A Phase I Study of OncoVEX$^{GM\text{-}CSF}$, a Second-Generation Oncolytic Herpes Simplex Virus Expressing Granulocyte Macrophage Colony-Stimulating Factor

Jennifer C.C. Hu,[1] Robert S. Coffin,[5] Ceri J. Davis,[6] Nicola J. Graham,[6] Natasha Groves,[5] Peter J. Guest,[6] Kevin J. Harrington,[2] Nicholas D. James,[6] Colin A. Love,[5] Iain McNeish,[1] Louise C. Medley,[6] Agnieszka Michael,[3] Christopher M. Nutting,[2] Hardev S. Pandha,[3] Claire A. Shorrock,[5] Julie Simpson,[6] Jan Steiner,[7] Neil M. Steven,[6] Dennis Wright,[4] and R. Charles Coombes[1]

Abstract

Purpose: To conduct a phase I clinical trial with a second-generation oncolytic herpes simplex virus (HSV) expressing granulocyte macrophage colony-stimulating factor (Onco VEX$^{GM\text{-}CSF}$) to determine the safety profile of the virus, look for evidence of biological activity, and identify a dosing schedule for later studies.

Experimental Design: The virus was administered by intratumoral injection in patients with cutaneous or s.c. deposits of breast, head and neck and gastrointestinal cancers, and malignant melanoma who had failed prior therapy. Thirteen patients were in a single-dose group, where doses of 10^6, 10^7, and 10^8 plaque-forming units (pfu)/mL were tested, and 17 patients were in a multidose group testing a number of dose regimens.

Results: The virus was generally well tolerated with local inflammation, erythema, and febrile responses being the main side effects. The local reaction to injection was dose limiting in HSV-seronegative patients at 10^7 pfu/mL. The multidosing phase thus tested seroconverting HSV-seronegative patients with 10^6 pfu/mL followed by multiple higher doses (up to 10^8 pfu/mL), which was well tolerated by all patients. Biological activity (virus replication, local reactions, granulocyte macrophage colony-stimulating factor expression, and HSV antigen-associated tumor necrosis), was observed. The duration of local reactions and virus replication suggested that dosing every 2 to 3 weeks was appropriate. Nineteen of 26 patient posttreatment biopsies contained residual tumor of which 14 showed tumor necrosis, which in some cases was extensive, or apoptosis. In all cases, areas of necrosis also strongly stained for HSV. The overall responses to treatment were that three patients had stable disease, six patients had tumors flattened (injected and/or uninjected lesions), and four patients showed inflammation of uninjected as well as the injected tumor, which, in nearly all cases, became inflamed.

Conclusions: Onco VEX$^{GM\text{-}CSF}$ is well tolerated and can be safely administered using the multidosing protocol described. Evidence of an antitumor effect was seen.

Oncolytic virus therapy is a promising approach to cancer treatment, particularly for the locoregional control of solid tumors. Here, viruses that selectively replicate in tumor compared with normal cells are used such that tumor cells are killed by lytic virus replication and normal cells are spared. Various viruses have been tested for use as oncolytic agents, including adenovirus, Newcastle disease virus, and vesticular stomatitis virus with promising preclinical, and, in some cases, clinical results. However, although safety has been shown, it is evident that improvements to oncolytic potency would be beneficial.

Herpes simplex virus (HSV) is a highly lytic virus in which deletion of the extensively studied gene encoding ICP34.5 provides tumor selectivity (see below). ICP34.5-deleted HSV infects and replicates in a broad range of human tumor cells, apparently irrespective of the genetic aberration resulting in their transformation. HSV thus has the potential to be a useful oncolytic virus that could be used in the treatment of a broad spectrum of solid tumor types.

Thus far, oncolytic HSVs have been tested in several clinical trials with encouraging results. These viruses are all deleted for one or both copies of ICP34.5 (virus strains 1716 and NV1020; refs. 1–3), or ICP34.5 and ICP6 (virus strain G207; refs. 4, 5).

Authors' Affiliations: [1]Department of Cancer Medicine, Imperial College School of Medicine; [2]Royal Marsden Hospital; [3]Department of Oncology, St Georges Hospital Medical School; [4]University College London, London, United Kingdom; [5]Biovex, Inc., Cambridge, Massachusetts; [6]Institute for Cancer Studies, The University of Birmingham, Birmingham, United Kingdom; and [7]Oxford Therapeutics Consulting, The Magdalen Centre, Oxford, United Kingdom

Received 3/28/06; revised 6/1/06; accepted 6/22/06.

Grant support: Biovex, Inc.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Requests for reprints: Robert S. Coffin, Biovex, Inc., 34 Commerce Way, Woburn, MA 018010. E-mail: rcoffin@biovex.com.

©2006 American Association for Cancer Research.

doi:10.1158/1078-0432.CCR-06-0759

Downloaded from http://aacrjournals.org/clincancerres/article-pdf/12/22/6737/1921819/6737.pdf by guest on 04 December 2024

Table 1. Patient characteristics and responses

Patient	Dose (pfu/mL)	Age/sex	HSV sero status	Pathology	Prior therapy
101	10^6	50/F	Positive	Breast	S, CXT, RXT
102	10^6	74/F	Negative	Breast	H, S, CXT
103	10^6	55/F	Positive	Breast	CXT, S, RXT
104	10^6	70/F	Positive	Colorectal	S, CXT
201	10^7	80/F	Positive	Melanoma	S, CXT, laser, thalidomide
202	10^7	57/F	Negative	Breast	S, CXT, RXT, TNF Autovac trial
203	10^7	40/F	Negative	Breast	CXT, S
204	10^7	59/F	Positive	Breast	S, H, CXT
205	10^7	31/F	Negative	Melanoma	S, CXT, I, TAM Glivec, thalidomide
301	10^8	30/M	Positive	Melanoma	S, CXT, RXT, I, isolated limb perfusion, thalidomide
302	10^8	61/F	Positive	Breast	S, CXT, TAM, H, RXT
303	10^8	63/F	Positive	Breast	TAM, H, CXT, RXT
304	10^8	67/F	Positive	Breast	S, TAM, H, RXT
401	10^6, 10^7, 10^7	39/F	Negative	Breast	S, high-dose CXT with stem cell transplant, CXT, RXT
402	10^6, 10^7, 10^7	61/F	Negative	Head and neck	S, RXT
403	10^6, 10^7, 10^7	60/F	Negative	Breast	TAM, CXT, RXT
601	10^6, 10^8, 10^8	48/M	Negative	Colorectal	CXT, S
602	10^6, 10^8, 10^8	47/F	Negative	Breast	S, H, TAM, CXT, RXT
603	10^6, 10^8, 10^8	43/F	Negative	Breast	CXT, herceptin
604	10^6, 10^8, 10^8	45/F	Negative	Melanoma	S, dendritic cell trial
501	10^8, 10^8, 10^8	50/F	Positive	Melanoma	S, CXT, I, laser
502	10^8, 10^8, 10^8	59/F	Positive	Melanoma	S
503	10^8, 10^8, 10^8	62/M	Positive	Melanoma	CXT, RXT, I, S
701	10^6, 10^8, 10^8	55/F	Positive	Head and neck	S, CXT, RXT
702	10^6, 10^8, 10^8	57/F	Positive	Head and neck	S, CXT, RXT
703	10^6, 10^8, 10^8	50/M	Positive	Head and neck	CXT, RXT, S
704	10^6, 10^8, 10^8	53/M	Positive	Head and neck	S, CXT, RXT
705	10^6, 10^8, 10^8	49/M	Positive	Melanoma	S, CXT, RXT
706	10^6, 10^8, 10^8	56/M	Positive	Melanoma	S
707	10^6, 10^8, 10^8	80/F	Positive	Breast	S, TAM, H, CXT

NOTE: Patients had progressive disease unless otherwise noted.
Abbreviations: S, surgery; H, hormonal therapy; I, immunotherapy; TAM, tamoxifen; CXT, chemotherapy; RXT, radiotherapy; U, injected and/or uninjected lesion(s) ulcerated; V, vesicles; E, erythmatous/inflamed (degree: +, ++, +++); FI, flattening/shrinkage of injected lesion; FU, flattening/shrinkage of uninjected lesion(s); SD, stable disease; EU, uninjected lesion inflamed (degree: +, ++, +++); N, necrosis in tumor biopsy; A, apoptosis in tumor; H, HSV antigen detected in necrotic areas; P, pain in injected or uninjected lesion(s).
*Small biopsy, possibly not representative.
†Patient 401 also received three further doses over 7 weeks on a compassionate basis.

1716 and G207 have been tested in clinical trials in glioma, with some evidence of disease stabilization (6–8), and NV1020 has been tested followed by chemotherapy in 12 patients with colorectal cancer hepatic metastases with some minor responses (9). 1716 has also been tested in melanoma at very low dose (10). All the patients in these clinical trials tolerated the treatment well. Thus, safety of ICP34.5-deleted HSV has been shown in multiple clinical studies. However, although suggestions of efficacy have been obtained, greater potency viruses would likely improve the effectiveness of the treatment seen.

Therefore, we set out to build on this previous work to develop a higher-potency oncolytic HSV while retaining the safety profile of these previous viruses. This virus was also developed to induce a more potent antitumor immune response through the deletion of the HSV gene encoding ICP47, which blocks antigen presentation in HSV-infected cells (11) and the delivery of the gene encoding human granulocyte macrophage colony-stimulating factor (GM-CSF). Improved oncolysis was achieved through the use of a more potent clinical isolate of HSV for construction of the virus, and, in addition to the deletion of ICP34.5, which is well documented to provide tumor-selective virus replication (12–16), the expression of the *US11* gene as an immediate early rather than late gene that has previously been shown to boost tumor-selective virus replication (11, 17, 18). GM-CSF was chosen as it induces myeloid precursor cells to proliferate and differentiate, is a recruiter and stimulator of dendritic cells, has given promising preclinical and clinical results (19–23), and is used routinely in clinical practice. In addition, autologous tumor cell vaccines expressing GM-CSF have shown significant clinical benefit (24, 25). This virus (OncoVEX$^{GM\text{-}CSF}$), and the other viruses constructed during the development of OncoVEX$^{GM\text{-}CSF}$, have been tested in extensive preclinical studies showing significant antitumor effects. These include that both the use of a clinical isolate for virus construction and the increased expression of US11 greatly increase oncolytic potency and that expression of GM-CSF greatly improves the effects seen in tumors that have not themselves been subjected to virus injection (26).

Downloaded from http://aacrjournals.org/clincancerres/article-pdf/12/22/6737/1921819/6737.pdf by guest on 04 December 2024

Table 1. Patient characteristics and responses (Cont'd)

Patient	Dose (pfu/mL)	Diagnosis to trial (mo)	Evaluable/evaluable biopsy? (drop out week)	Local reaction	Response
101	10^6	31	Y/Y	E+, P	N, H
102	10^6	13	Y/Y	E++	FI, FU, EU+, N, H
103	10^6	102	Y/Y		SD
104	10^6	28	Y/Y*	E+, P	A
201	10^7	50	Y/Y	E+	FI, N, H
202	10^7	180	N/N (3)	E++, U, V	EU+
203	10^7	28	Y/Y	E+++, U, V, P	N, H
204	10^7	144	Y/N	E+	
205	10^7	151	Y/Y	E+, P	N, H, FI
301	10^8	67	Y/Y	E+, U, P	FI, EU+, N, H
302	10^8	71	Y/Y	E+	
303	10^8	35	Y/Y	E+, U	EU+, N, H
304	10^8	208	Y/Y	E+, U	N, H
401	10^6, 10^7, 10^7	36	Y†/Y	E++, U, V, P	FI, FU, EU+
402	10^6, 10^7, 10^7	34	Y/N	E+, P	EU+
403	10^6, 10^7, 10^7	13	Y/Y	E+, U	
601	10^6, 10^8, 10^8	10	Y/Y*	E++, P, U	EU++
602	10^6, 10^8, 10^8	78	Y/Y	E++, P	
603	10^6, 10^8, 10^8	6	Y/Y	E+, P	EU+, N, H
604	10^6, 10^8, 10^8	116	N/N (7)	E+, P	FU, EU++
501	10^8, 10^8, 10^8	69	Y/Y	E+	EU+, SD, FI, FU
502	10^8, 10^8, 10^8	14	Y/Y	E+, P	EU+
503	10^8, 10^8, 10^8	108	Y/N	E+, P	
701	10^6, 10^8, 10^8	148	N/N (1)	E+, P	
702	10^6, 10^8, 10^8	20	Y/Y	P	N, H
703	10^6, 10^8, 10^8	13	Y/Y	E+, U, P	N, H
704	10^6, 10^8, 10^8	77	N/N (1)		
705	10^6, 10^8, 10^8	8	Y/Y	E+, U, P	N, H
706	10^6, 10^8, 10^8	36	Y/Y	E++, U	SD, FI
707	10^6, 10^8, 10^8	39	Y/Y	E+	N, H

Materials and Methods

OncoVEX$^{GM\text{-}CSF}$. OncoVEX$^{GM\text{-}CSF}$ has been described previously (26). Briefly, the virus is based on HSV-1 strain JS-1 in which the genes encoding ICP34.5 and ICP47 have been completely deleted. Deletion of ICP47 also results in the *US11* gene being under the control of the ICP47 immediate early promoter, rather than the US11 late promoter that results in enhanced tumor-selective replication (26). The ICP34.5-encoding sequences have been replaced with a cassette consisting of the cytomegalovirus promoter, the gene encoding human GM-CSF, and the bovine growth hormone pA sequence.

Patients. Patients with refractory cutaneous or s.c. metastases from breast, gastrointestinal adenocarcinoma, malignant melanoma, or epithelial cancer of the head and neck were included. Inclusion criteria included that patients were >18 years old; had WHO performance status of 0, 1, or 2; had an interval of ≥4 weeks since exposure to chemotherapy or radiotherapy; and ≥6 weeks since exposure to nitrosoureas or mitomycin C. Eligibility requirements also included that patients had a white cell count $\geq 3.0 \times 10^9$/L, a platelet count $\geq 100 \times 10^9$/L, serum creatinine ≤0.14 mmol/L, bilirubin ≤1.5×, and aspartate aminotransferase/alanine aminotransferase/alkaline phosphatase ≤2× the upper limit of the reference range, unless secondary to malignancy when ≤5 times upper normal limit was acceptable.

Exclusion criteria included cases where tumors were adjacent to the trachea or a major blood vessel, pregnancy, major surgery, or a history of cardiac or autoimmune diseases. The study was approved by the local ethics committees, Gene Therapy Advisory Committee and the Medicines and Healthcare Products Regulatory Authority. Biovex. Inc., which developed OncoVEX$^{GM\text{-}CSF}$, funded the clinical trial.

Patient details are given in Table 1, including, due to the late disease stage of the patients enrolled, details of the often extensive prior therapy they had received.

Treatment and clinical evaluation. The first, single dose, part of the study evaluated three escalating dose levels of OncoVEX$^{GM\text{-}CSF}$ at 10^6, 10^7, and 10^8 plaque-forming units (pfu)/mL. At each dose level, four evaluable patients were to be recruited irrespective of their HSV serology status. However, only HSV-seropositive patients were dosed at 10^8 pfu/mL (see Results). Patients in the second part of the study were given three injections testing three dosing regimens of the virus dependent on their HSV serology status. For HSV-seronegative patients, the regimens tested were 10^6, 10^7, 10^7 pfu/mL and 10^6, 10^8, 10^8 pfu/mL and for HSV-seropositive patients 10^6, 10^8, 10^8 pfu/mL and 10^8, 10^8, 10^8 pfu/mL. The volume of virus injected depended on tumor size (see below).

Patients were assessed clinically, an electrocardiogram and a computed tomography scan were also done, as well as routine hematology and biochemistry and the other tests described below.

OncoVEX$^{GM\text{-}CSF}$ handling and injection. Virus was stored at 1×10^8 pfu/mL at −70°C, diluted before use when necessary. The volume of injectate was based on the diameter of the target lesion (≤1.5 cm, up to 1 mL; >1.5 to ≤2.5 cm, up to 2 mL; >2.5 cm, up to 4 mL).

Patient monitoring. In the single-dose phase of the study, urine and blood samples were obtained for vector detection by PCR predose and at 1, 4, 8, 24, and 48 hours after injection and also at each follow-up visit when biochemical and hematologic tests were also done and the dressing and the injected lesion were swabbed for virus. A fine needle aspirate (FNA) was obtained for reverse transcription-PCR detection of GM-CSF at 48 hours postinjection. Patients were then discharged if they were well and if the 24-hour swab was negative for

Downloaded from http://aacrjournals.org/clincancerres/article-pdf/12/22/6737/1921819/6737.pdf by guest on 04 December 2024

vector. Biopsies were obtained at 2 weeks postinjection and patients were seen for the last time at 6 weeks postinjection, unless otherwise clinically required. For the multidose phase of the study, follow-up was similar, but without taking an FNA for the detection of GM-CSF. Injections were given at intervals of 1 to 3 weeks provided that patients were clinically well, the injected site had recovered from the previous injection, and HSV-seronegative patients had seroconverted after the first injection.

Cytokines and serology. FNA samples were analyzed at Lark Technologies, Inc. (Houston, TX) using a quantitative reverse transcription-PCR assay for GM-CSF, giving relative mRNA levels (presented as the level relative to patient 101 in Fig. 1). Blood samples were assayed for multiple cytokines using BD Cytometric Bead Arrays (BD Biosciences, San Jose, CA) predose and at 1, 2, and 6 weeks. HSV serology was assayed by indirect ELISA (Kalon Biological, Ltd., Aldershot, United Kingdom). Results are expressed as a proportion of a cutoff-level reference serum (Fig. 2). Patients with levels below this reference serum were thus classified as HSV seronegative.

Virus detection. DNA was extracted from 200 μL blood (QIAmp DNA Blood Mini Kit) or urine (QIAmp Viral RNA Mini Kit) and tested by quantitative PCR using primers specific for the cytomegalovirus promoter region of the vector. Quantitative PCR was done by Covance, Ltd., Swabbing (Virocult, Medical Wire & Equipment Co., Ltd., Corsham, Wiltshire, United Kingdom), and plaque assay on BHK-21 cells was used to detect virus on injected tumors, dressings, and new lesions at the time points indicated above.

Histology. Where possible, excision biopsies were obtained from patients ~3 weeks after the last injection. Specimens were fixed in 10% neutral buffered formalin and assessed for the degree of necrosis, immune cell infiltration, and stained using a polyclonal anti-HSV antibody (DAKO, Glostrup, Denmark). Immune infiltrates in the virally treated tumors were also compared with those seen in archival specimens from a number of patients with breast cancer.

Results

Patient characteristics

Twenty-six of the 30 patients enrolled were evaluable. Patient details are given in Table 1. Eleven patients were seronegative for HSV at enrollment and 19 were seropositive, reflecting the serologic status of the general population. Four patients were nonevaluable due to disease progression, which in one case meant that a final biopsy could not be obtained. However, for the sake of completeness, data with regard to side effects, tumor responses, and other variables are included for these non-evaluable patients where possible.

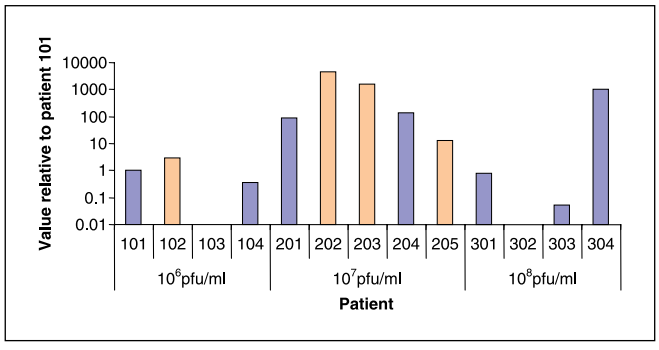

Fig. 1. Human GM-CSF RNA levels. Relative human GM-CSF – specific mRNA levels were measured in FNA samples taken from patient tumors by reverse transcription-PCR 48 hours postinjection with OncoVEX^{GM-CSF}. Blue columns, initially HSV-seropositive patients; pink columns, initially HSV-seronegative patients.

Side effects and adverse events

Single-dose group. The main side effects (see Table 2A) were grade 1 pyrexia and associated constitutional symptoms, which were more common and pronounced in HSV-seronegative patients. Only a single patient (patient 301) experienced grade 2 pyrexia, at 10^8 pfu/mL, associated with rigor, hypotension and tachycardia, and other constitutional symptoms. The other more common side effects were low-grade anorexia, nausea and vomiting, and fatigue. Two breast cancer patients developed abnormal liver function tests. In patient 102, this was abnormal predose that increased 5-fold 2 weeks after injection, remaining elevated at final follow-up. Both transaminases became more deranged (2-fold) at the same time point but returned to the predose level 2 weeks later. For patient 203, previously normal alkaline phosphatase had increased 2-fold, aspartate aminotransferase 9-fold, and alanine aminotransferase 20-fold at final follow-up, which may have been associated with disease progression.

The other main side effect was the local reaction at the injected tumor site (data included in Table 1). The injected lesion became inflamed in all but one patient starting 2 days after the injection and usually subsiding by 1 week for seropositive patients. For seronegative patients, inflammation and erythema was more pronounced, particularly at 10^7 pfu/mL, persisting for at least 2 weeks with extensive, moderate inflammation, and erythema of the surrounding tissue and sometimes areas distant from the injected lesion that did not have clinical disease. The injected lesion ulcerated in five patients (both seropositive and seronegative), generally healing some weeks later. Small vesicles appeared transiently, especially around the injected lesion, for 2 weeks after injection in three seronegative patients. These were negative for the presence of the virus as assessed by swabbing and plaque assay.

Due to the local reactions of inflammation and erythema in and surrounding injected lesions in seronegative patients at 10^7 pfu/mL (considered adverse events under the protocol), which were much less pronounced in seropositive patients, the maximum tolerated dose for seronegative patients was deemed to be 10^7 pfu/mL. The dose 10^8 pfu/mL was, therefore, only given to seropositive patients for whom no maximum tolerated dose was determined.

Multidose group. The initial protocol anticipated giving three doses of 10^8 pfu/mL to all patients. However, in view of the extensive inflammation and erythema in and around injected tumors seen in HSV-seronegative patients at 10^7 pfu/mL (see above), this was changed such that HSV-seronegative patients were initially given a dose of 10^6 pfu/mL to seroconvert (see Materials and Methods). Thus, in the multidose phase of the study, HSV-seronegative patients were given 10^6, 10^7, 10^7 pfu/mL and 10^6, 10^8, 10^8 pfu/mL, whereas HSV-seropositive patients were given10^8, 10^8, 10^8 pfu/mL and 10^6, 10^8, 10^8 pfu/mL. The 10^6, 10^8, 10^8 pfu/mL regimen was tested in HSV-seropositive patients with the intention of identifying a dosing regimen appropriate for all patients, irrespective of their prior HSV serostatus, which was achieved.

Each of the regimens tested was well tolerated with no dose-limiting toxicity seen. Particularly, in HSV-seronegative patients, the extensive and sometimes prolonged local

Downloaded from http://aacrjournals.org/clincancerres/article-pdf/12/22/6737/1921819/6737.pdf by guest on 04 December 2024

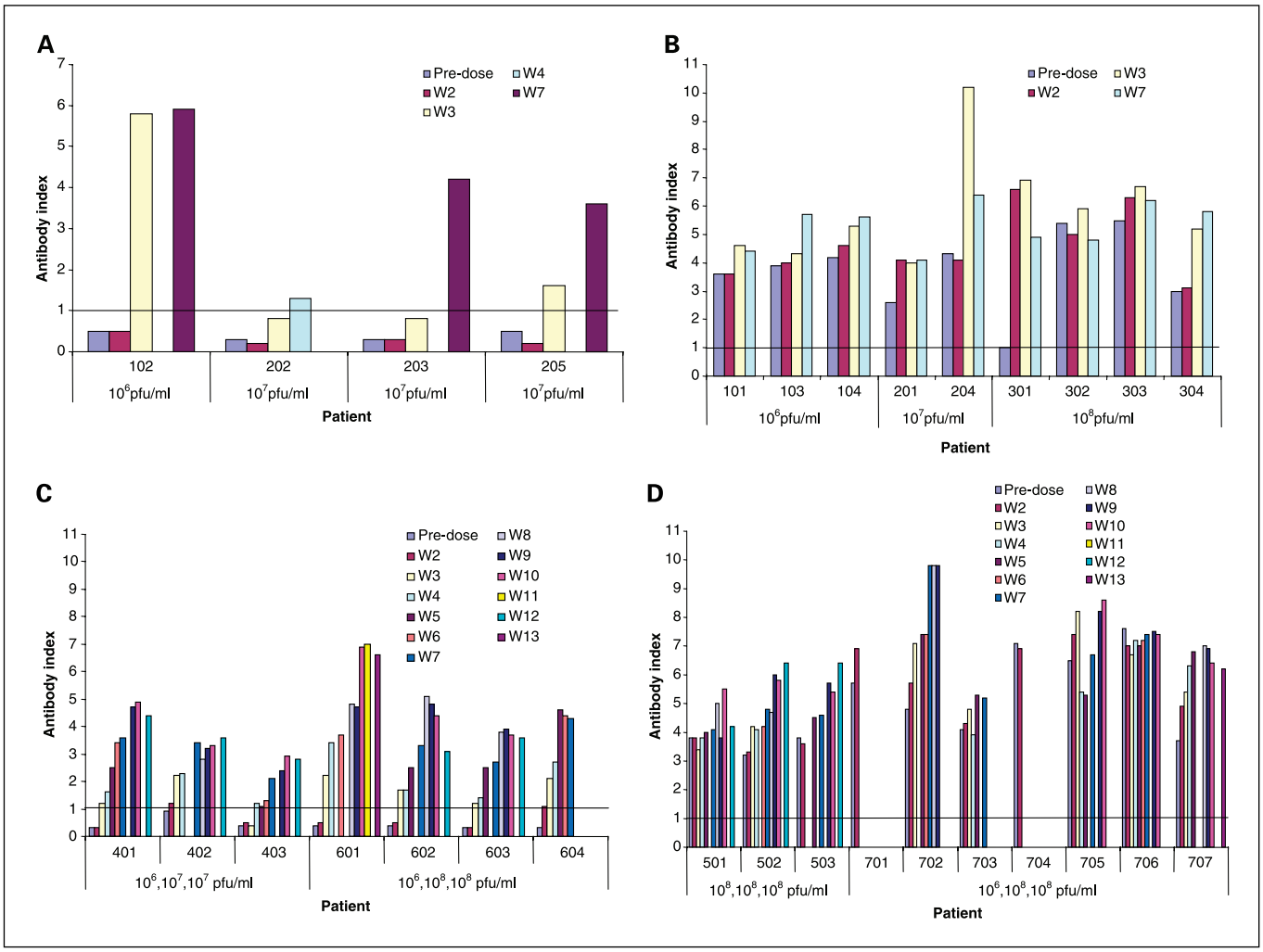

Fig. 2. Patient anti-HSV antibody levels before and after dosing. *A,* initially seronegative patients, single-dose group. *B,* initially seropositive patients, single-dose group. *C,* initially seronegative patients, multidose group. *D,* initially seropositive patients, multidose group. The anti-HSV antibody index is shown for each of the doses at the time points indicated.

reactions seen at 10^7 pfu/mL in the single-dose phase of the study were not evident, and febrile responses were reduced. Overall, however, the side effects in the multidose phase of the study (Table 2B) were similar to patients who received a single dose, although, as before, these were still more evident in patients who were initially HSV seronegative. Most injected lesions became inflamed after each dose. Two HSV-seronegative patients (patients 401 and 602) developed extensive inflammation of the surrounding tissue 1 week after the first injection, which lasted for a further week, although less severe and of shorter duration than seen with a 10^7 pfu/mL single dose. For patient 603, nearby uninjected lesions also became mildly inflamed as did a cervical lymph node distant from the injected lesion in patient 402. A subclinical noncontiguous lesion also became inflamed after the first injection in patient 501. Ulceration occurred in the injected lesion of six patients. Mild pain was experienced transiently in 11 patients, and also in noninjected adjacent lesions after the second and third dose in patient 703. Vesicles were only seen in one patient in the multidosing phase of the study (patient 401).

Other side effects, which may or may not have been treatment related, were noted in patient 502 consisting of red palms, swollen hands, and desquamation of palms and soles. These side effects were also noted in patient 601 (colorectal cancer, with liver metastases) whose already deranged alkaline phosphatase increased 2-fold after the second injection as did previously normal aspartate aminotransferase and alanine aminotransferase. These returned to predose levels by the end of the study.

Finally, patient 706, who had a history of cold sores, developed lesions on the lip reminiscent of HSV infection 48 hours after the second and third injection and also on the cheek after the third injection. These tested negative for HSV by plaque assay and PCR.

HSV serology

All seronegative patients strongly seroconverted 3 to 4 weeks after their first dose to a similar level to patients who were originally seropositive (Fig. 2). For seropositive patients, the general trend was an increase in anti-HSV antibody index with each injection, which eventually plateaued. Thus, with respect

Downloaded from http://aacrjournals.org/clincancerres/article-pdf/12/22/6737/1921819/6737.pdf by guest on 04 December 2024

Table 2. Side effects

(A) Single-dose patients

Adverse events	HSV seropositive (n = 9)		HSV seronegative (n = 4)	
	Grade 1/2	Grade 3/4	Grade 1/2	Grade 3/4
Constitutional				
Fever	1		3	
Rigor	1			
Sweating			1	
Fatigue	1		3	
Arthalgia	1			
Myalgia	1			
Cardiovascular				
Tachycardia	1		2	
Hypotension	1			
Anemia	3		1	
Transfusion reaction	1		1	
Hypertension	1			
Leg swelling			1	
Respiratory				
Dyspnea		1	1	1
Cough		1	2	
Pleural effusion				2
Pneumonia	1		1	
Gastrointestinal				
Anorexia	3		2	
Nausea	3		1	
Vomiting	1		1	
Constipation	2		1	
Diarrhea	3			
Ascites				1
Dehydration	1		1	
Abdominal pain			1	
Oral candidasis			1	
Aphthous ulcer	1			
Central nervous				
Headache			1	
Drowsiness	1			
Psychiatric				
Insomnia	5		1	
Endocrine				
Diabetes			1	
Musculoskeletal				
Back pain			1	
Cramp			1	
Cancer related				
Pain	2		1	
Progression	5	3	2	1

(Continued on the following page)

to the optimized dosing regimen described above, where patients are first given a lower dose of 10^6 pfu/mL before higher doses of 10^8 pfu/mL, the initial injection reliably induces a strong anti-HSV immune response, as evidenced by increase in antibody titer. However, although this reduces the side effects seen (particularly the local erythmatous reaction at the injection site), this does not appear to affect the extent of the other clinical responses seen (see below), including the degree of HSV antigen staining or necrosis observed at the injected tumor site.

GM-CSF expression

The level of GM-CSF mRNA was detected in injected tumors by reverse transcription-PCR from FNAs taken 48 hours after injection in the single-dose group. The results are shown in Fig. 1 where the signal relative to that observed in patient 101 is shown in each case, the results from the patients in the 10^6 pfu/mL group therefore providing a control for whether the signals detected in the higher dose groups are OncoVEX$^{GM\text{-}CSF}$ derived, there being no non-injected tumor biopsies available for comparison. The level of GM-CSF mRNA detected seemed to be dose related in the 10^6 and 10^7 pfu/mL dose groups (the highest signal in the 10^7 pfu/mL group being >1,000 times the highest signal in the 10^6 pfu/mL group), but without a further increase at 10^8 pfu/mL (the highest signal in the 10^7 and 10^8 pfu/mL group was similar, although with greater variability at 10^8 pfu/mL). The variability in expression levels within the groups could have been due to differences in either the tumor, injection, or biopsy procedure, or the HSV serology status of the

Downloaded from http://aacrjournals.org/clincancerres/article-pdf/12/22/6737/1921819/6737.pdf by guest on 04 December 2024

Table 2. Side effects (Cont'd)

(B) Multidose patients

Adverse events	HSV seropositive (n = 10)		HSV seronegative (n = 7)	
	Grade 1/2	Grade 3/4	Grade 1/2	Grade 3/4
Constitutional				
Fever	4	1	6	
Rigor	3		4	
Sweating			2	
Fatigue	3		2	
Arthalgia	2		2	
Myalgia	0		1	
Cardiovascular				
Tachycardia	1		1	
Hypotension			1	
Anemia	2		2	
Hypertension	1			
Leg swelling				
Lymphadema			1	
Gastrointestinal				
Anorexia			2	
Nausea	1		2	
Vomiting	1		3	
Constipation	1		1	
Diarrhea	1			1
Dehydration	1			
Abdominal pain	1		1	
Oral candidasis			1	
Respiratory				
Dyspnea		1	1	
Cough	1		1	
Pleural effusion			1	
Pneumonia				
Central nervous				
Headache	2		2	
Drowsiness				
Seizure		1		
Psychiatric				
Insomnia			1	
Depression	1			1
Musculoskeletal				
Back pain	2			
Ear, nose, and throat				
Conjunctivitis			1	
Mucositis			1	
Skin				
Red swollen palm	1			
Desquamation	1			
Eczema	1			
Allergy				
Dressing	1			
Drug			1	
Cancer related				
Pain			1	
Bleeding			2	
Progression	1	1	2	

patient, and probably also reflected the relatively small number of patients in the single dosing phase of the clinical trial. GM-CSF levels as assessed by ELISA were below the limit of detection in patient blood samples at all time points tested.

Cytokine and antinuclear antibody levels

No treatment-related changes in the levels of the cytokines measured were observed; thus, these results are not shown. This may have been due to the time points used (predose and 1, 2, and 6 weeks after dose); whereas other studies have found effects on cytokine levels, these have been earlier after injection than the earliest time point measured in the study reported here (27). In addition, no clinically significant effects on antinuclear antibody levels were observed (results not shown).

OncoVEX^{GM-CSF} biodistribution

Virus was assayed on the tumors, the occlusive dressing, and, where required, at other sites by swabbing followed by plaque assay. In the single dosing phase of the study, virus was detected at low levels on the tumor surface for up to 2

Downloaded from http://aacrjournals.org/clincancerres/article-pdf/12/22/6737/1921819/6737.pdf by guest on 04 December 2024

weeks in 3 patients, which, due to the fact that detectable virus levels initially reduced following injection, suggested virus replication. This, together with the timing of the local reactions seen, suggested that a 2- to 3-week interval between doses was appropriate, which was therefore used in the multidosing phase. In the multidosing phase, virus was only detected on the surface of the tumor of one patient at a single time point at a very low level (7.5 pfu per swab). This difference between the single and multidose patients was attributed to the optimized dosing regimens used (i.e., initially dosing with 10^6 pfu/mL before moving to higher doses), and the absence of a FNA, which prevented virus leakage through the puncture site. A vesicle adjacent to the tumor and under the occlusive dressing also tested positive at a similarly low level in this same patient, which could have been due to cross-contamination from the tumor as all other vesicles from this or other patients tested negative for the presence of the virus. Virus was not detected outside the occlusive dressing in any case during the study.

Virus DNA was not routinely detectable in blood or urine in any part of the study. In the single-dose phase of the study, virus was only detected in the blood of two patients between 8 hours and 1 week after injection. In the multidose phase of the study, virus was detected in the blood of eight patients 1 to 8 hours postdose, which was somewhat more evident in HSV-seronegative patients. Virus DNA was even more rarely detected in urine with only two patients testing positive at very low levels 8 hours to 1 week after injection in the single-dose group, and no patient tested positive in the multidose group. The injected virus, therefore, in most cases, seems to be retained at the injection site.

Clinical responses

There were no complete or partial responses in the study although stable disease was observed in three patients; in a number of cases, injected lesions either flattened or did not progress further. The responses observed are detailed below.

In the single-dose group, disease stabilized in patient 103 (breast cancer). For patient 102 (breast cancer), an extensive intradermal infiltration of breast carcinoma dissipated 2 weeks following dosing, which was maintained until the end of the study, although other lesions appeared. Other than where ulceration occurred as discussed above, and the histologic evidence of necrosis as discussed later, lesions were transiently flattened in three patients [patients 102 (breast cancer), 205 (melanoma), and 301 (melanoma); Fig. 3A].

In the multidose group, patients 501 and 706 with malignant melanoma had stable disease, but disease progressed in other patients. The one superficial lesion in patient 602 (breast cancer), which was injected, was stable until final follow-up, but disease progressed in the liver. Likewise, the injected lesion of patient 707 (breast cancer) remained stable but disease progressed elsewhere. As well as generalized stable disease, the injected and adjacent tumors of patient 501 also flattened as did the injected and adjacent tumors in patient 401 (breast cancer; Fig. 3B) and the injected lesion in patient 706. In Fig. 3B, it is evident that effects are observed up to ~6 cm from the injection site, which may have resulted from passage of virus through tumor tissue under the skin. These tumors in patient 401 did not regrow although disease progressed elsewhere. In patient 604 (melanoma), an uninjected lesion shrunk and disappeared, but other lesions continued to progress.

Interesting effects were seen in patient 705 (melanoma) in which the injected lesion was enlarged and engorged after the second injection, became fluctuant, and a large volume of fluid was aspirated, which was sterile. At excision, the lesion was found to be largely necrotic with only a rim of viable tumor cells (Fig. 4A). Disease progressed elsewhere.

Overall, there were no obvious differences in the clinical responses observed between HSV-seronegative or HSV-seropositive

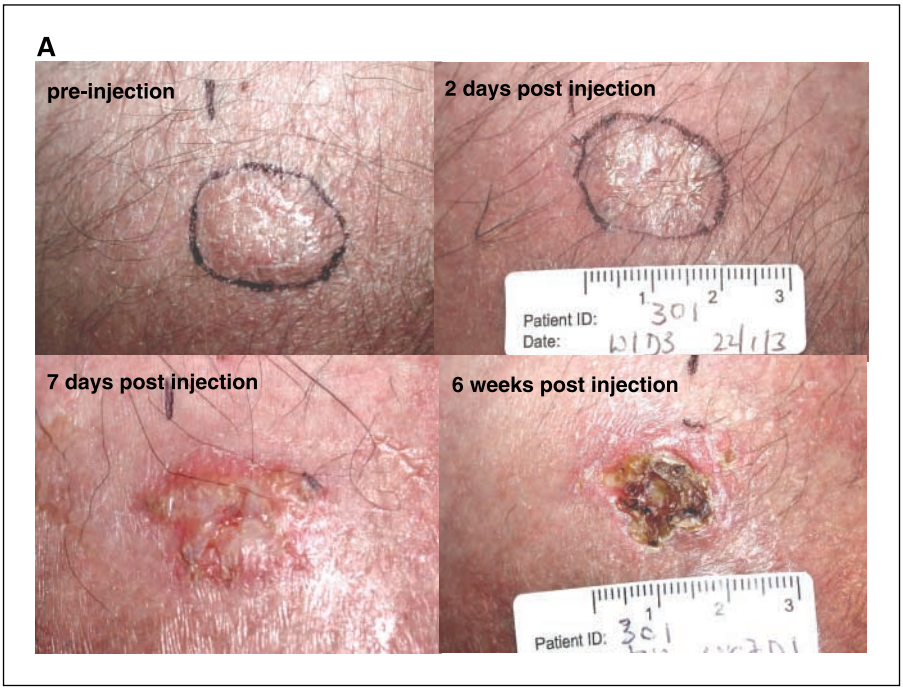

Fig. 3. Gross effects in injected tumors. *A,* patient 301. Melanoma, initially seropositive, dosed with a single dose of 10^8 pfu/mL.

Downloaded from http://aacrjournals.org/clincancerres/article-pdf/12/22/6737/1921819/6737.pdf by guest on 04 December 2024

patients, although side effects and virus detectable on the injected tumor surface were reduced in seronegative patients using the optimized dosing regimens, or between the different tumor types enrolled, which extended to the observations on the tumor biopsies described below.

Tumor biopsies

The final tumor biopsies were stained for the presence of T cells and macrophages. Although the degree of staining in each case was not obviously different between tumor types or dosage groups, in most cases, the degree of infiltration was concluded to be high by the examining histologist. However, the lack of preinjection biopsies generally prevented definitive conclusions being made other than for some of the breast cancer patients where preinjection biopsies were available. In these cases, it was evident that the level of infiltrating cells was generally higher postinjection than before (data not shown).

Biopsies were also examined for necrosis and the presence of HSV (Fig. 4). In most biopsies where tumor tissue was present, areas of necrosis (or in one case apoptosis) were seen (14 of 19 biopsies), which, in many cases, were considerable (Fig. 4A and B). Staining for HSV (Fig. 4C) showed the following observations: (*a*) in all biopsies where necrosis was present, HSV staining was also seen; (*b*) normal tissue showed no evidence of necrosis; (*c*) HSV staining was only rarely seen in nonnecrotic tumor tissue and even more rarely in nontumor tissue. Taken together, these suggest that HSV antigens were selectively expressed in tumor tissue, presumably accompanying virus replication, and thus that the virus is very likely to have caused the often very considerable necrosis seen.

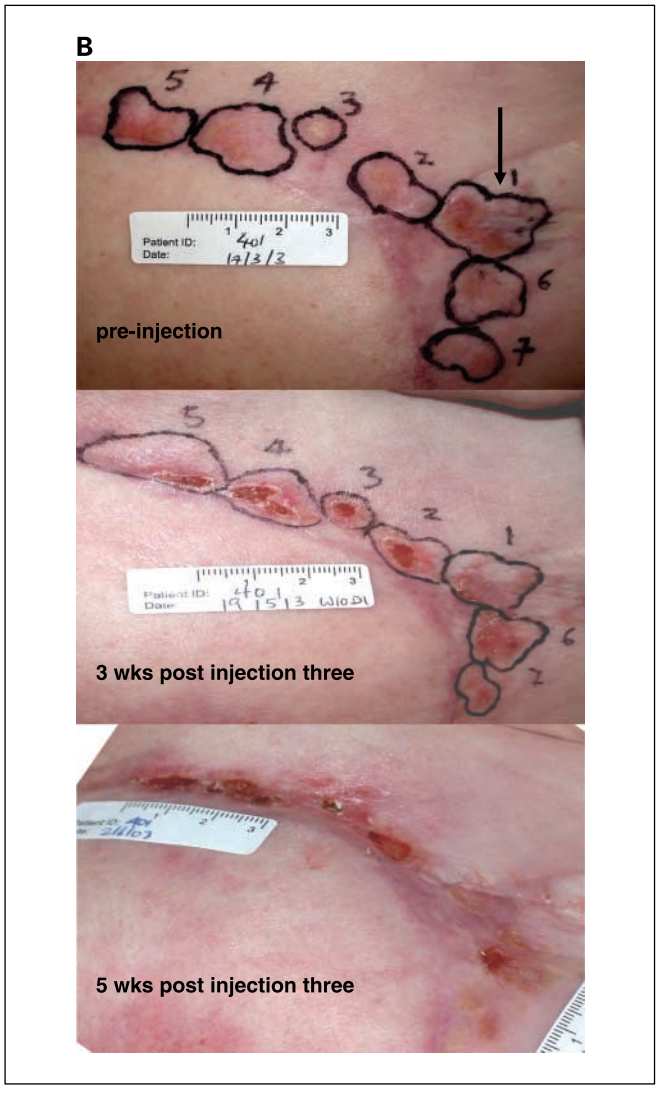

Fig. 3 *Continued. B,* patient 401. Breast cancer, initially seronegative, dosed with 10^6, 10^7, 10^7 pfu/mL. The injection was made into tumor 1 (*arrow*).

Discussion

This is the first reported clinical trial with an oncolytic HSV carrying a transgene or with a second-generation virus with improved oncolytic properties. The study was designed primarily to assess the safety and biodistribution of local delivery of the virus and secondarily to assess any level of biological activity.

We found that the virus was well tolerated, although side effects were more marked in patients who were previously seronegative for HSV, that GM-CSF was expressed, and that evidence of antitumor activity was seen.

The most common systemic side effects were low-grade constitutional/flu-like symptoms. This result, although similar in patients treated with all other oncolytic viruses, including adenovirus (28–30), reovirus (31), Newcastle disease virus (32), and vaccinia (33–35), contrasts with other clinical trials with oncolytic HSV where side effects were reported rarely. However, there are significant differences in these trials, such as the use of very low doses of virus in melanoma (10^3 pfu) or injection into the brain (6–8, 10, 36).

Most other studies with oncolytic viruses have not reported the extensive local reactions seen here, except for a study with a vaccinia virus incorporating GM-CSF in patients with melanoma (37). The local reactions seen could, therefore, at least in part, be GM-CSF mediated and were ameliorated through the multidosing regimen developed in which seronegative patients were first seroconverted with an initial dose of 10^6 pfu/mL. A regimen of one dose of 10^6 pfu/mL followed by multiple doses of 10^8 pfu/mL was identified as being well tolerated and appropriate for all patients and is thus intended for use in future studies. This dosing regimen also appeared to reduce the likelihood of detecting viral DNA in blood or urine, which was, in any case, relatively low, but did not seem to affect the degree of tumor necrosis seen following seroconversion to HSV. The timing of the local reactions, and in some cases indications of virus replication assessed by swabbing and plaque assay, suggested that injection every 2 to 3 weeks was appropriate, which is considerably less frequent than what has been found to be optimal with, e.g., oncolytic adenovirus, where dosing up to twice a day has been tested (38). In addition, in contrast to studies with oncolytic adenoviruses where the induction of antivirus antibodies has been thought undesirable, induction of antibodies in the study reported here was determined to be beneficial in reducing the side effects seen, but without obvious effects on the level of tumor necrosis generated.

Downloaded from http://aacrjournals.org/clincancerres/article-pdf/12/22/6737/1921819/6737.pdf by guest on 04 December 2024

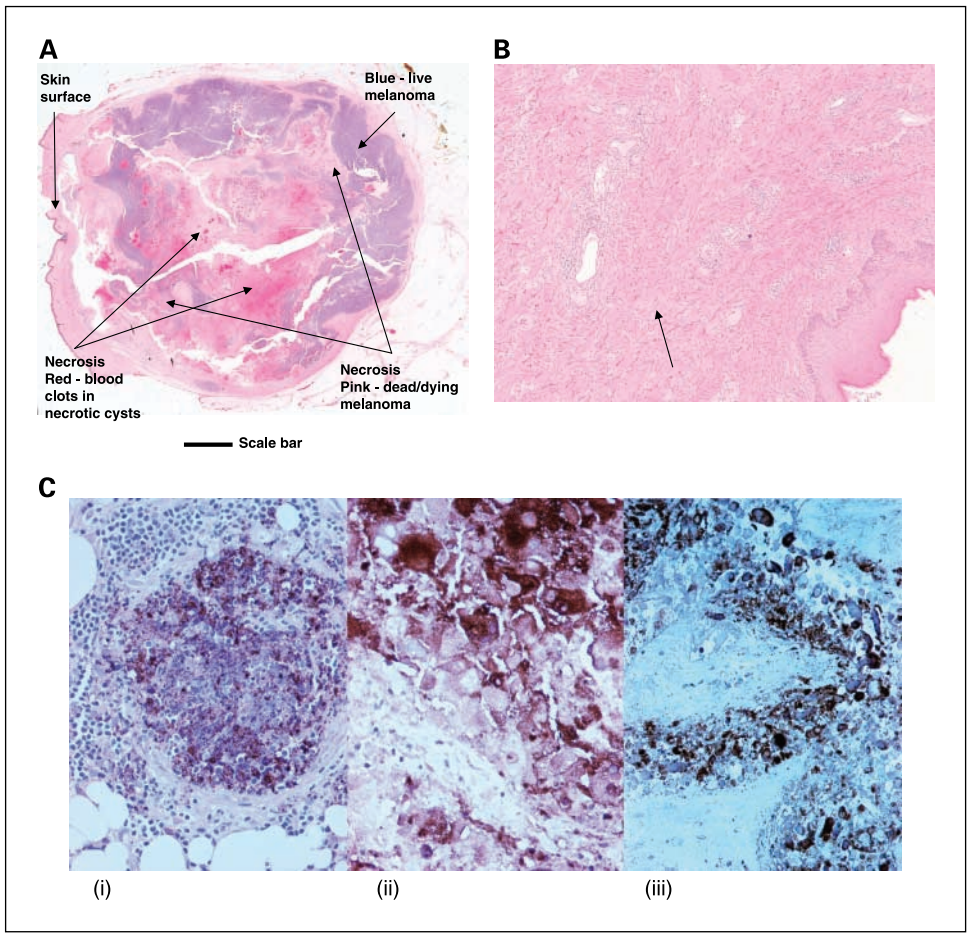

Fig. 4. Example histology, HSV antigen staining, and inflammatory infiltrates. *A,* whole-mount histology from patient 705 (melanoma) after excision of the tumor showing extensive necrosis. *B,* histology section from the same patient showing extensive necrosis and cystic degeneration (*pink, arrow*). *C,* example HSV antigen staining (*black/dark brown*) in necrotic (*i*) breast cancer (patient 201), (*ii*) melanoma (patient 205), and (*iii*) head and neck cancer (patient 702).

Bearing in mind the extensive local reactions in seronegative patients at 10^7 pfu/mL, and as these were associated with a low-grade fever, it was perhaps somewhat surprising that there was no associated effect on the levels of circulating inflammatory cytokines in the blood detected. In previous studies with the oncolytic adenovirus Onyx-015 (e.g., ref. 27), increases in inflammatory cytokines have been found associated with flu-like symptoms. However, in this case (27), blood samples were taken before injection and at 3 and 18 hours after injection, and elevated IFNγ, interleukin-6, and tumor necrosis factor were found at 3 hours after virus injection, which reduced toward baseline by 18 hours. However, in the study reported here, the first blood sample analyzed for cytokine levels was not taken until 1 week following OncoVEX$^{GM\text{-}CSF}$ injection. Thus, we would have been likely to miss any shorter-term changes in cytokine levels associated with OncoVEX$^{GM\text{-}CSF}$ injection in this case.

Histologic specimens from biopsies taken following injection showed inflammation and necrosis in the majority of cases where tumor was also detected (14 of 19 tumor biopsies, including one with apoptosis rather than necrosis noted). Necrosis was in some cases considerable, and we believe that the extent of necrosis observed has not been previously reported following oncolytic virus treatment, particularly when not combined with other therapy, such as chemotherapy or radiotherapy. Sections were also stained for the presence of HSV, and, wherever necrosis was seen, this colocalized for HSV. HSV was only rarely detected in nonnecrotic or nontumor tissue. Nontumor tissue also showed no evidence of necrosis, with or without associated staining for HSV. Taken together, these results strongly suggest that the virus caused the necrosis observed. Necrosis following administration of oncolytic HSV has previously been found in one patient with glioma (8) and in a study in melanoma with HSV 1716 (39). Necrosis has also been seen in clinical trials with Onyx-015, although only after frequent injection of high virus doses in conjunction with chemotherapy (38). This is again in contrast to one to three injections separated by 2 to 3 weeks used here.

In addition to the histologic effects, changes suggestive of clinical responses were observed in a number of patients, with three patients demonstrating stable disease and six patients showing flattening of injected tumors or uninjected tumors nearby. In a number of cases, injected tumors did not further progress, or first flattened and then did not further progress until at least the end of the follow-up period, although disease did progress elsewhere. However, as patients were only followed for 6 weeks following their final injection, longer-term conclusions cannot be made. Some evidence of a more distant, potentially immunomediated effect was observed as four cases of uninjected areas, including those with subclinical disease, became mildly inflamed.

In conclusion, OncoVEX$^{GM\text{-}CSF}$ is well tolerated using the multidosing protocol described, which has enabled the dosing

Downloaded from http://aacrjournals.org/clincancerres/article-pdf/12/22/6737/1921819/6737.pdf by guest on 04 December 2024

schedule for further studies to be defined. This indicates that dosing every 2 to 3 weeks is appropriate. Biological activity of the virus was observed, at doses as low as 10^6 pfu/mL, as evidenced by local reactions, tumor flattening, virus replication, GM-CSF expression, presence of inflammatory infiltrates, and often considerable HSV antigen-associated necrosis in tumor biopsies. Following these promising results, OncoVEX^{GM-CSF} is being entered into a number of phase II clinical trials in individual tumor types.

References

1. MacLean AR, ul-Fareed M, Robertson L, Harland J, Brown SM. Herpes simplex virus type 1 deletion variants 1714 and 1716 pinpoint neurovirulence-related sequences in Glasgow strain 17+ between immediate early gene 1 and the "a" sequence. J Gen Virol 1991;72:631–9.
2. Meignier B, Longnecker R, Roizman B. *In vivo* behavior of genetically engineered herpes simplex viruses R7017 and R7020: construction and evaluation in rodents. J Infect Dis 1988;158:602–14.
3. Meignier B, Martin B, Whitley RJ, Roizman B. *In vivo* behavior of genetically engineered herpes simplex viruses R7017 and R7020. II. Studies in immunocompetent and immunosuppressed owl monkeys (*Aotus trivirgatus*). J Infect Dis 1990;162:313–21.
4. Mineta T, Rabkin SD, Yazaki T, Hunter WD, Martuza RL. Attenuated multi-mutated herpes simplex virus-1 for the treatment of malignant gliomas. Nat Med 1995;1:938–43.
5. Kramm CM, Rainov NG, Sena-Esteves M, et al. Long-term survival in a rodent model of disseminated brain tumors by combined intrathecal delivery of herpes vectors and ganciclovir treatment. Hum Gene Ther 1996;7:1989–94.
6. Markert JM, Medlock MD, Rabkin SD, et al. Conditionally replicating herpes simplex virus mutant, G207 for the treatment of malignant glioma: results of a phase I trial. Gene Ther 2000;7:867–74.
7. Rampling R, Cruickshank G, Papanastassiou V, et al. Toxicity evaluation of replication-competent herpes simplex virus (ICP 34.5 null mutant 1716) in patients with recurrent malignant glioma. Gene Ther 2000;7:859–66.
8. Papanastassiou V, Rampling R, Fraser M, et al. The potential for efficacy of the modified (ICP 34.5(−)) herpes simplex virus HSV1716 following intratumoural injection into human malignant glioma: a proof of principle study. Gene Ther 2002;9:398–406.
9. Lin E, Nemunaitis J. Oncolytic viral therapies. Cancer Gene Ther 2004;11:643–64.
10. MacKie RM, Stewart B, Brown SM. Intralesional injection of herpes simplex virus 1716 in metastatic melanoma. Lancet 2001;357:525–6.
11. Mohr I, Gluzman Y. A herpesvirus genetic element which affects translation in the absence of the viral GADD34 function. EMBO J 1996;15:4759–66.
12. Leib DA, Harrison TE, Laslo KM, Machalek MA, Moorman NJ, Virgin HW. Interferons regulate the phenotype of wild-type and mutant herpes simplex viruses *in vivo*. J Exp Med 1999;189:663–72.
13. Leib DA, Machalek MA, Williams BR, Silverman RH, Virgin HW. Specific phenotypic restoration of an attenuated virus by knockout of a host resistance gene. Proc Natl Acad Sci U S A 2000;97:6097–101.
14. Haus O. The genes of interferons and interferon-related factors: localization and relationships with chromosome aberrations in cancer. Arch Immunol Ther Exp (Warsz) 2000;48:95–100.
15. Farassati F, Yang AD, Lee PW. Oncogenes in Ras signalling pathway dictate host-cell permissiveness to herpes simplex virus 1. Nat Cell Biol 2001;3:745–50.
16. Brown SM, MacLean AR, Aitken JD, Harland J. ICP34.5 influences herpes simplex virus type 1 maturation and egress from infected cells *in vitro*. J Gen Virol 1994;75:3679–86.
17. Cassady KA, Gross M, Roizman B. The herpes simplex virus US11 protein effectively compensates for the γ1 (34.5) gene if present before activation of protein kinase R by precluding its phosphorylation and that of the α subunit of eukaryotic translation initiation factor 2. J Virol 1998;72:8620–6.
18. Mulvey M, Poppers J, Ladd A, Mohr I. A herpesvirus ribosome-associated, RNA-binding protein confers a growth advantage upon mutants deficient in a GADD34-related function. J Virol 1999;73:3375–85.
19. Wong RJ, Patel SG, Kim S, et al. Cytokine gene transfer enhances herpes oncolytic therapy in murine squamous cell carcinoma. Hum Gene Ther 2001;12:253–65.
20. Toda M, Martuza RL, Rabkin SD. Tumor growth inhibition by intratumoral inoculation of defective herpes simplex virus vectors expressing granulocyte-macrophage colony-stimulating factor. Mol Ther 2000;2:324–9.
21. Parker JN, Gillespie GY, Love CE, Randall S, Whitley RJ, Markert JM. Engineered herpes simplex virus expressing IL-12 in the treatment of experimental murine brain tumors. Proc Natl Acad Sci U S A 2000;97:2208–13.
22. Bennett JJ, Malhotra S, Wong RJ, et al. Interleukin 12 secretion enhances antitumor efficacy of oncolytic herpes simplex viral therapy for colorectal cancer. Ann Surg 2001;233:819–26.
23. Andreansky S, He B, van Cott J, et al. Treatment of intracranial gliomas in immunocompetent mice using herpes simplex viruses that express murine interleukins. Gene Ther 1998;5:121–30.
24. Nemunaitis J. Vaccines in cancer: GVAX, a GM-CSF gene vaccine. Expert Rev Vaccines 2005;4:259–74.
25. Eager R, Nemunaitis J. GM-CSF gene-transduced tumor vaccines. Mol Ther 2005;12:18–27.
26. Liu BL, Robinson M, Han ZQ, et al. ICP34.5 deleted herpes simplex virus with enhanced oncolytic, immune stimulating, and anti-tumour properties. Gene Ther 2003;10:292–303.
27. Reid T, Galanis E, Abbruzzese J, et al. Hepatic arterial infusion of a replication-selective oncolytic adenovirus (dl1520): phase II viral, immunologic, and clinical endpoints. Cancer Res 2002;62:6070–9.
28. Reid T, Galanis E, Abbruzzese J, et al. Intra-arterial administration of a replication-selective adenovirus (dl1520) in patients with colorectal carcinoma metastatic to the liver: a phase I trial. Gene Ther 2001;8:1618–26.
29. Nemunaitis J, Cunningham C, Buchanan A, et al. Intravenous infusion of a replication-selective adenovirus (ONYX-015) in cancer patients: safety, feasibility and biological activity. Gene Ther 2001;8:746–59.
30. Ganly I, Kirn D, Eckhardt G, et al. A phase I study of Onyx-015, an E1B attenuated adenovirus, administered intratumorally to patients with recurrent head and neck cancer. Clin Cancer Res 2000;6:798–806.
31. Morris DG, Forsyth PA, Paterson KF, et al. A phase I clinical trial evaluating intralesional Reolysin (reovirus) in histologically confirmed malignancies [abstract]. J Clin Oncol 2002;92.
32. Pecora AL, Rizvi N, Cohen GI, et al. Phase I trial of intravenous administration of PV701, an oncolytic virus, in patients with advanced solid cancers. J Clin Oncol 2002;20:2251–66.
33. Machtiger NA, Pancake BA, Eberle R, Courtney RJ, Tevethia SS, Schaffer PA. Herpes simplex virus glycoproteins: isolation of mutants resistant to immune cytolysis. J Virol 1980;34:336–46.
34. Mastrangelo MJ, Maguire HC, Eisenlohr LC, et al. Intratumoral recombinant GM-CSF-encoding virus as gene therapy in patients with cutaneous melanoma. Cancer Gene Ther 1999;6:409–22.
35. Mukherjee S, Haenel T, Himbeck R, et al. Replication-restricted vaccinia as a cytokine gene therapy vector in cancer: persistent transgene expression despite antibody generation. Cancer Gene Ther 2000;7:663–70.
36. Harrow S, Papanastassiou V, Harland J, et al. HSV1716 injection into the brain adjacent to tumour following surgical resection of high-grade glioma: safety data and long-term survival. Gene Ther 2004;11:1648–58.
37. Mastrangelo MJ, Maguire HC, Lattime EC. Intralesional vaccinia/GM-CSF recombinant virus in the treatment of metastatic melanoma. Adv Exp Med Biol 2000;465:391–400.
38. Kirn D. Clinical research results with dl1520 (Onyx-015), a replication-selective adenovirus for the treatment of cancer: what have we learned? Gene Ther 2001;8:89–98.
39. McKie EA, MacLean AR, Lewis AD, et al. Selective *in vitro* replication of herpes simplex virus type 1 (HSV-1) ICP34.5 null mutants in primary human CNS tumours-evaluation of a potentially effective clinical therapy. Br J Cancer 1996;74:745–52.

Downloaded from http://aacrjournals.org/clincancerres/article-pdf/12/22/6737/1921819/6737.pdf by guest on 04 December 2024