Effective Intravenous Therapy of Murine Pulmonary Metastases with an Oncolytic Herpes Virus Expressing Interleukin 12

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ABSTRACT

Purpose: There currently is no therapy that enhances the survival of patients with distantly metastatic squamous cell carcinoma (SCC). Engineered herpes oncolytic viruses are effective therapeutic agents when delivered directly to tumors in animal models, but their efficacy in treating disseminated disease is poorly defined.

Experimental Design: We treated disseminated pulmonary SCC in mice with an interleukin (IL)-12-expressing oncolytic herpes virus (NV1042) or with the parent oncolytic virus (NV1023, IL-12 deficient) by i.v. tail vein administration.

Results: Lung IL-12 was 16.1 pg/mg and IFN-γ was 4.3 pg/mg at day 1 after a single dose of NV1042 (5 × 107 plaque-forming units); levels of both were undetectable for NV1023. 5-Bromo-4-chloro-3-indolyl-β-D-galactopyranoside histochemistry demonstrated viral infection of disseminated pulmonary tumor nodules by both vectors at day 1, with sparing of adjacent alveolar cells. NV1042-treated lungs showed no surface nodules at day 12, in contrast to NV1023-treated (92 ± 7 surface nodules) and PBS-treated (225 ± 9 surface nodules) lungs. Significantly enhanced survival was observed in NV1042-treated animals compared with NV1023- and PBS-treated animals (log rank < 0.05). In animals with a low tumor burden, 100% of NV1042-treated, 70% of NV1023-treated, and none of the control animals achieved long-term survival. NV1042 efficacy was similar to NV1023 efficacy in animals depleted of CD4/CD8 T lymphocytes, showing that IL-12 expression enhances oncolytic activity through immune effects. Histology showed no cytopathic effects in non-tumor-bearing lung, brain, spleen, liver, and pancreas after completion of viral therapy. No animals demonstrated any visible side effects attributable to viral therapy.

Conclusions: The i.v. delivery of an oncolytic herpes virus may achieve effective infection, oncolysis, and transgene expression at distant tumor sites. This approach to systemic therapy combining oncolysis with IL-12 immune stimulation led to significantly improved survival in animals with disseminated SCC.

INTRODUCTION

Squamous cell carcinoma (SCC) is a relatively common malignancy of epithelial cells that may arise from a variety of anatomical sites. SCC is the most common malignancy of the head and neck aerodigestive tract and cervix and often affects the esophagus, lungs, genitourinary system, and skin (1). Advanced-stage head and neck SCC may metastasize to distant sites, most commonly to the lungs. Lung metastases of SCC are generally considered an indicator of incurable disease and herald an extremely poor prognosis. To date, no treatment has been able to demonstrate a significantly improved survival in patients with head and neck SCC once distant metastases have been identified (2).

Attenuated, oncolytic herpes viruses have significant therapeutic effects when delivered directly by intratumoral injection to established tumors. In animal models, these vectors have shown efficacy in treating a wide variety of malignancies including brain, breast, colorectal, prostate, and head and neck cancers (3–7). We have constructed a series of replication-competent, attenuated herpes oncolytic viruses sharing a common viral structure. NV1023 is an oncolytic vector that is attenuated due to deletions in UL24, UL56, and the internal repeat sequences (joint region). NV1042 is identical to NV1023 except that the murine interleukin (IL)-12 gene, under control of a hybrid α4- thymidine kinase promoter, has been inserted into the deleted joint sequence. Although both vectors have equivalent oncolytic efficacy in lysing SCC in vitro, NV1042 administered intratumorally exhibited significantly enhanced therapeutic effects in mice bearing s.c. SCC tumors. IL-12 expression by NV1042 generated an antitumoral immune response that enhanced oncolytic efficacy (8).

As part of our preclinical investigation of these promising cancer therapy agents, we assessed their potential application for the treatment of disseminated disease. Our goal was to determine whether i.v. administration of these engineered herpes viruses could effectively treat a murine model of distant SCC metastases.
MATERIALS AND METHODS

Cell Lines. The murine SCC VII cell line is a cutaneous SCC that spontaneously arose from the C3H/HeJ mouse. SCC VII is a poorly immunogenic, rapidly dividing cell line with an estimated doubling time of 18 h (9–11). SCC VII cells were grown in vitro in MEM containing 10% FCS under standard cell culture conditions.

Viruses. NV1023 and NV1042 are attenuated, replication-competent, oncolytic herpes viruses whose construction has been described previously (8). NV1023 and NV1042 are derived from R7020, a herpes simplex virus (HSV)-1 vector designed as a HSV-1/2 vaccine candidate that was tested clinically (12, 13). R7020 contains deletions in UL23/4, UL56, and the internal inverted repeat (joint region). R7020 also contains an insertion of the HSV-2 HindIII L fragment and an insertion of the endogenous copy of UL23 (thymidine kinase) into the deleted joint region. NV1042 was constructed by replacing the exogenous copy of thymidine kinase with murine IL-12 under the control of a hybrid /H92514-thymidine kinase promoter and by repairing the UL23/4 locus. NV1023 was constructed by deleting the exogeneous copy of thymidine kinase and repairing the UL23/4 locus (8). Viruses were provided by MediGene, Inc. (San Diego, CA).

Murine Model of SCC Pulmonary Metastases. Animal procedures were approved by the Memorial Sloan-Kettering Institutional Animal Care and Use Committee (New York, NY). Six-week-old male C3H/HeJ mice (The Jackson Laboratory, Bar Harbor, ME) were used to develop a murine model of disseminated SCC pulmonary metastases. SCC VII cells were delivered by tail vein injection to C3H/HeJ mice at varying doses (5 × 10^4, 1 × 10^5, and 5 × 10^5 cells) in 200 μl of PBS. On day 12 after tail vein injections, animals were sacrificed by CO2 inhalation. To identify and quantitate surface pulmonary nodules, the chest cavity was exposed through a midline chest incision. The trachea was cannulated with a 20-gauge angiocatheter, and the lungs were slowly insufflated with 1.5 ml of India Ink (1:16 in PBS; Fig. 1A; Ref. 14). The trachea was ligated, and the lungs were excised en bloc. The lungs were immersed in Fekete’s solution (100 ml of 70% ethanol, 10 ml of 4% formaldehyde, and 5 ml of 100% glacial acetic acid) to destain the pulmonary nodules, making them appear white against a black background of normal lung parenchyma.

Intravenous Viral Therapy: IL-12 and IFN-γ Expression and 5-Bromo-4-chloro-3-indolyl-β-D-galactopyranoside (X-Gal) Histochemistry. Animals were treated 5 days after SCC VII inoculation (5 × 10^5 cells) with a single dose of i.v. NV1042 or NV1023 [5 × 10^7 plaque-forming units (pfu)] or PBS in 200-μl volume. Non-tumor-bearing animals receiving NV1042 were also studied. Animals were sacrificed at days 1, 3, and 7 after i.v. viral delivery (n = 3 animals/group). Lungs, brain, bone marrow, and blood were extracted. For assessment of IL-12 and IFN-γ expression, a sample of each tissue was weighed and homogenized in 1 ml of tissue lysis solution (T-PER; Pierce Biotechnology, Rockford, IL). Samples were...
centrifuged, and supernatants were isolated. IL-12 and IFN-γ levels were measured by ELISA (R&D Systems, Minneapolis, MN). Tumor-bearing lung parenchyma was used as a surrogate for lung tumor, due to the small size of the lung nodules.

Portions of lungs and brains were frozen in Tissue Tek, cut into 8-μm-thick sections, and stained with X-Gal at 37°C for 2 h as described previously for assessment of β-galactosidase expression (15). Counterstaining of background cells with nuclear fast red was performed. Virally infected cells expressing β-galactosidase were identified histologically as blue-stained cells.

**Intravenous Viral Therapy: H&E Histology.** Animals were treated 5 days after SCC VII inoculation (5 × 10^5 cells) with three serial doses of PBS, NV1023, or NV1042 (2 × 10^7 pfu) on days 1, 3, and 5. On days 6, 10, and 14, animals were sacrificed. Lungs were fixed in 10% buffered formalin, embedded in paraffin, sectioned, and stained with H&E. Slides were examined by a pathologist to assess the extent of lung tumor, identify tumor-infiltrating lymphocytes, and identify tumor necrosis. Lung, brain, liver, spleen, and pancreas tissue were harvested at day 14 to evaluate for potential organ cytopathology.

**Intravenous Viral Therapy: Pulmonary Nodule Assessment and Survival Analysis.** Animals were inoculated with SCC VII cells (1 × 10^5; brief trypsinization) by tail vein injection to establish disseminated disease. Animals were treated at days 1, 3, and 5 with PBS, NV1023, or NV1042 (2 × 10^7 pfu) in 200-μl volume by tail vein injection. On day 12, animals were sacrificed (n = 5 animals/group), and their lungs were insufflated with India Ink. The lungs were excised and fixed in Fekete’s solution, and surface pulmonary nodules were counted. In a separate experiment, similarly treated animals were followed clinically for long-term survival assessment (n = 10 animals/group). Animals with significant body weight loss, visible s.c. tumor nodules, or other morbidity were sacrificed by CO2 inhalation. All nonsurviving animals underwent autopsy for assessment of gross disease and quantification of pulmonary nodules.

An additional survival experiment was performed in animals that received injection with a lower pulmonary tumor burden. Intravenous inoculation of animals with SCC VII cells harvested after extended trypsinization resulted in fewer and smaller pulmonary nodules. Animals were inoculated with SCC VII cells (1 × 10^5; extended trypsinization) and treated with i.v. PBS, NV1023, or NV1042 as described above. These animals were followed clinically for long-term survival assessment (n = 8 animals/group).

**Animal CD4/CD8 Depletion Studies.** To assess the importance of T lymphocyte activity on NV1042 efficacy, C3H/HeJ mice were treated with GK1.5 (anti-CD4) and 53-6.72 (anti-CD8) antibodies to deplete CD4+ and CD8+ T lymphocytes as described previously (8). Each animal received 0.5 mg of each antibody delivered by i.p. injection on days 1, 3, and 10 and weekly thereafter. In a previous study, splenocytes isolated from both depleted and nondepleted animals on days 6 and 21 were stained with antimouse CD8 and CD4 monoclonal antibodies. Fluorescence-activated cell-sorting analysis confirmed the depletion of >98% CD4 and CD8 cells on both days (8).

CD4/CD8-depleted animals were inoculated with SCC VII cells (1 × 10^5; brief trypsinization) by i.v. tail vein injection on day 6. Animals were treated at days 7, 9, and 11 with PBS, NV1023 (2 × 10^7 pfu), or NV1042 (2 × 10^7 pfu) in 200-μl volume by tail vein injection. Animals were followed clinically for long-term survival assessment as described above and underwent postmortem surface lung nodule quantification.

**RESULTS**

**Murine Model of SCC Pulmonary Metastases.** Intravenous inoculation of SCC VII cells led to the reliable development of pulmonary surface nodules. Varying the dose of SCC VII inoculation (5 × 10^5, 1 × 10^5, or 5 × 10^3 cells) led to significant differences in nodule counts and sizes (Fig. 1, B–D). Animals inoculated with SCC VII cells harvested with extended trypsinization developed fewer and smaller pulmonary nodules than those inoculated with an identical number of cells harvested with brief trypsinization. Trypsinization of tumor cell lines in vitro has previously been noted to affect tumorigenicity in vivo (16). We observed that extended trypsinization caused SCC VII cells to form a single cell suspension, whereas brief trypsinization left some SCC VII cells clustered together in small groups. We selected three experimental models of pulmonary metastases to evaluate the effects of NV1023 and NV1042: (a) injection of 5 × 10^5 SCC VII cells as a model of heavy tumor burden; (b) injection of 1 × 10^5 SCC VII cells after brief trypsinization as a model of standard tumor burden; and (c) injection of 1 × 10^5 SCC VII cells after extended trypsinization as a model of low tumor burden.

**IL-12 Expression, IFN-γ Expression, and X-Gal Histochemistry after i.v. Viral Injection.** IL-12 levels for tumor-bearing, NV1042-treated animals at day 1 were measured in the lung (16.1 pg/mg), bone marrow (6.9 pg/mg), blood (5.5 pg/mg), and brain (<1.0 pg/mg; Fig. 2). Non-tumor-bearing animals had lower lung IL-12 expression (10.7 pg/mg) than tumor-bearing animals at day 1. IL-12 expression in tumor-bearing lungs decreased to 4.8 pg/mg at day 3 and was undetectable (<1 pg/mg) by day 7. IL-12 expression in the bone marrow, blood, and brain was undetectable at days 3 and 7. Intravenous NV1023 and PBS treatment resulted in undetectable levels of IL-12 in all tissues at all time points.

IFN-γ levels for tumor-bearing, NV1042-treated animals were measured in the lung at day 1 (4.3 pg/mg), day 3 (5.5 pg/mg), and day 7 (<1 pg/mg; Fig. 3). Non-tumor-bearing animals had lower lung IFN-γ expression (1.8 pg/mg) than tumor-bearing animals at day 1. After i.v. NV1023 treatment, IFN-γ was undetectable at day 1 and was 2.9 pg/mg at day 3. PBS treatment resulted in undetectable lung IFN-γ at all time points.

**X-Gal histochemistry was performed on excised lungs and brains at day 1 from animals with heavy SCC VII tumor burdens, treated with a single i.v. dose of PBS, NV1023, or NV1042 (5 × 10^7 pfu). Lung sections demonstrated mild blue staining of SCC VII nodules, with sparing of normal alveolar cells (Fig. 4, A–D). Brain tissue sections did not demonstrate any blue staining.**

**Intravenous Viral Therapy: Histology.** After i.v. therapy with PBS, NV1023, or NV1042 (2 × 10^7 pfu) on days 1, 3, and 5, lungs were examined histologically at days 6, 10, and 14. The lungs demonstrated significant differences in lung tumor
volume between experimental groups at each time interval. At day 10, the PBS-treated group displayed nearly confluent areas of tumor in the lung parenchyma, the NV1023-treated group displayed large nodules, and the NV1042-treated group displayed only small rare nodules (Fig. 4, E–G). There were a greater number of tumor-infiltrating lymphocytes in the NV1042-treated tumors (moderate) as compared with the NV1023-treated (mild) and PBS-treated (scant) groups on days 6 and 10 (Table 1). At day 14, both NV1042- and NV1023-treated tumors demonstrated a moderate number of tumor-infiltrating lymphocytes. Tumor-infiltrating neutrophils and macrophages were extremely rare for all groups at all time points. At day 14, tumor necrosis was evident in NV1023- and NV1042-treated lung tumors, but not in PBS-treated tumors.

Organs were examined histologically at day 14 for potential toxicities. Lung (non-tumor-bearing areas), brain, liver, spleen, and pancreatic tissue all appeared completely normal without any cytopathic effects for any of the groups (Fig. 5). PBS-treated, NV1023-treated, and NV1042-treated organs all appeared identical. Tumor was not identified within any organs other than the lung.

**Intravenous Viral Therapy: Pulmonary Nodules and Survival.** Surface pulmonary nodules were counted in animals 12 days after i.v. SCC VII inoculation followed by viral therapy (2 × 10⁷ pfu) on days 1, 3, and 5. The average number ± SE of surface nodules for PBS-treated controls (225 ± 9) was significantly higher than that for NV1023-treated animals (92 ± 27; \( P < 0.05 \), t test). No surface nodules were visible in any of the NV1042-treated animals (\( P < 0.05 \), t test compared with NV1023-treated animals; Fig. 6).

Significantly enhanced survival was observed with NV1042-treated animals compared with NV1023- and PBS-treated animals (log rank < 0.05). Median survival was 50, 27, and 25 days, respectively. Of the NV1042-treated animals, 40% demonstrated long-term survival exceeding 90 days, in contrast to none of the NV1023- or PBS-treated animals (Fig. 7A).

To explore the oncolytic activity of HSV in the model with low tumor burden, mice were inoculated with 1 × 10⁶ SCC VII cells after extended trypsinization. Striking differences were noted between both viral treatment groups and the control group (log rank < 0.05). Control animals treated with PBS had a median survival of 35 days, with 100% mortality by day 62. In contrast, 70% of animals treated with NV1023 and 100% of animals treated with NV1042 demonstrated long-term survival exceeding 90 days (Fig. 7B). Survival was significantly better with NV1042 therapy than with NV1023 therapy (log rank < 0.05).

Autopsies demonstrated that every nonsurviving animal, regardless of treatment group, had gross tumor present to varying degrees. Animals with shorter survivals generally died from widespread pulmonary parenchymal nodules, whereas those with intermediate survivals tended to manifest pleural, mediastinal, and soft tissue metastases. All long-term survivors remained completely free of disease, indicating that viral therapy for these animals was effective at all potential sites of tumor dissemination.

No animals developed any clinical signs that were attributable to NV1042 or NV1023 administration. Virally treated groups demonstrated less weight loss as compared with the control groups, most likely due to better control of tumor pro-
regation as compared with the control groups. In the NV1042- or NV1023-treated long-term survivors, there was no evidence of weight loss, poor grooming, neurotoxicity, mucosal ulcerations, or any other visible morbidity.

Animal CD4/CD8 Depletion Studies. To explore the effects of NV1042 immune-enhanced oncolysis, mice were depleted of CD4 and CD8 T lymphocytes. In these animals, the significant survival advantage seen previously with NV1042 therapy over NV1023 was largely reduced, with median surviv-

als of 32 and 30 days, respectively, and 100% mortality by days 43 and 35, respectively. NV1042 therapy remained only slightly improved compared with NV1023 therapy (log rank < 0.05). PBS-treated animals had a median survival of 21 days, 100% mortality by day 28, and significantly poorer survival as compared with the virally treated groups (log rank < 0.05; Fig. 7C). Postmortem pulmonary surface nodule counts of nonsurviving animals failed to demonstrate a statistically significant difference between NV1042 therapy (6.8 ± 2.1) and NV1023 therapy.
Control animals had a higher mean nodule count (98.7 ± 7.5) compared with either of the virally treated groups (P < 0.05, t test).

**DISCUSSION**

The identification of distant metastases in patients with SCC is an ominous finding that heralds an extremely poor prognosis (2). Distant metastases of head and neck SCC most often occur in the lungs and less commonly occur in the liver or bone. Unfortunately, patients with distant metastases are generally considered incurable and are usually offered either palliative chemotherapy or observation. Although a variety of chemotherapeutic agents have systemic activity against SCC, no treatment has been demonstrated to confer a significant survival advantage once distant metastases have been identified. Therefore, novel therapies that may effectively treat distant metastases of SCC are needed for clinical use.

Engineered herpes oncolytic viruses are novel therapeutic agents that have demonstrated potent antitumoral effects in a variety of preclinical studies (3–7). HSVs exhibit a natural ability to infect and lyse a wide variety of malignant tumor cells. HSVs have also been genetically modified to attenuate their toxicity to normal tissues while maintaining their oncolytic activity against malignant tumors (3). This combination is intended to enhance the safety of these viruses without sacrificing their efficacy for clinical use. Several replication-competent oncolytic herpes viruses have recently been studied in clinical trials. The G207 and HSV1716 herpes vectors are attenuated by deletions in both γ34.5 loci and were delivered to patients with malignant gliomas by direct injection in two separate Phase I trials (17, 18). Although adverse events were noted in some patients, no toxicity was unequivocally ascribed to G207 at doses up to 3 × 10⁹ pfu. The HSV1716 vector was administered at doses up to 10⁷ pfu without any adverse clinical symptoms. Although these results are preliminarily encouraging, more studies are necessary to fully evaluate the safety of these vectors for clinical application.

Many recent preclinical studies have focused on assessing therapeutic response after localized viral delivery at an established tumor site. Intratumoral injections of oncolytic viruses cause significant reductions of tumor volumes in experimental models of brain, colorectal, breast, prostate, and head and neck cancers (3–7). Similarly, viral delivery to tumor confined within an anatomical compartment, such as the peritoneal cavity, pleural cavity, or the bladder, also exhibits significant antitumoral effects (19–21). Studies have also shown that selective intra-
portal or intrasplenic delivery of an oncolytic virus may effectively treat animal models of liver tumors (22, 23). The potential of these agents to treat regionally metastatic nodal disease has also been demonstrated previously (24). Despite these successes in treating localized tumors, the efficacy of i.v., systemic oncolytic HSV therapy and its potential toxicities have not been well defined. Intravenous therapy with the G207 vector has been reported to have some activity in treating localized prostate and bladder tumors in animal models (6, 21).

The purpose of this study was to investigate the therapeutic efficacy of i.v. oncolytic HSV in treating disseminated SCC. Previous studies have suggested that systemic therapy with oncolytic herpes viruses may be problematic due to inhibitory immune effects. In a rat model, circulating IgM antibody and complement have been shown to impede the intravascular efficacy of an oncolytic herpes virus (25, 26). Suppression of IgM antibody with cyclophosphamide and inhibition of rodent plasma complement with cobra venom factor reversed these effects, enhancing viral survival and propagation. Furthermore, in animals previously exposed to an oncolytic herpes virus, i.v. delivery of the virus is less effective than intra-arterial delivery in treating liver nodules due to both humoral and cellular immune inhibition of virus (27). Viral inactivation by the host may therefore limit systemic herpes oncolytic efficacy. The goal of this study was to assess the efficacy of i.v. herpes oncolytic therapy in a murine model of disseminated pulmonary metastases. The i.v. delivery of herpes oncolytic vectors may be theoretically problematic due to viral clearance by circulating complement and neutralizing antibodies.

NV1023 and NV1042 are replication-competent, oncolytic herpes viruses that are attenuated through deletions of several HSV genes (8). For added safety, both vectors carry the thymidine kinase gene, making them sensitive to acyclovir as a potential means of eradicating the virus. The attenuated parent virus from which NV1023 and NV042 were derived, NV1020 (R7020), was originally designed as a potential vaccine and has a well-documented safety profile in Aotus monkeys, a primate exquisitely sensitive to wild-type herpes viral infections (12, 13). In these studies, NV1020 was found to cause significantly reduced toxicity at a 10,000-fold higher dose than that of the wild-type HSV-1 virus. The NV1020 vector is currently being studied in a trial for patients with hepatic colorectal metastases (28). Doses of up to $1.3 \times 10^7$ pfu were administered by hepatic infusion pump without dose-limiting toxicity. The only adverse effects possibly attributable to viral infusion were fever, nausea, and headache. The favorable safety profile of NV1020 has therefore encouraged our continuing investigation of its related vectors for possible clinical application.

In our study, virally treated animals suffered no clinically apparent side effects that may be attributable to viral administration for over a 3-month period. Histological examination demonstrated no evidence of any cytopathic effects to normal lung, brain, liver, spleen, and pancreas tissue after the completion three serial i.v. viral doses. Furthermore, viral infection appeared to be preferential for tumor tissue; X-Gal staining was identified within lung tumor nodules, with relative sparing of normal alveolar tissue. Despite these findings, additional preclinical studies are necessary to fully evaluate the safety of these vectors.

Fig. 7  A, in the model of standard pulmonary tumor burden, animals were i.v. treated with three serial doses of i.v. PBS, NV1023, or NV1042. NV1042-treated animals demonstrated enhanced survival (log rank < 0.05). B, in the model of decreased pulmonary tumor burden, NV1042 resulted in 100% long-term survival, in comparison with 70% for NV1023-treated animals and 0% for PBS-treated animals. C, in the model of standard pulmonary tumor burden, animals were depleted of CD4/CD8 T lymphocytes and treated with i.v. PBS, NV1023, or NV1042. CD4/CD8 depletion abrogated differences between NV1042 and NV1023 efficacy.
Treatment of Pulmonary Metastases with HSV and IL-12

Two models of differing pulmonary tumor burden were examined. Efficacy of both NV1023 and NV1042 was more pronounced in the model of decreased tumor burden, suggesting that these vectors may be more effective in treating microscopic or early distant disease rather than in treating more advanced sites of metastases. Pulmonary nodule counts decreased, and survival was enhanced with the purely oncolytic NV1023 virus, in comparison with PBS administration. Intravenous NV1042 oncolytic therapy with IL-12 demonstrated a more dramatic improvement: none of the NV1042-treated animals showed any visible pulmonary nodules at day 12; and significantly enhanced survival was demonstrated in both animal models. NV1042-treated tumors demonstrated more tumor-infiltrating lymphocytes at early time points. Antibody depletion experiments largely abrogated the enhanced efficacy of NV1042 over NV1023, suggesting that the IL-12 benefit was predominantly mediated through T lymphocyte activity.

IL-12 is a cytokine secreted by antigen-presenting cells such as dendritic cells, monocytes, macrophages, and B lymphocytes (29, 30). IL-12 acts as an important mediator in cell-mediated immunity by promoting helper T type 1 lymphocyte development and has been shown to stimulate antitumoral immune effects in murine models (31–33). NV1042-treated tumors demonstrated an increase in tumor-infiltrating lymphocytes as compared with the NV1023-treated tumors. IL-12 also stimulates the proliferation and activity of natural killer cells and has antiangiogenic effects mediated through IFN-γ (34). Lung IFN-γ levels were elevated in NV1042-treated animals but not in NV1023-treated ones, suggesting that antiangiogenic effects might also partially account for enhanced therapy by NV1042. Efficacy with NV1023 demonstrates that i.v. delivered HSV has a direct oncolytic effect on disseminated SCC. The improved therapeutic effect with NV1042 further shows that therapy may be significantly enhanced with antitumoral effects induced by IL-12 gene expression.

SCC VII is a rapidly proliferating, radiation-resistant, highly aggressive murine carcinoma that is poorly immunogenic and has both regional and distant metastatic potential (10, 11). A previous study demonstrated that i.v. seeding of SCC VII in mice resulted in significant pulmonary disease in all animals, with no improvement after adoptive T-cell immunotherapy with IL-2 (11). We found that NV1042 was effective in treating mice bearing systemic SCC VII, resulting in 100% long-term survival in comparison with 0% of the control animals. Because of the aggressive behavior of disseminated SCC VII disease, viral therapy was initiated 1 day after tumor cell inoculation. Although this model does not accurately reflect a clinical scenario of established metastatic disease, it does support the principle that systemically delivered oncolytic HSV has therapeutic effects on disseminated disease. Long-term survivors showed no clinical evidence of disease, whereas control and nonsurviving animals manifest diffuse disease in the pulmonary parenchyma, pleural cavity, mediastinum, and s.c. soft tissues. Viral therapy therefore demonstrated global therapeutic effects in treating disseminated SCC VII cells. Combined oncolytic and IL-12 therapy resulted in dramatically improved results as compared with a purely oncolytic approach in animals bearing this aggressive, disseminated malignancy.

In conclusion, these findings demonstrate that i.v. delivery of herpes oncolytic viruses (a) may infect distant metastatic deposits, (b) may express a cytokine transgene to enhance therapy, (c) may impede pulmonary nodule formation, and (d) may significantly enhance survival. IL-12 expression dramatically improved therapeutic efficacy through T lymphocyte activity. Histological examination of organs showed no cytopathic effects on normal organs after completion of three serial viral doses. Animals did not show any side effects attributable to viral therapy, although additional studies are necessary to fully investigate the safety profile of these vectors.

Patients with distant metastases from solid malignancies currently suffer a dismal prognosis and have few meaningful therapeutic options. Because engineered HSVs have potent oncolytic effects in numerous cancers, it is possible that i.v. efficacy may also be appreciated for other disseminated malignancies. The potential application of these novel antitumoral agents for systemic disease therefore carries implications for improving patient care. This study demonstrates that oncolytic herpes viruses may have systemic efficacy in treating disseminated SCC. The potential i.v. application of these agents for distinctly metastatic malignancies should be investigated in future studies.

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