Clinical Cancer Research

Phase I/II Study of Oncolytic HSV$^{GM\text{-}CSF}$ in Combination with Radiotherapy and Cisplatin in Untreated Stage III/IV Squamous Cell Cancer of the Head and Neck

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Abstract

Purpose: This study sought to define the recommended dose of JS1/34.5-/47-/GM-CSF, an oncolytic herpes simplex type-1 virus (HSV-1) encoding human granulocyte-macrophage colony-stimulating factor (GM-CSF), for future studies in combination with chemoradiotherapy in patients with squamous cell cancer of the head and neck (SCCHN).

Experimental Design: Patients with stage III/IVA/IVB SCCHN received chemoradiotherapy (70 Gy/35 fractions with concomitant cisplatin 100 mg/m^2 on days 1, 22, and 43) and dose-escalating (10^6, 10^6, 10^6, 10^6 pfu/mL for cohort 1; 10^6, 10^7, 10^7, 10^7 for cohort 2; 10^6, 10^8, 10^8, 10^8 for cohort 3) JS1/34.5-/47-/GM-CSF by intratumoral injection on days 1, 22, 43, and 64. Patients underwent neck dissection 6 to 10 weeks later. Primary end points were safety and recommended dose/schedule for future study. Secondary end points included antitumor activity (radiologic, pathologic). Relapse rates and survival were also monitored.

Results: Seventeen patients were treated without delays to chemoradiotherapy or dose-limiting toxicity. Fourteen patients (82.3%) showed tumor response by Response Evaluation Criteria in Solid Tumors, and pathologic complete remission was confirmed in 93% of patients at neck dissection. HSV was detected in injected and adjacent uninjected tumors at levels higher than the input dose, indicating viral replication. All patients were seropositive at the end of treatment. No patient developed locoregional recurrence, and disease-specific survival was 82.4% at a median follow-up of 29 months (range, 19-40 months).

Conclusions: JS1/34.5-/47-/GM-CSF combined with cisplatin-based chemoradiotherapy is well tolerated in patients with SCCHN. The recommended phase II dose is 10^6, 10^8, 10^8, 10^8. Locoregional control was achieved in all patients, with a 76.5% relapse-free rate so far. Further study of this approach is warranted in locally advanced SCCHN. *Clin Cancer Res; 16(15); 4005–15. ©2010 AACR.*

Squamous cell cancer of the head and neck (SCCHN) causes approximately 650,000 new cases and 350,000 deaths globally per annum (1). Locally advanced disease (stage III/IV) requires multimodality treatment involving radiotherapy with concomitant chemotherapy (CRT; refs. 2, 3). In patients with bulky cervical nodes, CRT is often followed by neck dissection (2, 4–11). Even with intensive treatment, 35% to 55% of patients develop locoregional or metastatic recurrence within two years (5, 12–15). New treatments that improve locoregional control are needed.

JS1/34.5-/47-/GM-CSF (OncoVEX$^{GM\text{-}CSF}$, BioVex Inc.) is a conditionally replication-competent herpes simplex type-1 virus (HSV-1) based on JS-1 HSV-1 (ECACC Accession Number 010102090; ref. 16). It has genetic deletions in ICP34.5, providing tumor-selective replication, and ICP47, which otherwise blocks antigen presentation to MHC class I and II molecules by inhibiting TAP1 and TAP2 transporters. ICP47 deletion also increases US11 expression, promoting tumor-selective growth. JS1/34.5-/47-/GM-CSF contains the human granulocyte/macrophage colony-stimulating factor (GM-CSF) coding sequence under the human cytomegalovirus immediate/early promoter (16). JS1/34.5-/47-/GM-CSF has two mechanisms of action: (*a*) direct oncolysis of infected tumor cells and (*b*) immune activation through tumor-associated antigen release and local virus-mediated GM-CSF expression (17).

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Translational Relevance

An oncolytic herpes simplex virus (HSV) vector expressing the human granulocyte-macrophage colony stimulating factor gene (JS1/34.5-/47-/GM-CSF) has been evaluated as a single agent in phase I (in a range of tumor types) and phase II (metastatic melanoma) studies. As a result, this agent is now undergoing phase III trial evaluation in metastatic melanoma. Oncolytic HSV also has the potential to enhance the therapeutic activity of conventional anticancer therapies, including radiation and chemotherapy. In this study, we show for the first time that JS1/34.5-/47-/GM-CSF can be safely combined with curative doses of chemoradiotherapy in patients with newly diagnosed head and neck cancers. The very high rates of pathologic complete response, the absence of locoregional recurrence, and the prolonged progression-free survival seen in two thirds of the patients strongly support further randomized phase II/III analysis of this approach. Such studies are currently being developed.

Local and distant antitumor immune responses have been reported in preclinical models and previous clinical studies (16, 18, 19). We hypothesized that these effects may cooperate with CRT to increase locoregional control in stage III/IV SCCHN. This premise has been supported by *in vitro* and *in vivo* studies in which cisplatin-based chemoradiotherapy combined with JS1/34.5-/47-/GM-CSF was safe and effective in immunocompetent mice bearing B16 and CT26 tumors.[6] Furthermore, given the fact that there is emerging consensus that the most likely future role for gene or viral therapies will lie in the context of combinations with standard modalities, such as radiotherapy and/or chemotherapy (20, 21), we felt that this study represented an important opportunity to test a novel viral agent in a curative treatment setting.

Intratumoral JS1/34.5-/47-/GM-CSF was tested in a phase I study in 30 patients with relapsed cutaneous/subcutaneous disease from various cancers (18). Biological activity was seen, such as tumor flattening/necrosis (including uninjected tumors), viral replication, GM-CSF expression, and virus-associated necrosis. An initial seroconverting dose of 10^6 pfu/mL, followed by subsequent doses of 10^8 pfu/mL, was defined as optimal. A phase II trial in melanoma using this regimen reported multiple objective responses (19), and a phase III study in melanoma is under way. In view of possible concerns regarding combining immunosuppressive chemotherapy and a replication-competent virus, we chose to undertake careful dose-escalation in this design (in line with our previous phase I single-agent study). Having considered the biological activity and safety profile of JS1/34.5-/47-/GM-CSF from previous studies (18, 19), we had no intention to dose-escalate to a maximum tolerated dose.

We have completed an open-label, ascending-dose phase I/II study of JS1/34.5-/47-/GM-CSF with CRT in stage III/IV SCCHN to define the safety and clinical activity of the combination.

Materials and Methods

Study design

This study was conducted in two centers in the United Kingdom. The primary objective was to establish the recommended phase II/III dose of JS1/34.5-/47-/GM-CSF with CRT. Other objectives were safety, tolerability, and clinical activity. The study (EudraCT2005-000777-21) was approved by the U.K. Gene Therapy Advisory Committee (GTAC105) and each center's independent ethics committee.

Key eligibility criteria

Patients were at least 18 years old, with histologically confirmed stage III/IVA/IVB SCCHN, Eastern Cooperative Oncology Group (ECOG) performance status of 0/1, and adequate renal, hepatic, and bone marrow function. Exclusion criteria were hearing impairment, platinum allergy, undifferentiated nasopharyngeal carcinoma, nodal disease that could threaten the airway in the event of swelling/inflammation postinjection, pregnancy, lactation, lack of effective contraception, major surgery within 14 days or intercurrent serious infections within 28 days of trial entry, HIV, hepatitis B/C or syphilis, and active autoimmune disease, herpes labialis, or dermatoses within 50 cm of target lesions. The protocol allowed patient withdrawal from virus treatment for Common Terminology Criteria for Adverse Events (CTCAE) v3 grade 3/4 treatment-related toxicity (except radiation-induced mucositis/skin reaction, alopecia, injection site reactions, fever, and vomiting).

Procedures

Preparation of JS1/34.5-/47-/GM-CSF. JS1/34.5-/47-/GM-CSF was produced in Vero cells and purified in accordance with clinical good manufacturing practice, stored at −70°C, and prepared for injection as described previously (18, 19).

Treatment. Cohorts of four patients received injections of up to four doses of virus into malignant cervical lymph node(s) using a dose escalation schedule (no injections were administered to mucosal disease): 10^6,10^6,10^6,10^6 pfu/mL (cohort 1); 10^6,10^7,10^7,10^7 pfu/mL (cohort 2); and 10^6,10^8,10^8,10^8 pfu/mL (cohort 3). An expansion/safety cohort of five patients then received the same dose schedule as cohort 3.

Maximum tolerated dose (MTD) was defined as the dose preceding that at which two or more dose-limiting toxicities (DLT) were recorded. DLT was defined as any ≥grade 3 nonhematologic toxicity except any grade injection site reaction, grade 3 arthralgia/myalgias, <1 week grade 3 fatigue, any grade alopecia, fever, or vomiting. Grade 3 or greater mucositis or skin reaction within radiation portals was not

[6] White et al., in preparation.

a DLT, unless it persisted >6 weeks after CRT and exceeded the anticipated reaction. JS1/34.5-/47-/GM-CSF–related nonhematologic toxicities (≥grade 2) requiring dose delays or treatment discontinuation were considered as DLT.

Tumors were injected through two needle punctures with injectate distributed along two to four tracks (depending on tumor size) during withdrawal. The same lesion(s) was injected on each occasion. If the lesion became impalpable, no more injections were given. Two patients (01/0011in cohort 3, 01/0013 in cohort 4) received injections in to two lymph nodes on each occasion. All injections were administered using only topical local anesthesia with lidocaine/prilocaine (Emla, Astra-Zeneca).

On days of JS1/34.5-/47-/GM-CSF administration, patients were admitted to the hospital and received radiotherapy, then viral injections in a side room, followed by cisplatin chemotherapy. Patients were hospitalized for at least 48 hours. This requirement was based on decisions taken by the Gene Therapy Advisory Committee and the local Genetically Modified Organisms Safety Committee. Swabs were taken from the injection site and exterior of the dressing at 24 and 48 hours and screened for JS1/34.5-/47-/GM-CSF. A negative 24-hour swab was needed for hospital discharge at 48 hours. A positive swab result led to repeats every 24 hours until two negatives were obtained. Patients were discharged from the hospital when their swabs were negative. Patients were monitored weekly where interval medical history, examination, and laboratory investigations were done.

Laboratory analyses

Swabs were tested for JS1/34.5-/47-/GM-CSF by plaque assay and blood by enzyme-linked immunosorbent assay (ELISA) to detect anti-HSV antibodies (18). Quantitative PCR (Q-PCR) was done on needle biopsies taken either during or after completion of study treatment in suitable patients. In practice, the relatively intense nature of the treatment schedule and the occurrence of chemoradiotherapy-related toxicity precluded needle biopsy during treatment in the majority of patients, with the main reason being patient choice. Surgical specimens were analyzed by H&E staining and immunohistochemistry for HSV-1 with a polyclonal antibody.

Analysis of surgical specimens. Tissue processing, embedding, section-cutting, and H&E staining were done using the standard procedures of the facilities. Immunohistochemical staining for HSV-1 used commercially sourced positive control tissue. Negative controls included staining without primary antibody (DakoCytomation B0114) and replacing primary HSV1 antibody with normal rabbit serum. An independent pathologist assessed slides for viable tumor and HSV-1 antigen. For Ki67 staining, deparaffinized formalin-fixed sections from seven cases were stained with monoclonal mouse antibody to Ki-67 (MIB1 clone, Dako) at 1:100 dilution, using heat-induced epitope retrieval, with appropriate positive and negative controls. Positive nuclear staining was scored, and cytoplasmic staining was disregarded.

Needle biopsy Q-PCR. Biopsies were snap-frozen postcollection. DNA was extracted using the Qiagen DNeasy kit. Amplification used AmpliTaq Gold DNA polymerase, AmpErase UNG, dNTPs passive reference 1 (ROX) buffer, and fluorescent primers specific to the cytomegalovirus (CMV) promoter. Positive controls were OncoVEX$^{GM\text{-}CSF}$ and plasmid pcDNA3-CMV-GFP, and negative control was human genomic DNA. Reaction conditions were: one cycle 50°C for 2 minutes, 95°C for 10 minutes, and then 40 cycles of 95°C for 15 seconds, 60°C for 1 minute.

Anti-HSV antibody analysis. Semiquantitative HSV-1 antibody measurement used indirect ELISA. Serum samples were added to HSV-1–coated microtiter plates. A peroxidise-conjugated antihuman immunoglobulin was used for detection. Optical densities (OD) were read and expressed as a proportion of reference: antibody index (AI) = average sample OD/average reference control OD, where seronegative = AI < 0.9, seropositive = AI > 1.1, and equivocal = AI 0.9 to 1.1.

Detection of replicating JS1/34.5-/47-/GM-CSF in swabs. Material from swabs and controls was applied to BHK cells and incubated (35-37°C, 16 hours). Cells were fixed and stained with primary rabbit α-HSV-1-FITC. The positive control was a virus reference standard (Onc904D) provided by the sponsor. Negative control was serum-free medium.

Tumor response evaluation

Initial assessment involved examination under anesthesia and contrast-enhanced computed tomography (CT) <3 weeks before commencing CRT. CT was repeated 3 weeks post-CRT. Response was assessed using Response Evaluation Criteria in Solid Tumors. Patients underwent repeat examination under anesthesia 6 to 8 weeks after CRT, at which time the primary tumor site was examined (and biopsied if abnormal) and a selective/modified radical neck dissection was done. Patients were followed for relapse and survival.

Statistics

In keeping with the standard design of phase I studies, descriptive statistics have been used to summarize toxicity, safety, and response end points. Survival analyses were based on the Kaplan-Meier method.

Results

Seventeen patients were enrolled and treated (Table 1). Study design is shown in Fig. 1. Seven of 15 (47%) patients with sufficient material for evaluation were positive for human papillomavirus (HPV) by *in situ* hybridization. There was insufficient material to confirm HPV status by p16 staining. Twelve patients completed cohorts 1, 2, and 3 without DLT. Five patients were then treated with the dose schedule of cohort 3, all without significant JS1/34.5-/47-/GM-CSF–associated toxicity. Fewer than four viral injections were delivered because of complete remission in six patients (35%): four patients received three injections (cohort 1, $n = 1$; cohort 2, $n = 2$; cohort 3/expansion, $n = 1$), one patient from cohort 2 received two

injections, and one patient from the expansion cohort received one injection. These data suggest a dose-related effect.

All patients experienced at least one treatment-emergent adverse event (AE): 86% were grade 1 or 2, but at least one grade 3 or 4 AE was observed in each patient. The investigators considered just two adverse events (pyrexia and fatigue) to be JS1/34.5-/47-/GM-CSF related and occurred in two or more patients. Across all cohorts and severity grades, the most frequent AEs were consistent with CRT delivery (Table 2). The grade 3 or 4 AEs observed in ≥2 patients were unrelated to JS1/34.5-/47-/GM-CSF dose (Supplementary Table S1).

All patients received full-dose radiotherapy without interruptions. One patient with ototoxicity switched from cisplatin to carboplatin (AUC5). The other 16 patients received three infusions of cisplatin; 5 had a single ≈25%

Table 1. Patient characteristics at baseline

Characteristic	JS1/34.5-/47-/GM-CSF dose group				All patients (*n* = 17)
	Cohort 1 (*n* = 4)	Cohort 2 (*n* = 4)	Cohort 3 (*n* = 4)	Cohort 4 (*n* = 5)	
	No.	No.	No.	No.	No. (%)
Age, years					
Median	59.0	54.5	61.0	47.0	58.0
Range	55-64	50-74	50-64	41-59	41-74
Sex					
Male	4	3	3	5	15 (88)
Female	0	1	1	0	2 (12)
Race					
White	4	4	4	5	17 (100)
Black	0	0	0	0	0 (0)
Other	0	0	0	0	0 (0)
ECOG performance status					
0	3	1	2	5	11 (65)
1	1	3	2	0	6 (35)
Primary tumor site (subsite)					
Oropharynx (palatine tonsil)	1	2	3	3	9 (53)
Oropharynx (base of tongue)	1	0	1	2	4 (24)
Supraglottis	1	1	0	0	2 (12)
Hypopharynx (pyriform fossa)	1	1	0	0	2 (12)
T stage					
T_0	0	0	0	0	0 (0)
T_1	0	0	0	0	0 (0)
T_2	2	3	2	4	11 (65)
T_3	1	1	1	1	4 (23)
T_4	1	0	1	0	2 (12)
N stage					
N_0	0	0	0	0	0 (0)
N_1	0	0	0	1	1 (6)
N_{2a}	0	0	1	0	1 (6)
N_{2b}	0	2	1	2	5 (29)
N_{2c}	3	2	1	1	7 (41)
N_3	1	0	1	1	3 (18)
Tumor stage					
III	0	0	0	1	1 (6)
IVA	3	4	3	3	13 (78)
IVB	1	0	1	1	3 (18)
IVC	0	0	0	0	0 (0)

NOTE: Cohort 1, 10^6 pfu/mL on four occasions, to a maximum of 4 mL per dose. Cohort 2, 10^6 pfu/mL on one occasion followed by 10^7 on three occasions, to a maximum of 4 mL per dose. Cohort 3, 10^6 pfu/mL on one occasion followed by 10^8 on three occasions, to a maximum of 4 mL per dose. Cohort 4, 10^6 pfu/mL on one occasion followed by 10^8 on three occasions, to a maximum of 8 mL per dose.

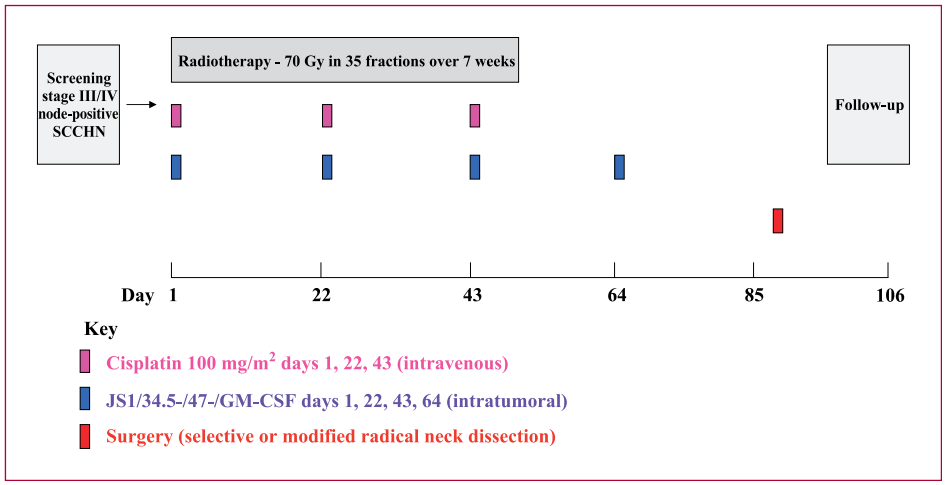

Fig. 1. Treatment schedule for cisplatin-based chemoradiation and concomitant intratumoral injections of JS1/34.5-/47-/GM-CSF, followed by planned neck dissection. Patients received full-dose radical chemoradiotherapy comprising 70 Gy in 35 daily fractions given 5 days per week (Monday to Friday) over 7 weeks with cisplatin given on days 1, 22, and 43 at a standard dose (100 mg/m^2 body surface area). Virus was injected on days 1, 22, 43, and 64 (this last dose was administered 2 weeks after the end of CRT). Injected volumes were based on the following algorithm: 0.5 mL for nodes of 0.5 to 1.5 cm longest dimension; 1 mL for nodes of 1.6 to 2.5 cm longest dimension; 2 mL for nodes >2.5 cm longest dimension. The maximum virus dose delivered on any one treatment day was 4 mL. Patients were scheduled to undergo resection of involved cervical lymph nodes 6 to 8 weeks after the end of CRT.

dose reduction for neutropenia ($n = 4$) or vomiting ($n = 1$). ECOG score decreased in 11 patients (65%), by one ($n = 10$) or two ($n = 1$) grades. Body weight fell in all patients (≤10%, $n = 6$; >10%-≤20%, $n = 10$; >20%, $n = 1$). These rates are slightly higher than those seen in 99 patients involved in four other studies of concurrent chemoradiotherapy in our unit during the same time, where the corresponding incidences were 56%, 40%, and 3% for <10%, 10% to 20%, and >20% body weight loss, respectively (22).[7]

Viral shedding from the injected site occurred in three patients. Patient 001-0006 was positive 24 and 96 hours (one of two injection site swabs) after the first virus dose. Swabs at 48, 72, 144, and 168 hours were negative. Patient 002-0002 was positive 24 hours after the second virus injection, but swabs at 48, 72, and 96 hours were negative. Patient 001-0014 was positive 48 (one of two swabs, 975 pfu/swab) and 144 hours (one of two swabs, 190 pfu/swab) after the third virus injection, but swabs were negative at 168, 192, and 216 hours. Positive swab results were never obtained from the exterior of the dressing.

Tumor samples were obtained for Q-PCR analysis from injected and uninjected nodes in 16 patients prior to surgery (needle biopsy; $n = 3$), at neck dissection ($n = 12$), or both ($n = 1$). HSV was detected by Q-PCR in 7 of 16 patients (Fig. 2). These samples were obtained at a median of 27 days (range, 0-96 days) after JS1/34.5-/47-/GM-CSF injection. Intervals between last injection and tissue sampling, sites of tissue sampling, and HSV genome copy number are provided in Supplementary Table S2. HSV was detected in two of four patients with presurgery samples, at 1.07×10^7, and 6.0×10^4 genome copies/μg at 21 and 14 days after last viral injection. This equates to 6.87×10^9 and 4.09×10^7 copies/g of tumor (assuming average DNA yield of 600 mg/g of tissue processed; range, 0.2-1.2 mg; Qiagen). For 13 patients sampled at neck dissection, HSV was detected in 5 (range, 2.27×10^3 to 6.02×10^6 genome copies/μg; 1.45×10^6 to 3.85×10^9/g of tumor) between 20 and 48 days after last viral injection. These DNA levels strongly suggest virus replication as a maximum of 3×10^9 genomes were administered per mL of tumor based on a particle:pfu ratio of 30.

Detailed histopathologic analysis of neck dissections is presented in Supplementary Table S3. On H&E staining, all showed extensive treatment-related changes. Viable tumor was seen in injected/uninjected nodal tissue in only 1 of 15 patients who underwent neck dissection (two patients with excellent responses refused surgery). This equates to a pathologic complete response rate of 93%. Nodes showed marked necrosis (Supplementary Fig. S1A, B, and D), often with tumor "ghost" cell outlines (Supplementary Fig. S1D, thin arrow) or fibrosis with chronic inflammation, including giant cell reaction (Supplementary Fig. S1C and D), and hemosiderin deposition/cholesterol clefting (Supplementary Fig. S1C). All cases additionally tested for Ki67, including those with ghost cells, showed complete absence of nuclear Ki67 staining (Supplementary Fig. S1E). This contrasted with moderate staining in small lymphocytes in surrounding inflammatory tissue and residual nodal parenchyma (Supplementary Fig. S1E, thin arrow), and with moderate/high staining in the positive control (normal node; Supplementary Fig. S1F).

Immunohistochemistry for HSV antigens was definitively positive in two cases (001-0001, 001-0009). HSV antigen staining was observed in both injected (Fig. 3A and B) and uninjected (Fig. 3C and D) nodes, indicating trafficking

[7] A. Miah, K.J. Harrington, C.M. Nutting, unpublished observations.

Table 2. Adverse events of any grade reported in ≥10% of patients during study treatment regardless of causality

Adverse event	JS1/34.5-/47-/GM-CSF dose group				All patients (*n* = 17)
	Cohort 1 (*n* = 4)	Cohort 2 (*n* = 4)	Cohort 3 (*n* = 4)	Cohort 4 (*n* = 5)	
	No. (%)	No. (%)	No. (%)	No. (%)	No. (%)
Any event	4 (100)	4 (100)	4 (100)	5 (100)	17 (100)
Weight decreased	3 (75)	4 (100)	4 (100)	5 (100)	16 (94)
Constipation	4 (100)	3 (75)	3 (75)	5 (100)	15 (88)
Mucosal inflammation	3 (75)	4 (100)	3 (75)	4 (80)	14 (82)
Radiation skin injury	1 (25)	4 (100)	4 (100)	5 (100)	14 (82)
Anemia	2 (50)	4 (100)	4 (100)	3 (60)	13 (76)
Nausea	4 (100)	4 (100)	2 (50)	3 (60)	13 (76)
Dysphagia	4 (100)	3 (75)	1 (25)	4 (80)	12 (71)
Pyrexia	3 (75)	2 (50)	1 (25)	4 (80)	10 (59)
Dehydration	1 (25)	1 (25)	4 (100)	3 (60)	9 (53)
Dry mouth	0 (0)	3 (75)	2 (50)	4 (80)	9 (53)
Neutropenia	2 (50)	3 (75)	2 (50)	1 (20)	8 (47)
Pharyngolaryngeal pain	2 (50)	2 (50)	1 (25)	3 (60)	8 (47)
Leukopenia	1 (25)	4 (100)	1 (25)	1 (20)	7 (41)
Fatigue	1 (25)	0 (0)	2 (50)	3 (60)	6 (35)
Odynophagia	1 (25)	2 (50)	2 (50)	1 (20)	6 (35)
Oral candidiasis	0 (0)	0 (0)	3 (75)	3 (60)	6 (35)
Vomiting	2 (50)	1 (25)	1 (25)	2 (40)	6 (35)
Anorexia	1 (25)	0 (0)	2 (50)	2 (40)	5 (29)
Diarrhea	3 (75)	1 (25)	0 (0)	1 (20)	5 (29)
Tinnitus	1 (25)	0 (0)	2 (50)	2 (40)	5 (29)
Dysgeusia	0 (0)	0 (0)	2 (50)	2 (40)	4 (24)
Insomnia	2 (50)	0 (0)	1 (25)	1 (20)	4 (24)
Mouth ulceration	0 (0)	1 (25)	1 (25)	2 (40)	4 (24)
Renal failure	1 (25)	2 (50)	1 (25)	0 (0)	4 (24)
Anxiety	2 (50)	0 (0)	0 (0)	1 (20)	3 (18)
Confusional state	0 (0)	2 (50)	1 (25)	0 (0)	3 (18)
Cough	1 (25)	0 (0)	1 (25)	1 (20)	3 (18)
Depressed mood	1 (25)	0 (0)	1 (25)	1 (20)	3 (18)
Dyspepsia	2 (50)	0 (0)	0 (0)	1 (20)	3 (18)
Febrile neutropenia	2 (50)	0 (0)	1 (25)	0 (0)	3 (18)
Hypokalemia	0 (0)	1 (25)	1 (25)	1 (20)	3 (18)
Hyponatremia	1 (25)	1 (25)	1 (25)	0 (0)	3 (18)
Lymphopenia	1 (25)	1 (25)	0 (0)	1 (20)	3 (18)
Rash	0 (0)	0 (0)	3 (75)	0 (0)	3 (18)
Stomatitis	1 (25)	0 (0)	1 (25)	1 (20)	3 (18)
Thrombocytopenia	0 (0)	1 (25)	2 (50)	0 (0)	3 (18)
Abdominal discomfort	0 (0)	0 (0)	1 (25)	1 (20)	2 (12)
Azotemia	1 (25)	1 (25)	0 (0)	0 (0)	2 (12)
Depression	0 (0)	2 (50)	0 (0)	0 (0)	2 (12)
Dizziness	0 (0)	0 (0)	2 (50)	0 (0)	2 (12)
Dysphonia	0 (0)	2 (50)	0 (0)	0 (0)	2 (12)
Erythema	1 (25)	0 (0)	1 (25)	0 (0)	2 (12)
Headache	0 (0)	1 (25)	0 (0)	1 (20)	2 (12)
Hypogeusia	0 (0)	0 (0)	1 (25)	1 (20)	2 (12)
Hypotension	0 (0)	1 (25)	1 (25)	0 (0)	2 (12)
Injection site hemorrhage	0 (0)	0 (0)	1 (25)	1 (20)	2 (12)

(*Continued on the following page*)

Table 2. Adverse events of any grade reported in ≥10% of patients during study treatment regardless of causality (Cont'd)

Adverse event	JS1/34.5-/47-/GM-CSF dose group				All patients (*n* = 17)
	Cohort 1 (*n* = 4)	Cohort 2 (*n* = 4)	Cohort 3 (*n* = 4)	Cohort 4 (*n* = 5)	
	No. (%)	No. (%)	No. (%)	No. (%)	No. (%)
Injection site reaction	0 (0)	0 (0)	1 (25)	1 (20)	2 (12)
Lower respiratory tract infection	1 (25)	1 (25)	0 (0)	0 (0)	2 (12)
Malaise	2 (50)	0 (0)	0 (0)	0 (0)	2 (12)
Pharyngitis	1 (25)	1 (25)	0 (0)	0 (0)	2 (12)
Stomach discomfort	0 (0)	0 (0)	1 (25)	1 (20)	2 (12)
Syncope	1 (25)	0 (0)	1 (25)	0 (0)	2 (12)

NOTE: Cohort 1, 10^6 pfu/mL on four occasions, maximum of 4 mL per dose. Cohort 2, 10^6 pfu/mL once followed by 10^7 on three occasions, maximum of 4 mL per dose. Cohort 3, 10^6 pfu/mL once followed by 10^8 on three occasions, maximum of 4 mL per dose. Expansion cohort, 10^6 pfu/mL once followed by 10^8 on three occasions, maximum of 8 mL per dose.

through the nodal chain. Equivocal positive staining was seen in six patients, including in uninjected nodal tissue (Supplementary Table S3). The prolonged interval between the last viral injection and sampling at neck dissection probably accounted for low HSV immunohistochemical detection rates. Notably, HSV antigen was detected only in patients treated at lower virus doses, where it might be anticipated that it would have taken longer to reach the maximum virus titer and antitumor effect. This may explain why virus was detected at the prolonged time points tested in the lower, but not higher, dose groups.

Seven patients were seronegative for anti-HSV antibody at screening, five of whom had seroconverted by week 3 and the remaining two were positive by week 6 (Fig. 4A). The mean (SD) antibody index values for treatment cohorts showed a dose-dependent increase.

Review of the end-of-treatment CT scans confirmed a response in 14 (82.3%) patients, with 4 (23.5%) complete and 10 (58.8%) partial responses. Representative images are shown for complete response (Fig. 4B and C and Supplementary Fig. S2A-D) and partial response (Supplementary Figs. S3 and S4).

Median follow-up was 29 months (range, 19-40 months; Supplementary Table S4). No patient developed recurrent mucosal or cervical nodal disease. Four patients (23.5% developed distant metastasis (lung, *n* = 3; bone, *n* = 1), one of whom also had new primary SCCHN. Two of these were in cohort 1, one in cohort 3, and one in cohort 4. Thus, only

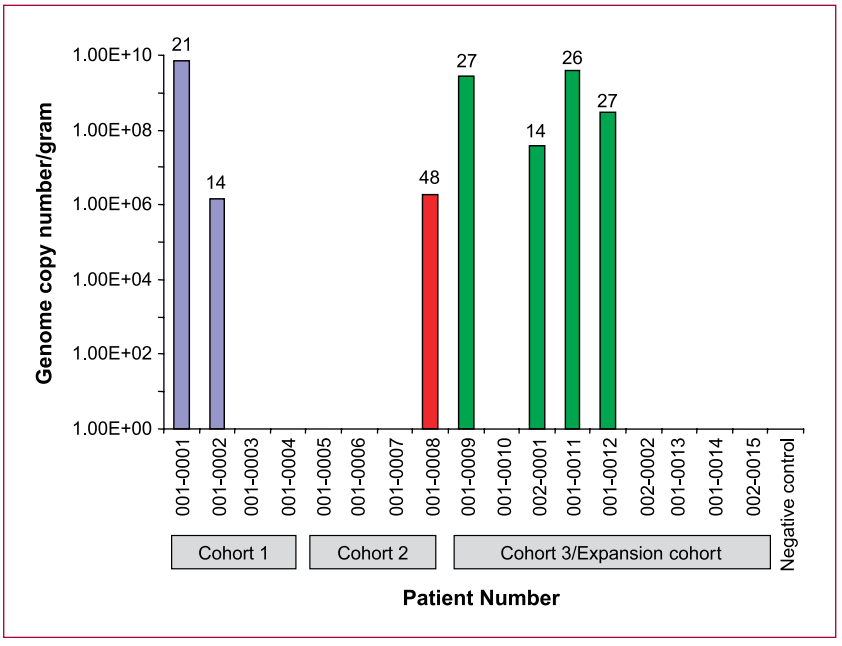

Fig. 2. Q-PCR from tumor-containing lymph node tissue showing viral recovery in seven patients. Note that patient 001-0006 did not provide a presurgery or surgical biopsy specimen. Patient study numbers and the cohort in which they were treated are indicated. HSV detection is expressed as genome copy number per gram of tumor tissue (see text for details). The number above each bar represents the interval (in days) between the last injection of JS1/34.5-/47-/GM-CSF and sample collection.

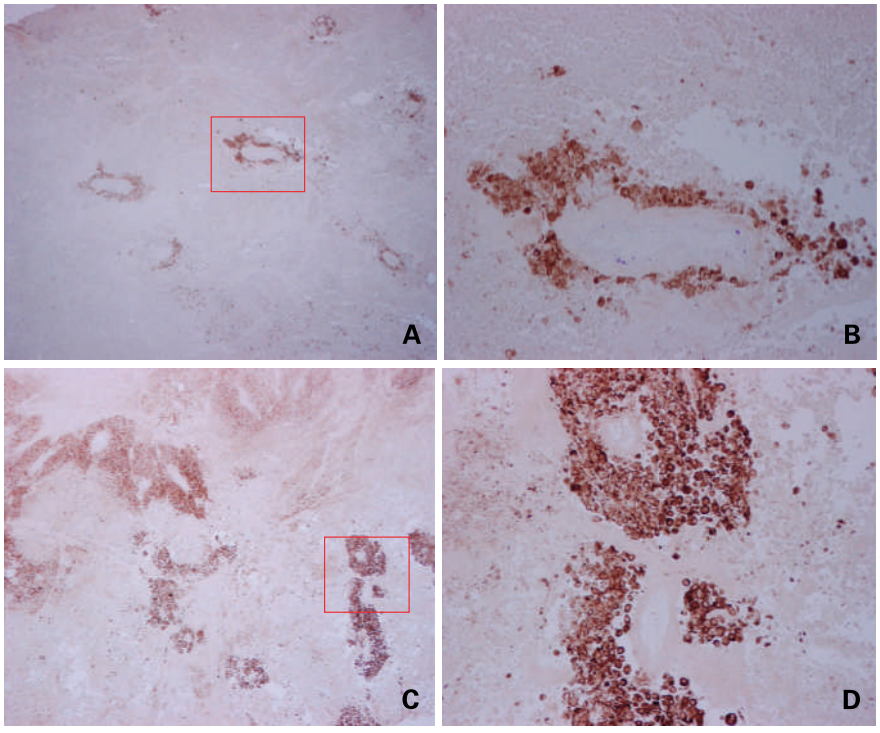

Fig. 3. Immunohistochemical staining for HSV-1 (B0114 polyclonal antibody) in injected and uninjected nodal tissue. A, injected lymph node showing perivascular and scattered parenchymal HSV-1-positive staining (×4). B, expanded view of injected node in A (red box; ×40). C, uninjected lymph node showing similar pattern of perivascular and scattered parenchymal HSV-1–positive staining (×4). D, expanded view of uninjected node in C (red box; ×40).

2 of 13 patients (15%) dosed at 10^7 pfu/mL, or above, relapsed. Two patients died of intercurrent disease: one of cardiorespiratory failure and the other in his sleep. The latter patient had no sign of recurrence. An autopsy was not done; thus, although presumed cardiovascular, the cause was not confirmed. Therefore, 100% of patients remained free of locoregional disease, disease-specific survival was 82.4%, relapse-free rate was 76.5%, and overall survival was 70.5% (Fig. 4D).

Discussion

This study confirms that oncolytic HSV can be safely combined with full-dose CRT in newly diagnosed SCCHN. Up to four intratumoral viral injections were well tolerated without study drug-related DLT or exacerbation of the anticipated side effects of CRT. The previously defined administration schedule (18), involving an initial seroconverting 10^6 pfu dose, was associated with robust virus dose-dependent anti-HSV antibody responses, even in patients receiving potentially immunosuppressive CRT. None of the patients experienced a delay in radiotherapy or chemotherapy schedule, in keeping with the observed tolerability of the combination. Injection site reaction and/or hemorrhage occurred in only two (12%) patients and did not delay ongoing radiotherapy. These observations are of critical importance to this approach and show for the first time that oncolytic viruses can be included in standard curative anticancer regimens.

Selective or modified radical neck dissection was done as part of the protocol approximately four weeks after the last intratumoral virus injection, with the extent of surgery left to the discretion of the surgeon based on his clinical judgment. Although there is no currently accepted standard of care for the management of the neck in patients with stage III/IV node-positive SCCHN, many centers recommend routine neck dissection postchemoradiotherapy in patients with N_2 and N_3 disease. The advantage of including neck dissection as part of this protocol was that it also allowed us to obtain material for pharmacodynamic studies.

Analysis of virus shedding revealed transient injection site leakage in three patients for up to six days. Virus was never detected on the swab from the external surface of the dressing. These data mirror findings from previous studies of single-agent virus administration (18, 19). The lack of virus shedding in patients receiving concomitant CRT is extremely important because it allows examination of this approach in large-scale multicenter trials. Indeed, JS1/34.5-/47-/GM-CSF has already been administered to outpatients in a phase II trial in 50 patients with melanoma (19), and a phase III study using this approach is under way.

Pharmacodynamic analyses showed virus infection of tumor cells in injected and uninjected nodes. HSV was detected definitively (immunohistochemistry or Q-PCR)

in seven patients, with a further three patients showing equivocal immunohistochemical staining. Both patients with definitively positive immunohistochemical staining for HSV also had virus detected by Q-PCR. In seven patients, it was possible to derive viral yields based on the amount of HSV DNA in biopsy tissue (Fig. 2B). In five patients, calculated viral levels were >50,000 copies/μg suggesting viral replication in tumor-bearing nodal tissue. Such quantitative analysis is not possible using immunohistochemistry (even for markers of early or late viral replication) because their presence is not conclusive evidence of production of intact virions. It is noteworthy that HSV staining and Q-PCR detection of viral genomes was seen in uninjected nodes (Fig. 3C and D). This strongly suggests

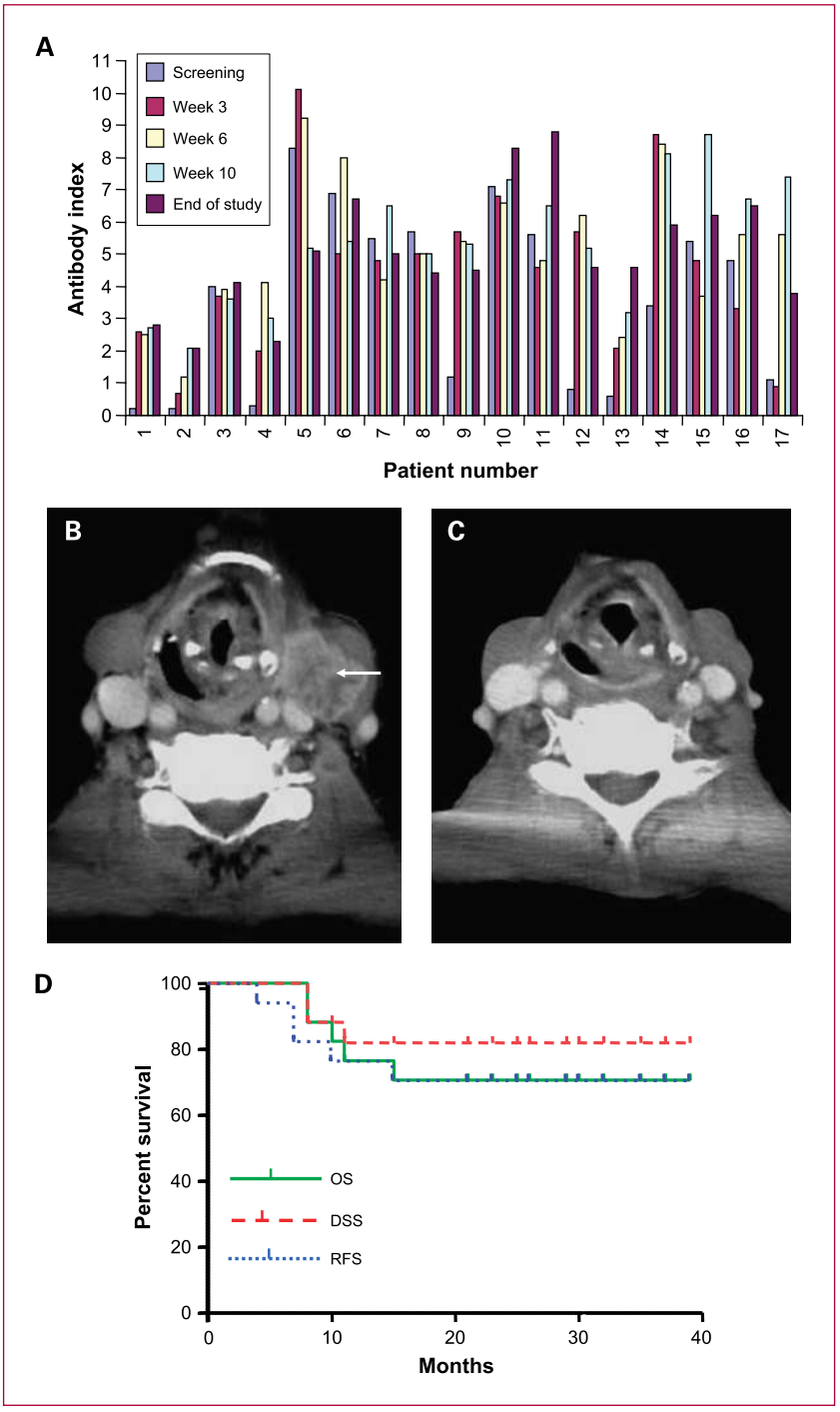

Fig. 4. A, neutralizing antibody response to JS1/34.5-/47-/GM-CSF. Data are expressed in terms of the antibody index (AI): seronegative = AI <0.9; seropositive = AI >1.1; equivocal = AI 0.9-1.1. Seven patients were seronegative at the start of treatment, all of whom seroconverted during treatment. There was a dose-dependent increase in mean (SD) AI: cohort 1, 2.85 (0.54); cohort 2, 5.53 (0.58); cohort 3, 6.49 (1.60). B and C, pretreatment and posttreatment CT images of patient 001-0007 in cohort 2. The large left level 3 nodal mass (arrow) seen pretreatment (B) completely resolved posttreatment (C). D. Kaplan-Meier survival curves showing overall (OS), disease-specific (DSS) and progression-free survival (PFS).

that JS1/34.5-/47-/GM-CSF can traffic through lymphatic channels from initial injection sites. These pharmacodynamic data must be interpreted in light of the study design, in which the primary focus was to deliver curative treatment. As such, opportunities to obtain biopsy material from injected sites were limited by cumulative side effects of CRT and the desire to avoid iatrogenic treatment delays. Therefore, most tissue samples were obtained at neck dissection, long after the last injection of JS1/34.5-/47-/GM-CSF.

The clinical, radiologic, and pathologic response rates attest to the activity of this regimen. At a median follow-up of 29 months, locoregional control, disease-specific survival, and overall survival were 100%, 82.4% and 70.6%, respectively. These compare favorably with previous phase II and III series (5, 11–14). It should be borne in mind, however, that routine use of elective neck dissection in this protocol may have contributed to the locoregional control rates. Of particular interest is the extremely high (94%) pathologic complete response rate and the fact that no locoregional relapses have occurred. These values exceed those in historical control series (4–10), in which rates of 60% to 71% for histopathologic complete response and 30% to 50% for two-year locoregional relapse (5, 11–14) are typical. For the 13 patients who were treated at the 1×10^7 or 1×10^8 pfu/mL dose levels, only 2 developed a relapse at any site (systemic) for a disease-specific survival rate of 84.6%. However, this was a small study with only a small number of patients treated at the target dose. It is not possible at present to judge the nature of the interaction (if any) between JS1/34.5-/47-/GM-CSF and a radical chemoradiotherapy regimen. Therefore, the results must be interpreted with caution pending large-scale studies of this approach.

In addition, the response, disease-specific survival, and overall survival rates must be interpreted in light of the fact that 47% of tumors in which there was sufficient material for assessment were HPV positive by *in situ* hybridization. It is well established that patients with HPV-positive tumors have better response rates and improved prognosis than those with HPV-negative tumors (23). The small number of patients in this study renders subgroup analysis of HPV-positive versus HPV-negative patients futile, but it will certainly be important to ensure balance for this variable between study arms in future randomized phase III trials of JS1/34.5-/47-/GM-CSF. This can be achieved by using HPV status as one of the stratifications in the study design.

In conclusion, further evaluation of the combination of JS1/34.5-/47-/GM-CSF with chemoradiation in a randomized setting for the treatment of locally advanced SCCHN is warranted. A phase III clinical trial is currently being planned.

Disclosure of Potential Conflicts of Interest

K. Harrington discloses research funding from BioVex Inc. R. Coffin and H. Goldsweig are employees of BioVex plc.

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