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In Vivo Evaluation of a Cancer Therapy Strategy Combining HSV1716-Mediated Oncolysis with Gene Transfer and Targeted Radiotherapy

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Oncolytic herpes viruses show promise for cancer treatment. However, it is unlikely that they will fulfill their therapeutic potential when used as monotherapies. An alternative strategy is to use these viruses not only as oncolytic agents but also as a delivery mechanism of therapeutic transgenes to enhance tumor cell killing. The herpes simplex virus 1 deletion mutant HSV1716 is a conditionally replicating oncolytic virus that selectively replicates in and lyses dividing tumor cells. It has a proven safety profile in clinical trials and has demonstrated efficacy as a gene-delivery vehicle. To enhance its therapeutic potential, we have engineered HSV1716 to convey the noradrenaline transporter (NAT) gene (HSV1716/NAT), whose expression endows infected cells with the capacity to accumulate the noradrenaline analog metaiodobenzylguanidine (MIBG). Thus, the NAT gene–infected cells are susceptible to targeted radiotherapy using radiolabeled ^{131}I-MIBG, a strategy that has already shown promise for combined targeted radiotherapy–gene therapy in cancer cells after plasmid-mediated transfection. **Methods:** We used HSV1716/NAT as a dual cell lysis–gene delivery vehicle for targeting the NAT transgene to human tumor xenografts in vivo. **Results:** In tumor xenografts that did not express NAT, intratumoral or intravenous injection of HSV1716/NAT induced the capacity for active uptake of ^{131}I-MIBG. Administration of HSV1716/NAT and ^{131}I-MIBG resulted in decreased tumor growth and enhanced survival relative to injection of either agent alone. Efficacy was dependent on the scheduling of delivery of the 2 agents. **Conclusion:** These findings support a role for combination radiotherapy–gene therapy for cancer using HSV1716 expressing the NAT transgene and targeted radionuclide therapy.

Key Words: HSV1716; ^{131}I-MIBG; targeted radiotherapy; cancer; gene transfer

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Herpes simplex virus type 1 (HSV-1) is an attractive vector for cancer gene therapy because of its broad tropism, high infectivity in nondividing and dividing cells, lack of host genome integration, and ease of preparation of high-titer stocks (*1*). Furthermore, 30 kb of the HSV genome can be replaced by transgenes, allowing the combination of oncolytic therapy with transgene delivery.

Generation of safe, selectively replication-competent viruses has been predominantly accomplished by disruption of the HSV virulence factor ICP34.5 (*1,2*). Several such viruses have been used in clinical trials (*3,4*) and have demonstrated efficacy in preclinical studies (*5*). One such conditionally replicating virus is HSV1716 (*6*), a mutant lacking both copies of the RL1 gene that encodes the protein ICP34.5. It selectively replicates in and lyses dividing but not terminally differentiated cells (*7*) and has been shown to be safe after injection into the normal animal brain (*8*). On administration, HSV1716 significantly increased survival times in mice bearing human tumor cell xenografts (*9–12*). HSV1716 has been used in phase 1 clinical trials to treat patients with malignant glioma (*13–15*), refractory melanoma (*16*), and advanced squamous cell carcinoma of the head and neck (*17*). These trials demonstrated the safety and limited toxicity of HSV1716 and provided proof of principle that the virus is capable of directly destroying human tumor cells while leaving normal cells intact.

Although encouraging results have been obtained using conditionally replicating HSV-1 mutants as oncolytic monotherapies, the optimal application of these viruses will be in combination with cytotoxic agents. Accordingly, second-generation viruses are being developed. These combine the oncolytic activity and safety of the first-generation HSV-1 mutants with the delivery of tumoricidal transgenes (*2,4,5*). Because of the difficulty in targeting every cell within a tumor, either for oncolysis or for transgene delivery, a successful cancer gene therapy strategy will mediate killing of infected and noninfected bystander tumor cells.

Targeted radionuclide therapy is the selective irradiation of tumor cells by radionuclides conjugated to tumor-seeking

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molecules. One such agent, metaiodobenzylguanidine (MIBG), has high affinity for the noradrenaline transporter and is used for imaging and treatment of noradrenaline transporter (NAT)–expressing neuroendocrine tumors. Unfortunately, few tumor types have radiotargetable features such as endogenous NAT expression. However, we have demonstrated the possibility of rendering various tumor types susceptible to ^{131}I-MIBG by transfer of the NAT gene, endowing the cells with the capacity for uptake of ^{131}I-MIBG and resulting in toxicity to tumor cells in vitro (*18*) and in vivo (*19*).

A limitation to cancer gene therapy is the low efficiency of gene transfer to malignant cells in tumors. Therefore, an attractive feature of the combination of targeted radiotherapy with gene transfer is the cross-fire irradiation of surrounding untargeted cells, from cells that have accumulated radiopharmaceutical. Furthermore, intracellular concentrated radionuclides induce a potent radiation-induced biologic bystander effect (RIBBE) (*20*), which results from the processing of the physical radiation insult into toxic factors that impinge on neighboring cells (*20*). RIBBEs are especially significant as a response of cells to low-dose and dose-rate radiation (*21*) characteristics of targeted radiotherapy (*22*).

To develop NAT gene transfer to enable ^{131}I-MIBG therapy for nonneural crest–derived tumors, we constructed an HSV1716 recombinant virus containing the NAT gene. Previously, we reported that infection of glioma cells in vitro with HSV1716/NAT (recombinant HSV1716 virus expressing the NAT transgene) resulted in expression of a functional transporter and uptake of ^{131}I-MIBG in a time- and viral dose–dependent manner, indicating the effectiveness of HSV1716 as a transgene delivery vehicle (*23*). Here we report the utility of this virus in vivo.

MATERIALS AND METHODS

Viral Constructs

HSV1716 was derived from HSV Glasgow strain 17+ as previously described (*6*). HSV1716/NAT was constructed, characterized, and titrated in BHK21/13C as previously described (*23,24*).

No-Carrier-Added Synthesis of ^{131}I-MIBG

Chemicals were purchased from Aldrich Chemical Co. High-performance liquid chromatography–grade solvents were obtained from Rathburn Chemicals. Carrier-free sodium ^{131}I was purchased from GE Healthcare. No-carrier-added ^{131}I-MIBG was synthesized by electrophilic iododesilylation of trimethylsilylbenzylguanidine, as described previously (*25*).

Cells

Xenografts were established from the human glioma cell line UVW and the human malignant melanoma cell line SK-MEL-3 (American Type Culture Collection). UVW cell lines were maintained in Eagle minimum essential medium (Invitrogen) with 10% (v/v) fetal bovine serum, penicillin and streptomycin (100 U/mL), fungizone (2 μg/mL), and glutamine (200 mM) (Invitrogen). The SK-MEL-3 cell line was maintained in McCoy 5a medium (Invitrogen) supplemented as described for the UVW cell lines. The cells were cultured at 37°C in an atmosphere of 5% CO_2.

Experimental Animals

Six-week-old female congenitally athymic nude mice of strain MF1 *nu/nu* were obtained from Charles River. In vivo experiments were performed in accordance with the U.K. Coordinating Committee for Cancer Research guidelines on experimental neoplasia in animals (*26*).

Tumor Xenografts

Xenografts were established in nude mice by subcutaneous injection of 3×10^6 UVW or SK-MEL-3 cells freshly harvested at 60%–70% confluency. After 8 d (for UVW cells) or 16 d (for SK-MEL-3 cells), mice bearing tumors of approximately 100 mm^3 in volume were randomized into treatment groups and placed in the biohazard containment facility for use of category 2 virus and targeted radionuclides as determined by local safety regulations.

To monitor potential toxicity, body weight was measured daily and experimental animals were evaluated for signs of distress using standard guidelines (*27*). Mice whose xenografts reached 1,900 mm^3 were euthanized.

Biodistribution of ^{131}I-MIBG

Biodistribution experiments were undertaken to determine whether HSV1716/NAT conferred on UVW or SK-MEL-3 tumor xenografts the ability to take up ^{131}I-MIBG.

Mice with tumors of approximately 250 mm^3 were randomized into treatment groups of 12 mice per group. In the groups that received intratumoral injection of the virus, each mouse received 1×10^6 plaque-forming units (PFU) of either HSV1716 or HSV1716/NAT virus diluted in 50 μL of phosphate-buffered saline (PBS) or 50 μL of PBS alone. In the groups that received an intravenous injection of virus, each mouse received 1×10^7 PFU HSV1716/NAT diluted in 100 μL of PBS or 100 μL of PBS alone. Mice then received intraperitoneal injection of either PBS or ^{131}I-MIBG (2 MBq) either simultaneously with or 24 h after virus or PBS delivery.

Twenty-four or 48 h after radiopharmaceutical administration, mice were euthanized. Tumor, heart, lung, adrenal glands, kidney, spleen, and liver were excised and weighed, and the associated radioactivity was measured in an automated γ-counter (Packard Biosciences Ltd.). The activity of each sample, in counts per minute, was converted to an absolute value in megabecquerels, by comparison with standards of known activity. From this value, the weight of the tissue sample, and the activity of ^{131}I-MIBG administered, the activity in each organ and the tumor was expressed as the percentage of the injected dose per gram of tissue. Correction was made for radioactive decay since the time of injection.

Biodistribution of HSV1716/NAT

An assay for the presence of infectious HSV in mice bearing SK-MEL-3 tumors was performed by homogenizing tissue samples 3 and 7 d after HSV1716/NAT injection. Briefly, tissue samples were homogenized using an Omni TH-02 homogenizer in 1 mL of PBS before titration on BHK21/13C cells as described previously (*24*).

Immunohistochemical analysis for the presence of HSV was performed on 3-μm-thick paraffin sections of tissue samples from mice bearing UVW and SK-MEL-3 tumors using a monoclonal antibody recognizing an unspecified epitope of HSV1 strain Stoker (Dako). Sections were pretreated by microwaving for antigen retrieval. Bound antibody was visualized using the enVision system (Dako). Tissue sections were imaged using a Zeiss Axioplan 2 microscope and ISIS software (Metasystems) for capturing images. Negative controls were sections from tumors treated with PBS.

Tumor Therapy

Mice with tumors of approximately 100 mm^3 diameter were randomized into treatment groups of 12 mice each. Groups of animals received 1×10^5, 1×10^6, or 1×10^7 PFU of either HSV1716 or HSV1716/NAT diluted in 50 μL of PBS by intratumoral injection. Control animals received injection of 50 μL of PBS. Virus injection was followed by intraperitoneal injection of 10 MBq of ^{131}I-MIBG simultaneously with or 24 h after virus or PBS injection.

To determine the efficacy of systemic administration HSV1716/NAT on tumor growth, groups of animals received an intravenous injection of 1×10^7 PFU of HSV1716/NAT diluted in 100 μL of PBS or PBS alone, followed by an intraperitoneal injection of 10 MBq of ^{131}I-MIBG 24 h after virus or PBS injection.

Subcutaneous tumors were measured with calipers immediately before treatment and every 2 or 3 d thereafter. On the assumption of ellipsoidal geometry, diameter measurements were converted to an approximate volume by multiplying half the longest diameter by the square of the mean of the 2 shorter diameters. Mice whose xenograft volume reached 1,900 mm^3 were euthanized. For every animal, relative tumor volume (volume at any time point divided by volume immediately before treatment) was plotted against time, and the area under the time–volume curves was determined by trapezoidal approximation. The area under the time–volume curves was used as a measure of treatment effectiveness for the purpose of comparison between groups. For animals that were euthanized because of fast tumor growth, before the termination of the experiment, the tumor volume curve was extrapolated at constant volume from the time of euthanasia.

Statistical Analysis

One-way ANOVA was used to compare the magnitude of radiopharmaceutical uptake after various administration schedules of virus. Post hoc testing used Bonferroni correction for multiple comparisons. A P value of less than 0.05 was considered significant for all results.

To test for differences in tumor growth between experimental therapy groups, the Kruskal–Wallis test was used, with post hoc testing by the Mann–Whitney U test with Bonferroni correction.

RESULTS

Biodistribution of ^{131}I-MIBG After Virus Administration

NAT expression in UVW and SK-MEL-3 xenografts and normal organs, after administration of HSV1716/NAT, was assessed using a functional ^{131}I-MIBG uptake assay.

The concentration of ^{131}I-MIBG (in both tumor types) from animals treated with the radiopharmaceutical and virus via 2 different schedules was compared: ^{131}I-MIBG was administered either simultaneously with 1×10^6 PFU of HSV1716, HSV1716/NAT, or PBS or with virus–PBS preceding ^{131}I-MIBG administration by 24 h (Fig. 1A; Supplemental Fig. 1A [supplemental materials are available online only at http://jnm.snmjournals.org]). HSV1716 and PBS were used to determine the biodistribution of ^{131}I-MIBG in the absence of the NAT transgene. One-way ANOVA revealed no significant difference in the accumulation of radioactivity between xenografts infected with HSV1716, compared with PBS administration, in any schedule examined. However, in both xenografts, there was a statistically significant difference in ^{131}I-MIBG uptake after administration of HSV1716/NAT, compared with HSV1716 or PBS, regardless of the delivery schedule ($P < 0.001$, 1-way ANOVA). Post hoc analysis showed that in both xenografts, tumor uptake of ^{131}I-MIBG was significantly enhanced when HSV1716/NAT was administered 24 h before ^{131}I-MIBG, relative to uptake after simultaneous administration ($P < 0.001$). There was no significant difference between radiopharmaceutical retention in either tumor xenograft at 24 and 48 h after radiopharmaceutical administration within each schedule, indicating that ^{131}I-MIBG was retained within the tumor.

[**Fig. 1**]

To determine whether intratumoral injection of HSV1716/NAT or PBS delivered by either schedule affected the biodistribution of ^{131}I-MIBG in other organs, we compared the uptake in liver, spleen, adrenals, kidney, lung, and heart in UVW and SK-MEL-3 tumor–bearing mice 48 h after radiopharmaceutical administration (Fig. 1B; Supplemental Fig. 1B). Compared with PBS, HSV1716/NAT did not significantly affect the accumulation of radioactivity in any of the organs examined (1-way ANOVA, $P > 0.05$), regardless of the delivery schedule. Furthermore, the biodistribution of ^{131}I-MIBG between the 2 tumor types was similar.

Braidwood et al. (*28*) previously showed that an intravenous injection of 1×10^7 PFU of HSV1790 (HSV1716 with nitroreductase gene) resulted in significant accumulation of virus within UVW tumor xenografts. Therefore, to assess the effect of intravenous administration of HSV1716/NAT on ^{131}I-MIBG uptake in SK-MEL-3 and UVW xenografts, 1×10^7 PFU of virus was administered 24 h before radiopharmaceutical by tail vein injection. Uptake of ^{131}I-MIBG into the tumor was significantly higher after an intravenous injection of HSV1716/NAT than of PBS ($P < 0.01$, 1-way ANOVA) (Fig. 2). No difference was found in the accumulation of ^{131}I-MIBG in any other organs investigated after HSV1716/NAT administration, compared with PBS (data not shown).

[**Fig. 2**]

No toxicity was observed in any animal after intratumoral injection of 1×10^6 PFU of HSV1716/NAT or HSV1716 with or without ^{131}I-MIBG administration or indeed with any other virus titers or treatment schedules administered throughout the experimental cohort.

Biodistribution of HSV1716/NAT

The biodistribution of HSV1716/NAT in UVW and SK-MEL-3 tumor xenografts and major organs was determined by immunohistochemistry 24 h after intratumoral injection or intravenous administration of the virus. Figures 3A and 3B show representative images of HSV staining in UVW tumor sections after intratumoral injection of the virus. Figure 3A shows several areas with HSV-positive cells (brown). Figure 3B shows an area with a typical HSV infection, characterized by giant multinucleated cells and holes, in which the cells have been killed. In all animals treated with an intratumoral injection of HSV1716/NAT, evidence of HSV was found in the tumors, whereas there was no indication of positive staining in any other organ investigated.

[**Fig. 3**]

After intravenous administration, however, HSV was undetectable in the tumor or any other tissues, as determined

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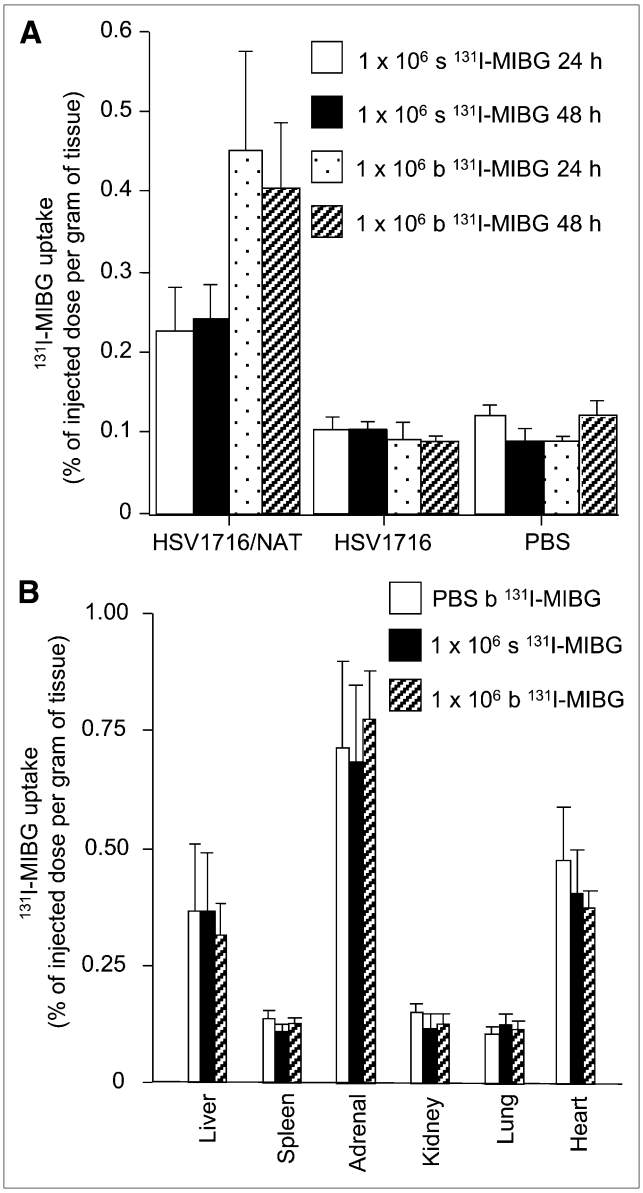


FIGURE 1. Effect of scheduling of viral delivery on accumulation of ^{131}I-MIBG by human UVW glioma tumor xenografts (A) and normal organs (B). HSV1716/NAT or HSV1716 (1×10^6 PFU) was injected intratumorally 24 h before (b) or simultaneously (s) with intraperitoneal administration of 2 MBq of ^{131}I-MIBG. Radiation accrual was measured 24 or 48 h after radiopharmaceutical administration in excised tumors and 48 h after in normal organs. Data are means and SDs of 12 determinations.

by immunohistochemistry (data not shown). Therefore, we measured the level of infectious virus present 3 and 7 d after virus administration (intravenous or intratumoral injection) by titration of virus from SK-MEL-3 tumors and major organ samples onto BHK21/13C cells. Infectious virus was detected in tumor samples from animals injected with HSV1716/NAT intravenously or intratumorally 3 d after virus injection (Fig. 3C). Higher titers than injected were measured on days 3 and 7 after intratumoral injection of 1×10^6 PFU of HSV1716/NAT, indicating virus replication within the tumor. The lower amount of virus titrated 3 d after intravenous administration (Fig. 3C) is consistent with the indirect administration route. The failure to detect virus by titration on day 7 after intravenous injection suggests that little HSV1716/NAT lodged in this tumor. No infectious virus was found in any of the other organs examined after either route of infection (Fig. 3C).

Tumor Growth Delay After Intratumoral Administration of HSV1716/NAT and ^{131}I-MIBG

Before proceeding to the assessment of combination therapy, it was important to determine a concentration of HSV1716/NAT alone that did not cure tumors and thus allowed any additional benefit of radiopharmaceutical to be determined. We further wished to confirm the absence of additional tumor growth delay in the presence of ^{131}I-MIBG but in the absence of the NAT transgene (HSV1716 and PBS as controls). We therefore assessed tumor growth delay that was attributable to intratumoral injection of HSV1716/NAT alone at different viral titers (1×10^5 to 1×10^7 PFU) and the effect on tumor growth delay of HSV1716 or PBS alone or in combination with ^{131}I-MIBG (10 MBq) administered 24 h after viral injection (Fig. 4A). [**Fig. 4**]

Comparisons by the Kruskal–Wallis test of differences between growth curves for UVW tumor xenografts injected with PBS or HSV1716 or with HSV1716/NAT at different viral titers indicated that there were significant differences in the growth of tumors between these groups ($P < 0.001$). Pairwise comparisons revealed that 1×10^5 PFU of HSV1716/NAT produced significantly greater growth delay than did PBS treatment ($P < 0.02$). Administration of 1×10^6 PFU of HSV1617/NAT significantly reduced tumor growth relative to that achieved by 1×10^5 PFU of the virus ($P < 0.02$). Similarly, the tumor growth rate after treatment with 1×10^7 PFU of the virus was significantly slower than that of mice treated with 1×10^6 PFU ($P < 0.02$). When

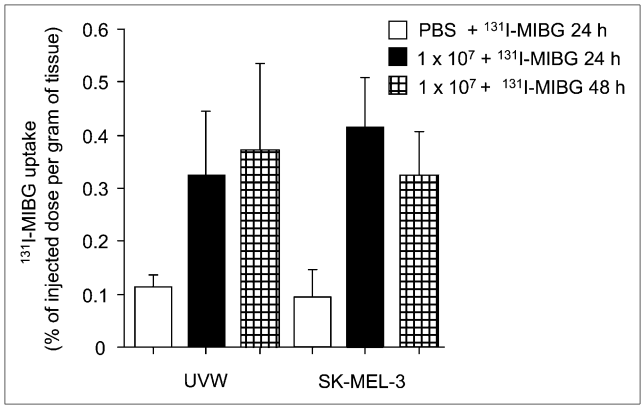
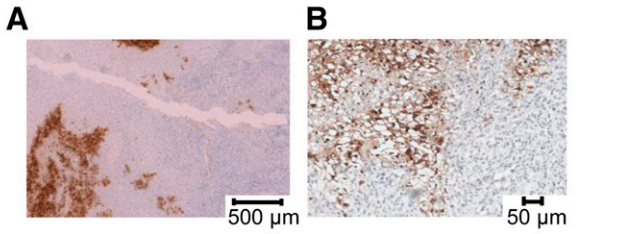


FIGURE 2. Effect of intravenous injection of HSV1716/NAT on accumulation of ^{131}I-MIBG in human UVW glioma and SK-MEL-3 tumor xenografts. HSV1716/NAT (1×10^7 PFU) was administered 24 h before intraperitoneal administration of 2 MBq of ^{131}I-MIBG. Radioactivity in excised tumors was measured 24 or 48 h after radiopharmaceutical administration. Data are means and SDs of 12 determinations.

RGB



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Tissue	Titer day 3 IV	Titer day3 IT	Titer day 7 IV	Titer day 7 IT
SK-MEL-3 tumor	$5.7 \times 10^4 \pm 1.2 \times 10^4$	$2.3 \times 10^8 \pm 2.2 \times 10^8$	0	$3.2 \times 10^7 \pm 4.0 \times 10^6$
Liver	0	0	0	0
Spleen	0	0	0	0
Adrenal glands	0	0	0	0
Kidney	0	0	0	0
Lung	0	0	0	0
Heart	0	0	0	0

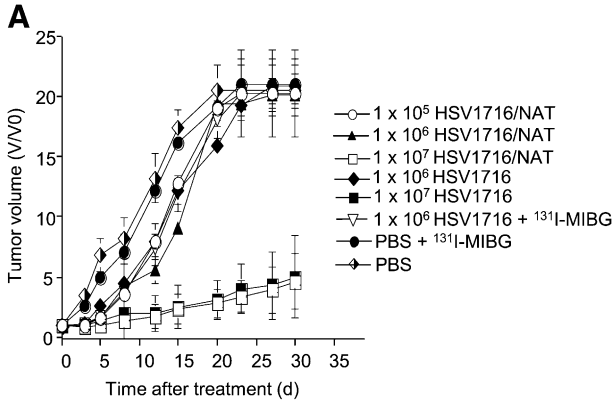
FIGURE 3. (A and B) Representative images of immunohistochemical staining of HSV (brown) in UVW xenograft tumors 24 h after intratumoral administration of 1×10^6 PFU of HSV1716/NAT ($n = 3$). (C) Titer of infectious virus (PFU/mL) present 3 and 7 d after administration of HSV1716/NAT (intratumor, 1×10^6 PFU; intravenous, 1×10^7 PFU) in tumor samples from mice bearing melanoma tumors. Data are means and SDs of 3 determinations. IT = intratumorally; IV = intravenously.

comparing tumor growth between animals treated with the same titer of HSV1716 (lacking the NAT transgene) or HSV1716/NAT (with the NAT transgene), no significant difference in tumor growth delay was evident ($P = 0.3$). Compared with the virus alone, when HSV1716 (lacking the NAT transgene) was administered in combination with ^{131}I-MIBG, no additional effect on tumor growth delay was evident. PBS treatment followed by ^{131}I-MIBG injection, compared with injection of PBS alone, also showed no significant effect on tumor growth delay ($P > 0.05$). These experiments show that in the absence of the HSV1716-delivered NAT transgene, treatment with ^{131}I-MIBG did not affect tumor growth. Tumor cure rates are shown in Figure 4B. Tumor cures occurred only after the injection of 1×10^7 PFU of HSV1716 or HSV1716/NAT, indicating that virus titers lower than 1×10^7 PFU (1×10^5 and 1×10^6 PFU) should be used for subsequent evaluation of combination therapy.

Comparison of Treatment Schedules for Tumor Growth Delay After Intratumoral Administration of HSV1716/NAT and ^{131}I-MIBG

In the combination schedules assessed, virus was injected intratumorally into UVW or SK-MEL-3 xenografts either 24 h before or simultaneously with ^{131}I-MIBG (Figs. 5A and 5B). [**Fig. 5**]

In control animals, PBS was injected 24 h before ^{131}I-MIBG administration. For both tumor types, there were significant differences in growth between tumors treated with 1×10^5 PFU of HSV1716/NAT alone or in combination with 10 MBq of ^{131}I-MIBG either simultaneously or 24 h later ($P < 0.001$, Kruskal–Wallis test). Pairwise comparisons revealed significant differences between the growth of tumors treated with 1×10^5 PFU of HSV1716/NAT alone and with each of the ^{131}I-MIBG schedules ($P < 0.001$), indicating that the addition of ^{131}I-MIBG caused greater tumor cell sterilization than was achieved by the virus alone. Similar statistically significant differences were observed after injection of 1×10^6 PFU of HSV1716/NAT with or without ^{131}I-MIBG ($P < 0.001$) for both tumors. For both tumor types, there was also a statistically significant enhancement of cell kill when HSV1716/NAT at 1×10^5 or 1×10^6 PFU was administered 24 h before rather than simultaneously with ^{131}I-MIBG



B

Treatment	Cure rate (%)
1 x 10^5 PFU HSV1716/NAT	0
1 x 10^6 PFU HSV1716/NAT	0
1 x 10^7 PFU HSV1716/NAT	58
1 x 10^6 PFU HSV1716	0
1 x 10^7 PFU HSV1716	50
1 x 10^6 PFU HSV1716 + ^{131}I-MIBG	0
PBS + ^{131}I-MIBG	0
PBS	0

FIGURE 4. Determination of appropriate dose of virus to use in combination therapies: 1×10^5, 1×10^6, and 1×10^7 PFU of HSV1716/NAT and 1×10^6 and 1×10^7 PFU of HSV1716 were administered and assessed against growth (A) and cure (B) of human glioma xenografts. As control for absence of NAT transgene delivery, treatment with ^{131}I-MIBG (10 MBq) was preceded by intratumoral delivery of HSV1716 or PBS. Cure was defined as failure of xenografts to grow over experimental period (days 0–30). Data are means and SDs of 12 determinations.

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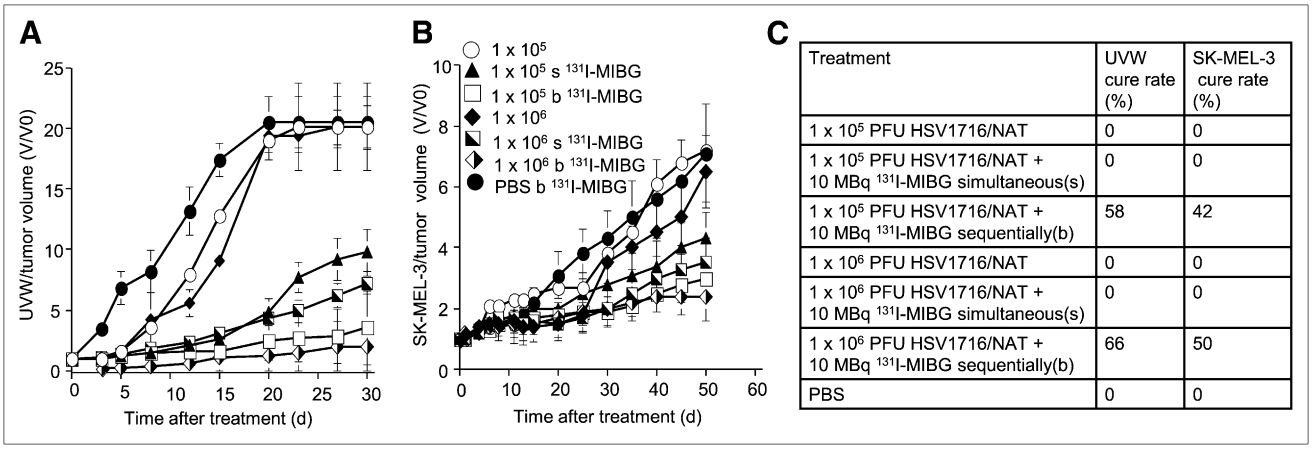

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Treatment	UVW cure rate (%)	SK-MEL-3 cure rate (%)
1 x 10^5 PFU HSV1716/NAT	0	0
1 x 10^5 PFU HSV1716/NAT + 10 MBq ^{131}I-MIBG simultaneous(s)	0	0
1 x 10^5 PFU HSV1716/NAT + 10 MBq ^{131}I-MIBG sequentially(b)	58	42
1 x 10^6 PFU HSV1716/NAT	0	0
1 x 10^6 PFU HSV1716/NAT + 10 MBq ^{131}I-MIBG simultaneous(s)	0	0
1 x 10^6 PFU HSV1716/NAT + 10 MBq ^{131}I-MIBG sequentially(b)	66	50
PBS	0	0

FIGURE 5. Effect of intratumoral administration of various titers of HSV1716/NAT alone or in combination with ^{131}I-MIBG treatment (10 MBq) on growth of UVW (A) and SK-MEL-3 (B) xenografts. Radiopharmaceutical was administered either simultaneously with (s) or 24 h after (b) virus injection. (C) Cure of UVW and SK-MEL-3 xenografts after treatment schedules was defined as failure of xenografts to grow over experimental period. Data are means and SDs of 12 determinations.

($P < 0.001$ for both tumor types), demonstrating the importance of appropriate scheduling to achieve maximum therapeutic benefit.

Tumor cures were achieved in both xenografts by combination of HSV1716/NAT and ^{131}I-MIBG only when HSV1716/NAT was injected 24 h before the radiopharmaceutical (Fig. 5C).

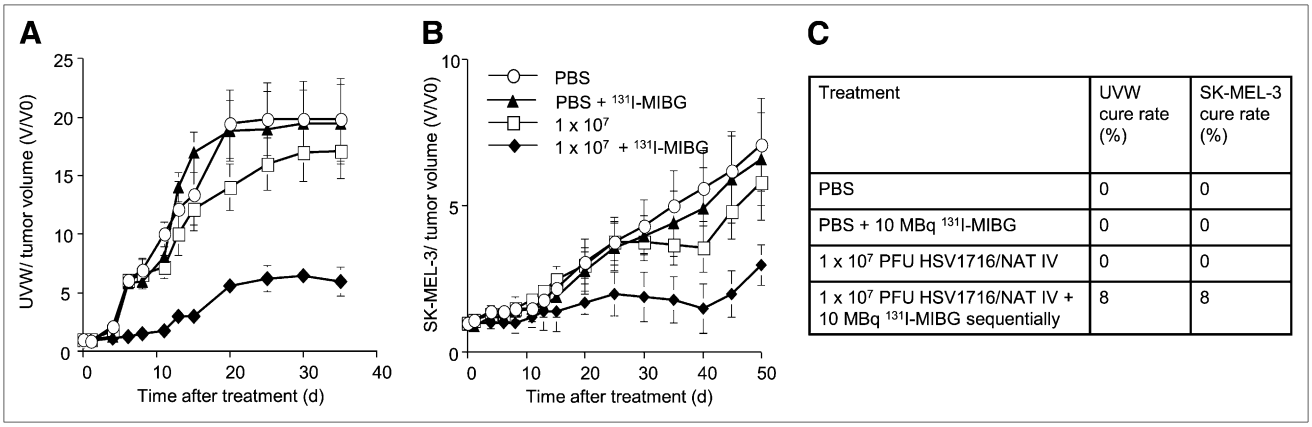
Tumor Growth Delay After Intravenous Administration of HSV1716/NAT and ^{131}I-MIBG

We also investigated the effect of intravenous injections of 1×10^7 PFU of HSV1716/NAT and ^{131}I-MIBG combination therapy on the growth of UVW and SK-MEL-3 xenografts [**Fig. 6**] (Figs. 6A and 6B, respectively). Greatest growth delay was previously observed (Fig. 4) when virus was administered 24 h before ^{131}I-MIBG; therefore, this schedule was used. For both xenografts, there were significant differences in growth between tumors treated with an intravenous injection of HSV1716/NAT alone or in combination with 10 MBq of ^{131}I-MIBG ($P < 0.01$). Tumor cures (although modest) were achieved in both xenografts only with the combination of HSV1716/NAT and ^{131}I-MIBG (Fig. 6C).

DISCUSSION

Radiotherapy efficacy is limited by normal-tissue toxicity. One way to increase the therapeutic ratio is by targeted radionuclide therapy. ^{131}I-MIBG is one of the most promising radiopharmaceuticals currently used for the imaging and treatment of neural crest–derived tumors, because of its high affinity for the noradrenaline transporter (*29*). Unfortunately, this approach is limited to a few tumors possessing molecular characteristics rendering them amenable to targeting (*29*).

We describe the combination of oncolytic virus therapy and cancer gene therapy incorporating delivery of the NAT gene, which endowed human glioma and melanoma cancer cells grown as xenografts in athymic mice with the capacity for uptake of ^{131}I-MIBG, an ability that these cells do not normally possess.



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Treatment	UVW cure rate (%)	SK-MEL-3 cure rate (%)
PBS	0	0
PBS + 10 MBq ^{131}I-MIBG	0	0
1 x 10^7 PFU HSV1716/NAT IV	0	0
1 x 10^7 PFU HSV1716/NAT IV + 10 MBq ^{131}I-MIBG sequentially	8	8

FIGURE 6. Effect of intravenous administration of HSV1716/NAT (1×10^7 PFU) or PBS alone or in combination with ^{131}I-MIBG treatment (10 MBq) on growth of xenografts derived from UVW glioma (A) and SK-MEL-3 melanoma (B) cells. Radionuclide was administered 24 h after injection of virus. (C) Cure of UVW and SK-MEL-3 xenografts after treatment was defined as failure of xenografts to grow over experimental period. Data are means and SDs of 12 determinations.

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The results corroborate that HSV1716 alone has significant antitumor activity. We demonstrated, first, that virally induced inhibition of tumor growth is enhanced by the administration of ^{131}I-MIBG after infection with the recombinant HSV1716 expressing the NAT transgene; second, that therapeutic benefit of the application of HSV1716/NAT and radiopharmaceutical is scheduling-dependant; and third, that cure is achievable after the combination of the 2 modalities.

Intratumoral and intravenous administration of HSV1716/NAT enhanced the accumulation of ^{131}I-MIBG in xenografts but did not affect uptake of the radiopharmaceutical by normal tissues. Intratumoral virus injection 24 h before radiopharmaceutical, compared with the simultaneous administration of the 2 agents, resulted in approximately twice the concentration of ^{131}I-MIBG in tumors. In the absence of the NAT transgene (injection of PBS or HSV1716), ^{131}I-MIBG accumulation was negligible, indicating that ^{131}I-MIBG did not accrue in tumor tissue without the introduction of the NAT transgene via HSV1716/NAT. Furthermore, the greater accumulation achieved by sequential application of virus and ^{131}I-MIBG was reflected in a measurable increase in the inhibition of tumor growth and an enhanced cure rate, probably because of the enabling of HSV1716/NAT infection of tumor cells with concomitant expression of the NAT before ^{131}I-MIBG uptake.

The preferred route of administration of HSV1716 in clinical trials has been by intratumoral injection. This, however, would not benefit patients with metastatic disease. We have demonstrated in these proof-of-principle studies that intravenous injection of HSV1716/NAT results in NAT expression and active uptake of ^{131}I-MIBG within tumor tissue, with no evidence of virus or additional uptake of ^{131}I-MIBG in normal tissues.

For several decades, viruses and their replication-selective counterparts have been investigated as a means of killing tumor cells (*30*) and have shown considerable promise for cancer treatment (*5*). HSV1716, an avirulent HSV-1 mutant (*31*), does not replicate in nondividing or terminally differentiated cells (*7*) or cause encephalitis after intracerebral inoculation of mice (*6*), but replication and lysis readily occur in dividing tumor cells (*7*). These characteristics render HSV1716 a promising candidate for tumor-specific cell lysis, in particular for brain tumors, which rapidly proliferate within a background of nondividing cells.

The safety of intratumoral injection of HSV1716 has been demonstrated in clinical studies (*13–17,32*). Although HSV1716 has shown promise as a monotherapy, enhanced therapeutic potential is achievable via combination with conventional treatments, in which the expression of several different targets in the rapidly evolving cells of tumors is exploited. Because the mode of action of HSV toxicity is distinct from conventional chemotherapy and radiotherapy, the benefit of the combination with chemotherapeutic drugs or radiation is implied (*33,5*). Another strategy used to enhance HSV efficacy has been the creation of HSV variants expressing prodrug activation enzymes that, compared with virus alone, have increased antitumor efficacy (*34*).

Radiation in combination with HSV-1 mutants has demonstrated enhanced kill in tumor models (*35–39*). Moreover, there are indications that the interaction between HSV-1 and radiation is synergistic, possibly because of increased viral replication and improved dissemination of the virus within tumors (*36,39*).

We demonstrated that HSV1716 can be modified to express the NAT transgene and endow cells with the capacity for uptake of ^{131}I-MIBG, allowing tumor cells to be targeted both by the oncolytic activity of HSV1716 and by the selective concentration of radionuclide without increased toxicity. Because of the physical limitations to efficient delivery of viruses, it is likely that most of the tumor cells are uninfected by initial virus inoculums. Although subsequent viral replication cycles result in increased tumor cell infection, it is improbable that all the infected cells will be killed, and additional modalities will be required to ensure 100% tumor cell kill. Furthermore, variations in cell state and cell cycle have a determining impact on the replication capacity of the virus, and HSV1716 may not enter its lytic replication cycle in all infected cells.

An attractive feature of oncolytic viral therapy combined with targeted radiotherapy is an intrinsic radiologic bystander effect derived from the transfer of radiation-induced toxic factors to, and cross-fire irradiation of, noninfected cells (*21*). Consequently, control of tumor growth may result from virus replication resulting in direct lysis of cells and the expression of the NAT transgene in other cells whose state does not allow a full virus replication. NAT gene expression allows the accumulation of ^{131}I-MIBG, resulting in direct radiation kill of such cells plus toxicity to neighboring, nontargeted cells (which have not been infected with virus) via cross-fire irradiation and RIBBE.

CONCLUSION

Scheduling experiments to further optimize delivery of virus and uptake of radionuclide by tumor cells are in progress. It is evident from these data that the delivery of HSV1716/NAT, followed by treatment with ^{131}I-MIBG, could have exciting clinical applications. Third-generation versions of the virus are under construction with tumor-targeting moieties for enhanced infection rates after systemic administration.

DISCLOSURE STATEMENT

The costs of publication of this article were defrayed in part by the payment of page charges. Therefore, and solely to indicate this fact, this article is hereby marked "advertisement" in accordance with 18 USC section 1734.

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REFERENCES

1. Shen Y, Nemunaitis J. Herpes simplex virus 1 (HSV-1) for cancer treatment. *Cancer Gene Ther.* 2006;13:975–992.
2. Grandi P, Peruzzi P, Reinhart B, Cohen JB, Chiocca EA, Glorioso JC. Design and application of oncolytic HSV vectors for glioblastoma therapy. *Expert Rev Neurother.* 2009;9:505–517.
3. Cassady KA, Parker JN. Herpesvirus vectors for therapy of brain tumours. *Open Virol J.* 2010;4:103–108.
4. Friedman GK, Pressey JG, Reddy AT, Markert JM, Gillespie GY. Herpes simplex virus oncolytic therapy for pediatric malignancies. *Mol Ther.* 2009;17:1125–1135.
5. Kanai R, Hiroaki W, Tooba C, Rabkin SD. Oncolytic herpes simplex virus vectors and chemotherapy: are combinatorial strategies more effective for cancer? *Future Oncol.* 2010;6:619–634.
6. 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;72:631–639.
7. Brown SM, Harland J, Maclean AR, Podlech J, Clements JB. Cell type and cell state determine differential in vitro growth of non virulent ICP34.5-negative herpes simplex virus. *J Gen Virol.* 1994;75:2367–2377.
8. McKie EA, Brown SM, MacLean AR, Graham DI. Histopathological responses in the CNS following inoculation with a non-neurovirulent mutant (1716) of herpes simplex virus type 1 (HSV 1): relevance for gene and cancer therapy. *Neuropathol Appl Neurobiol.* 1998;24:367–372.
9. Randazzo BP, Bhat MG, Kesari S, Fraser NW, Brown SM. Treatment of experimental subcutaneous human melanoma with a replication restricted herpes simplex mutant. *J Invest Dermatol.* 1997;108:933–937.
10. Coukos G, Makrigiannakis A, Kang EH, Rubin SC, Albelda SM, Molnar-Kimber KL. Oncolytic herpes simplex virus-1 lacking ICP34.5 induces p53-independent death and is efficacious against chemotherapy-resistant ovarian cancer. *Clin Cancer Res.* 2000;6:3342–3353.
11. Toyoizumi T, Mick R, Abbas AE, Kang AE, Kaiser LR, Molnar-Kimber KL. Combined therapy with chemotherapeutic agents and herpes simplex type 1 ICP34.5 mutant (HSV1716) in human non small cell lung cancer. *Hum Gene Ther.* 1999;10:3013–3029.
12. Thomas DL, Fraser NW. HSV-1 therapy of primary tumours reduces the number of metastases in an immune competent model of metastatic breast cancer. *Mol Ther.* 2003;8:543–551.
13. Rampling R, Cruickshank G, Papanastassiou V, et al. Toxicity evaluation of replication competent herpes simplex virus (ICP34.5 null mutant 1716) in patients with recurrent malignant glioma. *Gene Ther.* 2000;7:859–866.
14. Papanastassiou V, Rampling R, Fraser M, et al. The potential for efficacy of the modified (ICP34.5-) herpes simplex virus HSV1716 following intratumoural injection into human malignant glioma: proof of principle study. *Gene Ther.* 2002;9:398–406.
15. 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;11:1648–1658.
16. MacKie RM, Stewart B, Brown SM. Intralesional injection of herpes simplex virus 1716 in metastatic melanoma. *Lancet.* 2001;357:525–526.
17. Mace AT, Ganly I, Soutar DS, et al. Potential for efficacy of the oncolytic herpes simplex virus 1716 in patients with oral squamous cell carcinoma. *Head Neck.* 2008;30:1045–1051.
18. Boyd M, Mairs RJ, Mairs SC, et al. Expression in UVW glioma cells of the noradrenaline transporter gene, driven by the telomerase RNA promoter, induces active uptake of [131]MIBG and clonogenic cell kill. *Oncogene.* 2001;20:7804–7808.
19. McCluskey AG, Boyd M, Ross SC, et al. [^{131}I]meta-iodobenzylguanidine and topotecan combination treatment of tumours expressing the noradrenaline transporter. *Clin Cancer Res.* 2005;11:7929–7937.
20. Boyd M, Mairs SC, Stevenson K, et al. Transfectant mosaic spheroids: a new model for the evaluation of bystander effects in experimental gene therapy. *J Gene Med.* 2002;4:567–576.
21. Mothersill C, Seymour CB. Radiation induced bystander effects: past history and future directions. *Radiat Res.* 2001;155:759–767.
22. Carlsson J, Aronsson EF, Hietala S-O, Stigbrand T, Tennvall J. Tumour therapy with radionuclides: assessment of progress and problems. *Radiother Oncol.* 2003;66:107–117.
23. Quigg M, Mairs RJ, Brown SM, et al. Assessment *in vitro* of a novel therapeutic strategy for glioma, combining herpes simplex virus HSV1716-mediated oncolysis with gene transfer and targeted radiotherapy. *Med Chem.* 2005;1:423–429.
24. Harland J, Brown SM. HSV growth, preparation, and assay. In: Brown SM, MacLean AR, eds. *Methods in Molecular Medicine—Herpes Simplex Virus Protocols*. Totowa, N.J.: Humana Press Inc.; 1997:1–8.
25. Hunter DH, Zhu X. Polymer-supported radiopharmaceuticals: [^{131}I]MIBG and [^{123}I]MIBG. *J Labelled Comp Radiopharm.* 1999;42:653–661.
26. Workman P, Twentyman P, Balkwill F, et al. United Kingdom Co-ordinating Committee on Cancer Research (UKCCCR) guidelines for the welfare of animals in experimental neoplasia (second edition). *Br J Cancer.* 1998;77:1–10.
27. Morton DB, Griffiths PHM. Endpoints in animal study protocols. *Vet Rec.* 1985;116:431–436.
28. Braidwood L, Dunn PD, Hardy S, Evens TR, Brown SM. Antitumor activity of a selectively replication competent herpes simplex virus (HSV) with enzyme prodrug therapy. *Anticancer Res.* 2009;29:2159–2166.
29. Mairs RJ, Boyd M. Optimizing MIBG therapy of neuroendocrine tumors: preclinical evidence of dose maximization and synergy. *Nucl Med Biol.* 2008;35(suppl 1):S9–S20.
30. Asada T. Treatment of human cancer with mumps virus. *Cancer.* 1974;34:1907–1928.
31. McKie EA, Hope RG, Brown SM, MacLean AR. Characterization of the herpes simplex virus type 1 strain 17+ neurovirulence gene RL1 and its expression in a bacterial system. *J Gen Virol.* 1994;75:733–741.
32. Hammill AM, Conner J, Cripe TC. Oncolytic virotherapy reaches adolescence. *Pediatr Blood Cancer.* 2010;55:1253–1263.
33. Mace AT, Harrow SJ, Ganly I, Brown SM. Cytotoxic effects of the oncolytic herpes simplex virus HSV1716 alone and in combination with cisplatin in head and neck squamous cell carcinoma. *Acta Otolaryngol.* 2007;127:880–887.
34. Guffey MB, Parker JN, Luckett WS, et al. Engineered herpes simplex virus expressing bacterial cytosine deaminase for experimental therapy of brain tumors. *Cancer Gene Ther.* 2007;14:45–56.
35. Dai MH, Zamarin D, Gao SP, et al. Synergistic action of oncolytic herpes simplex virus and radiotherapy in pancreatic cancer cell lines. *Br J Surg.* 2010;97:1385–1394.
36. Jarnagin WR, Zager JS, Hezel M, et al. Treatment of cholangiocarcinoma with oncolytic herpes simplex virus combined with external beam radiation therapy. *Cancer Gene Ther.* 2006;13:326–334.
37. Blank SV, Rubin SC, Coukos G, Amin KM, Albelda SM, Molnar-Kimber KL. Replication-selective herpes simplex virus type 1 mutant therapy of cervical cancer is enhanced by low dose radiation. *Hum Gene Ther.* 2002;13:627–639.
38. Advani SJ, Sibley GS, Song PY, et al. Enhancement of replication of genetically engineered herpes simplex viruses by ionizing radiation: a new paradigm for destruction of therapeutically intractable tumors. *Gene Ther.* 1998;5:160–165.
39. Adusumilli PS, Stiles BM, Chan MK, et al. Radiation therapy potentiates effective oncolytic viral therapy in the treatment of lung cancer. *Ann Thorac Surg.* 2005;80:409–416.