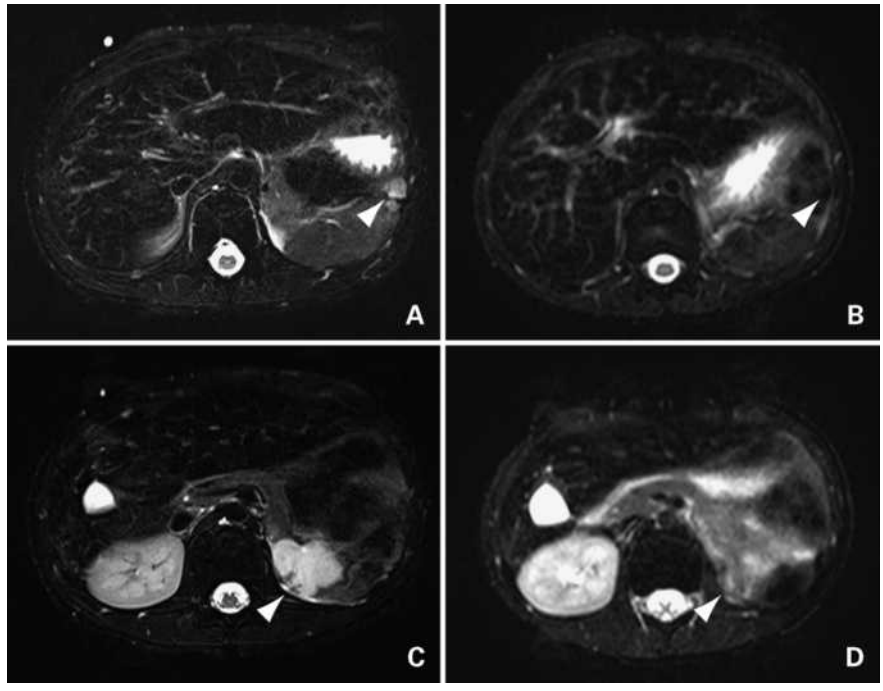

Fig. 1. For Wilms tumor patient U157, magnetic resonance images are shown 14 d before (A and C) and 36 d after (B and D) treatment to demonstrate the clinical effect of ICOVIR-7. The reduction in the overall sum of tumor diameters according to RECIST was 37%, indicating partial response. A and B, tumor lesion at the left side of the abdominal cavity wall in the proximity of spleen. This lesion was completely eradicated by ICOVIR-7. C and D, tumor lesion at the left kidney area (left kidney had been removed previously). Tumor size before treatment was 36 × 30 mm, and after treatment 24 × 5 mm. The viral dose was 1×10^{11} and both lesions were injected. Arrowheads, the locations of the tumors.

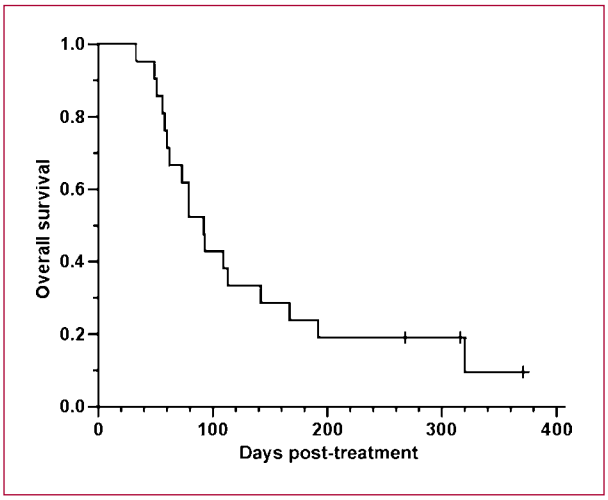

Fig. 2. Patient survival after ICOVIR-7 treatment.

of treatment efficacy is the lack of suitable evaluation methods. RECIST criteria, originally developed for chemotherapy agents, may give incorrect results because viral replication might cause local inflammation, enlarging tumors and thus leading to false conclusions of progression. The same applies to tumor marker analysis, as tumor cell lysis due to oncolytic replication might misleadingly increase tumor markers temporarily (5). One proposed mechanism for marker surge is the activation of the CEA promoter by viral replication (45). To reduce regulatory T-cells, some of our patients received metronomic low-dose cyclophosphamide in addition to ICOVIR-7 (Table 4; refs. 30, 31). In this nonrandomized series, it cannot be excluded that cyclophosphamide per se influenced treatment outcome. However, antitumor activity was also seen in patients not treated with cyclophosphamide.

In summary, our data suggests that ICOVIR-7 is safe for the treatment of human cancer and that there might be antitumor activity. Further increases in efficacy could be obtained by treating patients with less advanced disease, combining the virus with standard therapies (4, 6, 25, 27), and by treating patients with more than one injection. Randomized trials are needed to ultimately determine the safety and efficacy of the approach.

Disclosure of Potential Conflicts of Interest

A. Hemminki is cofounder and shareholder in Oncos Therapeutics Inc. The other authors had no conflicts to disclose.

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