Systemic Therapy of Spontaneous Prostate Cancer in Transgenic Mice with Oncolytic Herpes Simplex Viruses

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Abstract

Oncolytic viruses are an innovative therapeutic strategy for cancer, wherein viral replication and cytotoxicity are selective for tumor cells. Here we show the efficacy of systemically administered oncolytic viruses for the treatment of spontaneously arising tumors, specifically the use of oncolytic herpes simplex viruses (HSV) administered i.v. to treat spontaneously developing primary and metastatic prostate cancer in the transgenic TRAMP mouse, which recapitulates human prostate cancer progression. Four administrations of systemically delivered NV1023 virus, an HSV-1/HSV-2 oncolytic recombinant, to TRAMP mice at 12 or 18 weeks of age (presence of prostate adenocarcinoma or metastatic disease, respectively) inhibited primary tumor growth and metastases to lymph nodes. Expression of interleukin 12 (IL-12) from NV1042 virus, a derivative of NV1023, was additionally effective, significantly reducing the frequency of development of prostate cancer and lung metastases, even when the mice were treated after the onset of metastasis at 18 weeks of age. NV1042-infected cells, as detected by 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside staining for Lac Z expressed by the virus, were present in prostate tumors 1 week after the final virus injection and viral DNA was detected at 2 weeks after final virus injection by real-time PCR in primary and metastatic tumors but not in liver or blood. No toxicity was observed in any of the treated mice. The efficacy of the IL-12–expressing NV1042 virus in this aggressive prostate cancer model using a clinically relevant treatment paradigm merits its consideration for clinical studies. [Cancer Res 2007;67(19):9371–9]

Introduction

Oncolytic viruses designed to differentially target cancer cells while sparing normal tissues have advanced in the past decade to the forefront of innovative strategies for cancer treatment (1, 2). Since the conception in 1991 of using mutated herpes simplex virus (HSV), a neurotropic virus, to treat brain tumors (3), the unique forms of the disease in which hormone- and radiation-refractory prostate cancer irrespective of hormone status or radiosensitivity is a nerve-sparing virus (12, 13). This overcomes a current challenge of conventional treatments such as surgery and radiation therapy, which are associated with risks of nerve damage. (b) Oncolytic HSV mutants, including G207 and NV1020, have shown efficacy against human prostate cancer xenografts and mouse prostate cancers following intraneplastic or i.v. administration (14–17). (c) G207 and other vectors are effective against human prostate cancer irrespective of hormone status or radiosensitivity (14, 16, 18)—a major advantage in its application for advanced forms of the disease in which hormone- and radiation-refractory tumor is an inevitable progression.

To date, however, all efficacy studies with HSV vectors for prostate cancer have used implanted tumor models, which are artificial systems with respect to their milieu and lymphovascular supply. Whereas these implanted models are easily amenable to therapeutic manipulation, they do not truly reflect the in situ cancer situation and may affect the outcome of the therapy being investigated. Genetically engineered mouse models that develop prostate cancer spontaneously are currently the most representative models to conduct efficacy studies. Therefore, we have used the transgenic TRAMP mouse, which develops prostate cancer spontaneously with progression to metastatic disease (19, 20). In TRAMP mice, the rat probasin promoter regulated by androgens drives SV40 T antigen expression, restricting its expression to the liver and has deletions of one copy of 34.5, the internal repeat, and UL24 and UL56 genes, as well as the addition of gI, gG, US2, and US3 genes from HSV-2 (21, 22). NV1023 has an additional...
insertion of LacZ and deletion of ICP47, US11, and US10 genes (21). Comparison of efficacy in s.c. or metastatic lung TRAMP-C2 tumors showed that the murine interleukin 12 (IL-12)–expressing NV1042 virus was superior to its parent, NV1023, and G207 (17, 23). We also showed that IL-12 expression from NV1042 resulted in both immune and antiangiogenic effects (17, 23).

Based on the above findings, we investigated the utility of systemically administered NV1023 and NV1042 to treat spontaneously arising prostate cancer and metastasis in TRAMP mice. Results show that both NV1023 and NV1042 significantly inhibited the growth of primary tumors in prostate and metastasis in periaortic lymph nodes. NV1042 was additionally effective in reducing the frequency of the development of prostate carcinoma and lung metastasis.

Materials and Methods

Mice. TRAMP (C57Bl/6 background) breeder pairs (female TRAMP and male C57Bl/6) were purchased from The Jackson Laboratory and bred in-house at the Center for Comparative Medicine facility at Massachusetts General Hospital. Female transgenic F1 pups were crossed with male FVB/N mice obtained from National Cancer Institute to generate TRAMP mice on an FVB/N background. The pups were genotyped at 3 weeks of age using MHC class I alleles and obtained from the Collaborative Biomedical Resources Program at Massachusetts General Hospital. The pups were genotyped at 3 weeks of age using SV40 large T antigen primers (5'-CACGAGCAATGGTGAGGTTG-3' and 5'-ACACCAAACAACTAGTCG-3') for PCR of tail genomic DNA isolated using phenol-chloroform extraction (24). F1 male TRAMP mice obtained from this cross-breeding were used for all experiments described below. Mice were housed in a pathogen-free facility and all animal procedures were conducted with approval from the Massachusetts General Hospital Subcommittee on Research Animal Care. All animal studies were blinded.

Viruses. Purified virus stocks of NV1023 and NV1042 were obtained from MediGene, Inc. Construction of NV1023 and NV1042 has previously been described (21). NV1023, derived from NV1020 (R7020), a HSV-1/HSV-2 intertypic recombinant developed as a vaccine strain (22), contains an insertion of LacZ into the ICP47 locus, deleting ICP47, US11, and US10 (21). NV1042 is NV1023 with an insertion of murine IL-12 cDNA (p35 and p40 as a single polypeptide separated by elastin motifs) expressed from a hybrid α4-TK promoter (21). The viruses were individually titered on Vero (African green monkey kidney) cells by plaque assay. NV1042-infected TRAMP-C2 cells secreted 52 ng/ml of IL-12 (17).

Virus treatment and efficacy evaluation. Twelve-week-old (n = 8–9 per group) or 18-week-old (n = 17 per group) male TRAMP mice were inoculated via tail vein with 2 × 10⁷ plaque-forming units (pfu) of NV1023 or NV1042 or virus buffer consisting of 10% glycerol in PBS (mock) in a 200-μl volume on days 0, 3, 7, and 10. By day 14 after initiation of treatment, anti-HSV serum antibody was detectable (data not shown). Mice were monitored twice weekly and sacrificed if moribund. At 24 weeks, all mice were sacrificed, terminating the experiment. Prostate and seminal vesicles were removed en bloc, weighed, and photographed. Formalin-fixed sections of prostate, periaortic lymph nodes, and lungs were evaluated by H&E staining and the frequency of carcinoma was scored in a blinded manner by the collaborating pathologist (G.P.N.). Histologic grading of prostate samples was done as previously published (25).

Virus biodistribution studies. TRAMP mice were treated with 2 × 10⁷ pfu of NV1042 on days 0, 3, 7, and 10. Mice were sacrificed at predetermined days and various tissues were evaluated for β-galactosidase by 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside (X-gal) staining and immunohistochemistry and for the presence of HSV-1 DNA by real-time PCR. β-Galactosidase staining. Tissue cryostat sections of prostate and seminal vesicles, periaortic lymph nodes, lung, liver, and brain obtained from three mice each sacrificed on days 11, 13, and 17 (or 1, 3, and 7 days after the final treatment) were analyzed by X-gal staining at pH 7.2, as previously described. For senescence-associated β-galactosidase staining, we carried out X-gal histochemistry at pH 6.0 (26). Sections were counterstained with eosin or H&E. For immunohistochemistry, sections were washed with 0.2% Triton X-100 in PBS, 0.3% hydrogen peroxide in PBS, and 1% and then 10% goat serum in PBS; incubated with rabbit anti-E. coli β-galactosidase (1:1000; Abcam, Inc.) overnight at 4°C; washed in PBS; and incubated with biotinylated goat anti-rabbit immunoglobulin G (Vector Laboratories). Immunoreactive material was detected with Vectastain Elite ABC and diaminobenzidine kits (Vector Laboratories).

Real-time PCR. NV1042-treated mice were sacrificed on days 11, 13, and 24 (or 1, 3, and 14 days after the final treatment) and various tissues (prostate and seminal vesicles, periaortic lymph nodes, lung, liver, brain, and blood) were removed aseptically and immediately snap frozen on dry ice with isopentane. Tissues were resuspended in nucleic acid lysis buffer (Applied Biosystems) and homogenized using a Mixer Mill (Qiagen). Total DNA was extracted from the homogenate using ABI Prism 6100 Nucleic Acid PrepStation (Applied Biosystems). Absolute quantification of viral DNA was conducted by real-time TaqMan PCR using HSV-1 gB primer sequences (forward primer, 5'-TGTGTCATATGTCGTTTGTACG-3'; reverse primer, 5'-GGCTAGAGCGGTCAACCT-3'; probe, 5'-ACACAGCTACGGCGGC-3') synthesized using Assays-by-Design service (Applied Biosystems). Mouse glyceraldehyde-3-phosphate dehydrogenase (GAPDH) primers (Applied Biosystems) were used as endogenous control for input DNA. Strain F genomic DNA served as positive control and was used to generate a standard curve from 15 to 2.4 × 10⁵ copies.

Statistical analysis. Statistical analyses were conducted by comparing NV1023- or NV1042-treated mice with mock, or NV1023-treated with NV1042-treated mouse group. Because the experimental data of prostate tumor weight from the efficacy studies did not follow a normal Gaussian distribution, nonparametric Mann-Whitney tests (two-tailed) were used to analyze significance between treatment groups. The frequency of carcinoma in prostate, periaortic lymph nodes, and lungs between treatment groups was found by contingency analysis using Fisher's exact two-sided test. Kaplan-Meier survival data were analyzed using χ² log-rank test. z levels for all analyses were P < 0.05; n values and exact P values are indicated in the text and legends. All statistical analyses were done using GraphPad Prism v.4.

Results

Spontaneous primary and metastatic prostate cancer development. Because the TRAMP mice were bred in-house, we determined the time line of prostate cancer and metastasis development before using them in viral therapy studies by analyzing 125 male transgenic TRAMP mice, and observed reproducibility with tumor development and progression. As illustrated in Fig. 1, TRAMP mice on the FVB/N background display PIN by 8 weeks of age, which progresses to carcinoma by 12 weeks and to metastases in periaortic lymph nodes and lung by 18 weeks of age. For comparison, histology of normal prostate from a nontransgenic littermate is shown. Systemic treatment with NV1023 or NV1042 was initiated at an age when mice first exhibit either primary prostate carcinoma (12 weeks) or metastasis (18 weeks). The mice were sacrificed at 24 weeks of age when untreated mice become moribund from disease.

Efficacy of systemic oncolytic HSV therapy on primary prostate cancer. Mouse cells are more resistant to HSV infection, and in our prior studies with implanted mouse prostate TRAMP-C2 cells in C57Bl/6 mice, we had noted that four intraneoplastic injections were significantly more effective than two treatments (17). Additionally, with TRAMP-C2 tumors metastatic to lung, we had observed that four i.v. administrations were significantly more effective than two treatments (17). With TRAMP-C2 tumors metastatic to lung, we had observed that four i.v. administrations were significantly more effective than two treatments.
prostate cancer growth in virus-treated mice when compared with mock mice as illustrated in the gross photograph (Fig. 2A). Because multifocal tumors also arise in the seminal vesicles of these mice and often coalesce with the prostate gland by sacrifice (24 weeks), the carcinomatous mass containing both prostate and seminal vesicles was excised as one unit and weighed. Distribution of prostate and seminal vesicle tumor weights (Fig. 2B) illustrates that mock-treated mice harbored tumors with a mean weight of 10.17 g, NV1023 with 3.98 g ($P = 0.026$, versus mock, Mann-Whitney test), and NV1042 with 2.79 g ($P = 0.003$, versus mock, Mann-Whitney test). For comparative purposes, the average weight of prostate and seminal vesicles from nontransgenic TRAMP mice is 0.78 g. In this experiment, two of nine mice from both the mock- and NV1023-treated groups died within 2 days of the 24-week sacrifice and one mouse from the NV1042 group died 1 week after treatment (at ~14 weeks of age). Histologic analysis of prostates from these dead mice showed that those from the mock and NV1023 treatment groups had large prostate tumors comprising of carcinoma, whereas the single NV1042-treated mouse did not display any evidence of cancer and therefore likely died from unrelated causes. H&E analysis of prostates showed consistent histologic grades among the treatment groups and included glands with normal histology, PIN, and invasive carcinoma of undifferentiated type. Whereas 8 of 9 (89%) mock mice had progressed to undifferentiated invasive carcinoma, only 6 of 9 (67%) NV1023-treated and 2 of 8 (25%) NV1042-treated mice ($P = 0.015$, versus mock, Fisher’s exact test) progressed to invasive carcinoma (Table 1). Correspondingly, PIN was the highest grade observed in the prostates of 3 of 9 (33%) NV0123-treated and 5 of 8 (63%) NV1042-treated mice, suggesting an inhibition of tumor progression in these treated mice. Thus, whereas both NV1023 and NV1042 were equally effective in inhibiting the growth of primary tumors (as assessed by tumor weight) when treated at 12 weeks, only NV1042 was effective in inhibiting tumor progression (as assessed by histologic grading) compared with mock or NV1023. Representative H&E-stained prostates, based on the most frequently observed histologic stage, from various treatment groups are illustrated in Fig. 2C. The largest tumors were highly necrotic (seen as pink areas in Fig. 2C, mock) with islands of tumor cells closely apposed to blood vessels within the necrotic areas.

Both NV1023 and NV1042 also inhibited primary prostate tumor growth as compared with mock treatment in mice treated at 18 weeks of age ($n = 17$ per group), when they begin to exhibit metastasis (Fig. 3A). There was a significant difference in the number of animals surviving to 24 weeks, with 8 of 17 mock dying or being sacrificed between 22 and 24 weeks due to tumor burden, as compared with 3 of 17 NV1023 and 2 of 17 NV1042 ($P = 0.003$, NV1042 versus mock, log-rank test). All of these mice harbored carcinoma within the prostate as determined by histologic analysis. Comparison of prostate and seminal vesicles weights in mice sacrificed at 24 weeks (Fig. 3B) shows a mean weight of 12.25 g in mock versus 6.54 g in NV1023 ($P = 0.04$ versus mock; Mann-Whitney test) and 3.66 g in NV1042 ($P = 0.002$, versus mock, Mann-Whitney test). Histologic analysis revealed that 16 of 17 (94%) mock mice, 13 of 17 (76%) of NV1023, and 10 of 17 (59%) of NV1042 ($P = 0.039$, versus mock, Fisher’s exact test) harbored invasive carcinoma, which were either well-differentiated adenocarcinoma or undifferentiated carcinoma (Table 1). Treatment at 18 weeks of age also resulted in inhibition of tumor progression within the prostate gland, with 35% of NV1042-treated mice displaying PIN as the highest grade without any advancement to carcinoma. Thus, when treated at 12 or 18 weeks, only NV1042 was effective in
inhibiting tumor growth within the prostate (as measured by prostate weight) and development of invasive carcinoma (as assessed by histologic grading).

**Efficacy of systemic oncolytic HSV therapy on metastasis.**

Treatment of TRAMP mice with NV1023 or NV1042 at 12 weeks of age, when they begin to develop prostate carcinoma, resulted in a significant reduction of metastatic frequency in periaortic lymph nodes from 86% in mock-treated to 14% in NV1023-treated mice (\( P = 0.03 \), versus mock, Fisher’s exact test) and to 25% in NV1042 (\( P = 0.04 \), versus mock, Fisher’s exact test). Whereas there was a

<table>
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<tr>
<th>Site/histology</th>
<th>Treatment at 12 wk of age, ( n ) (%)</th>
<th>Treatment at 18 wk of age, ( n ) (%)</th>
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<tbody>
<tr>
<td></td>
<td>Mock ((n = 7s + 2d))</td>
<td>NV1023 ((n = 7s + 2d))</td>
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<tr>
<td>Prostate—normal</td>
<td>1/8 (12.5)</td>
<td>1/17 (6)</td>
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<tr>
<td>Prostate—PIN</td>
<td>1/9 (11)</td>
<td>3/9 (33)</td>
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<tr>
<td>Prostate—invasive carcinoma: well-differentiated adenocarcinoma</td>
<td>1/7 (14)*</td>
<td>2/8 (25)*</td>
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<td>6/9 (67)</td>
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<tr>
<td>Periaortic lymph node carcinoma</td>
<td>6/7 (86)</td>
<td>1/7 (14)*</td>
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<tr>
<td>Lung carcinoma</td>
<td>4/7 (57)</td>
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NOTE: NV1023 or NV1042 \((2 \times 10^7\) pfu) or virus buffer (mock) was administered systemically on days 0, 3, 7, and 10 in TRAMP mice at either 12 or 18 wk of age. Mice were sacrificed at 24 wk and various tissues were processed for H&E staining and histologically graded. \( N \) values are shown in parentheses under each group (s, number of mice sacrificed at 24 wk of age; d, number of mice dead before 24 wk of age).

*\( P < 0.05 \), versus mock.

*Combined total of the two types of invasive carcinoma for statistical analysis.
reduction in metastatic frequency in the lungs, it was not significant (Table 1).

To examine whether oncolytic viral therapy could treat metastasis after their onset, TRAMP mice were treated at 18 weeks of age and sacrificed 6 weeks later at 24 weeks (Table 1). A significant reduction in frequency of metastasis to the periaortic lymph nodes was observed with NV1023 ($P = 0.04$, versus mock, Fisher’s exact test) and NV1042 ($P = 0.013$, versus mock, Fisher’s exact test), similar to the observations for treatment at 12 weeks of age. Interestingly, when scored for lung metastasis, a significant reduction was observed in the NV1042-treated mice, with only 1 of 14 (7%) mice exhibiting carcinoma ($P = 0.014$, versus mock, Fisher’s exact test) as compared with 5 of 9 (56%) mock-treated and 5 of 14 (36%) NV1023-treated mice.

**Virus biodistribution in NV1042-treated mice.** Both NV1023 and NV1042 harbor the *E. coli* LacZ gene, which acts as a reporter to track these viruses following systemic administration. TRAMP mice treated for 12 weeks with four doses of $2 \times 10^7$ pfu of i.v. NV1042 were sacrificed 1, 3, and 7 days after the last treatment, and various tissues (prostate, lung, liver, and brain) were removed following the final virus injection. X-gal staining was observed in a few isolated cells in the liver and lung and none in the brain (brain data not shown). However, by days 3 and 7, no staining was observed in the lungs, liver, or brain, whereas in the prostate, significant amounts of staining were detected. Thus, systemically administered NV1042 was able to persist at least for 7 days after treatment in the cancerous prostates but not in the normal organs. X-gal staining seen in hyperplastic glands on day 1 was distinct from senescence-associated β-galactosidase (Fig. 4C) that has been reported in prostate hyperplasia (27, 28). To further confirm that X-gal histochemistry was identifying LacZ-expressing cells, immunohistochemistry-positive cells were seen in the same region as X-gal–staining cells (Fig. 4B).

Biodistribution of NV1042 in 18-week-old TRAMP mice treated systemically on days 0, 3, 7, and 10 was assessed by real-time PCR using HSV gB sequences. DNA was isolated from organs (prostate and seminal vesicles, periaortic lymph nodes, lung, liver, brain, and blood) harvested on days 11, 13, and 24. Results shown in Fig. 5 illustrate that viral DNA was detected until day 24 (last day tested) in those organs that harbor primary and metastatic cancers (prostate, periaortic lymph nodes, and lungs). In contrast, whereas many viral copies were detected in the liver and blood on day 1, the level decreased to nondetectable (negative) by day 24, suggesting clearance of virally infected cells or degradation of viral DNA. No viral DNA was detected in the brain of any animal on any day tested.

**Discussion**

Prostate cancer in TRAMP mice arises from the targeted expression of SV40 T antigen within the epithelial cells of the prostate (19) and is influenced by the local prostate milieu. Studies using TRAMP mice would therefore be expected to be superior to implanted tumor models for a number of reasons: (a) Unlike implanted tumors generated from cultured cells, which are usually of a homogenous clonal phenotype, prostate tumors in TRAMP mice arise multifocally and are heterogeneous in nature, similar to the clinical situation. Such differences make treatment of these multiclonal autochthonous TRAMP tumors more difficult as opposed to implanted tumors. (b) Cells grown *in vitro* tend to accumulate additional alterations distinct from the original tumor, potentially influencing the outcome of therapies, thus affecting clinical translation. In contrast, evaluation of autochthonous *in vivo* tumors would minimize such external influences. (c) *In situ* prostate cancer development as observed in TRAMP mice is a dynamic process between transformed cells and the surrounding stroma and vasculature (29–31), whereas implanted tumors are in an artificial environment with respect to stroma, vasculature, and lymphatic supply, and therefore may respond to therapies more effectively, especially when initiated at a short interval after implantation when the tumor and local stromal cells have not become responsive to one another.

The TRAMP mice used in this study were bred on a FVB/N background. We also attempted to breed the TRAMP mice obtained from The Jackson Laboratory on a C57BL/6 background, but none of the 250+ F1 mice advanced from prostate adenoma to carcinoma even at death, which varied from 40 to 52 weeks. This lack of carcinoma development in the C57BL/6 background is at variance with the original report of TRAMP mice (20) but could be attributed to genetic polymorphisms (32) or dietary and environmental influences (33, 34). Nevertheless, when the F1 pups from the C57BL/6 background were crossed with the FVB/N background, the pups from this cross simulated the time line of prostate cancer progression reported previously (35). Even these TRAMP × FVB/N pups exhibited variations in cancer development that differed from previous reports: (a) a majority (~75%) of our mice survived only

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*Figure 3. Efficacy of systemic oncolytic virus in TRAMP mice treated at 18 wk of age. A, photograph of representative prostate and seminal vesicles excised en bloc from various treatment groups illustrating prostate tumors. B, distribution of weights of prostate and seminal vesicles from each treatment group. Mean is denoted by the line in each group; mean weight ± SE for each group are as follows: mock, 12.25 ± 2.3 g; NV1023, 6.54 ± 1.20 g ($P = 0.04$, versus mock, Mann-Whitney test); NV1023, 3.96 ± 0.72 g ($P = 0.002$, versus mock, Mann-Whitney test).*
until 25 weeks as opposed to a previously reported range of 24 to 39 weeks (36); (b) none of the 80 mice more than 18 weeks of age displayed bone metastases (20), which may have been due to our inability to detect the occasional incidence of bone metastasis reported in these mice. We also observed some litter/colt variation, which included (a) the rate of penetrance of prostate carcinoma, with some cohorts displaying 100% penetrance whereas others showing less (90%), and (b) the survival rate of mice to 24 weeks (as described in the results), with 22% death in the 12-week-old treatment experiment and 47% in the 18-week-old treatment experiment. Such variations highlight the difficulty in conducting treatment studies with spontaneous tumor models, and in fact, most of the literature using TRAMP mice has focused on prevention as opposed to treatment studies (37).

Viruses were administered systemically in this study for several reasons: (a) Because we had chosen a regimen of four treatments with the viruses based on our prior studies (17), repeated laparatomies for virus delivery into the prostate would have greatly increased the risk of procedure-related toxicity. (b) Systemic administration would be the most effective method to reach various metastatic sites. (c) I.v. administration is much more amenable than a surgical procedure from a translational perspective. In this study, NV1042-infected cells were detected within prostate tumors and viral DNA was detected in the cancerous prostates, periaortic lymph nodes, and lungs, suggesting that systemically administered virus reached and persisted in tumor-bearing organs but not in normal organs. Multiple injections of virus did not seem to be toxic, and tumor progression accounted for observed morbidity.

Both NV1023 and NV1042 treatment of TRAMP mice resulted in a significant reduction of primary prostate tumor weight irrespective of the age (12 or 18 weeks) at which treatment was administered systemically for several reasons: (a) Because we had chosen a regimen of four treatments with the viruses based on our prior studies (17), repeated laparatomies for virus delivery into the prostate would have greatly increased the risk of procedure-related toxicity. (b) Systemic administration would be the most effective method to reach various metastatic sites. (c) I.v. administration is much more amenable than a surgical procedure from a translational perspective. In this study, NV1042-infected cells were detected within prostate tumors and viral DNA was detected in the cancerous prostates, periaortic lymph nodes, and lungs, suggesting that systemically administered virus reached and persisted in tumor-bearing organs but not in normal organs. Multiple injections of virus did not seem to be toxic, and tumor progression accounted for observed morbidity.

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**Figure 4.** Biodistribution of NV1042 virus following i.v. injection of 12-week-old TRAMP mice. A, tissues harvested 1, 3, and 7 d after four viral injections were sectioned and stained with X-gal to detect LacZ expression from the virus. Top, sections from prostate, liver, and lungs obtained from mice sacrificed 1 d after treatments showing areas of staining in prostate glands with low-grade PIN and a few X-gal–positive cells in liver and lung (arrows); middle, tissues from 3 d after virus injections showing X-gal staining in prostate but not in liver; bottom, only prostate tissue stained at 7 d after virus injections. B, prostate tumor sections from a different mouse at 7 d after virus injections stained with X-gal (left) and anti–β-galactosidase antibody (right). Note the overlapping LacZ immunohistochemical and X-gal histochemical staining (open arrowheads) from nearby sections. C, senescence-associated β-galactosidase staining in mock-treated prostate tumors. Frozen prostate sections from a mock-injected TRAMP mouse at 12 wk (as in A) were histochemically stained at the same time for senescence-associated β-galactosidase (SA-β-gal; left) and X-gal (right). Cells staining blue are positive. Senescence-associated β-galactosidase was not detected in high-grade PIN or adenocarcinoma. In one mock-treated mouse, small clusters of positive-staining cells were seen in prostate tumors after X-gal histochemistry, likely due to endogenous activity (48).
initiated; however, only NV1042 was effective in inhibiting the progression from PIN to invasive prostate carcinoma. Both NV1023 and NV1042 also significantly inhibited the frequency of metastasis in periaortic lymph nodes independent of the age (12 or 18 weeks) at which treatment was initiated; however, again, only NV1042 but not NV1023 was significantly effective against metastasis in the lung when treated after the onset of metastasis at 18 weeks of age. Thus, whereas both NV1023 and NV1042 viruses were equally effective against primary tumor growth, only NV1042, and not NV1023, significantly inhibited the progression of primary and metastatic cancer compared with mock. This is an important outcome in determining which virus would be more effective for therapy because mortality in prostate cancer patients is associated with progression to metastatic disease.

We have previously compared the efficacies of NV1042 and NV1023 in s.c. and lung metastatic models using implanted TRAMP-C2 tumor cells and observed varying results, with NV1042 more efficacious in extending survival than NV1023 in the metastatic lung tumor compared with the s.c. model, although the s.c. tumors were directly injected. In the bilateral s.c. tumor model, NV1042 had only a minimal effect on noninoculated tumor growth (17) whereas in the metastatic lung model the enhanced efficacy of NV1042 over NV1023 was abrogated in athymic mice (23). NV1042 has previously been shown to be significantly better than NV1023 at inhibiting tumor growth in squamous cell, hepatic, and colorectal carcinomas (21, 38, 39). Augmented efficacy of IL-12 expression in other oncolytic HSV vectors has also been reported (40, 41).

NV1042 was significantly more effective than mock in the TRAMP mice in almost all of the outcomes measured, whereas NV1023 was only significantly better in less than half. Reasons for the absence of a more significant difference between NV1042 and NV1023 in this study as compared with prior studies (17, 18) include the following: (a) Nature of the tumor type: As compared with TRAMP-C2 tumors, the spontaneous TRAMP tumors are multifocal, independently arising in individual glands, and are heterogeneous masses that are continually progressing to more malignant phenotypes with time, along with associated changes in the microenvironment and immune phenotype. (b) Exposure of the tumor to IL-12: The in situ spontaneous prostate tumors are enclosed within a well-defined capsule, potentially limiting the diffusion of IL-12 expressed from NV1042 virus. (c) Less than optimal dosing of the virus: For this highly aggressive spontaneous model, it is possible that more frequent dosing may have increased the differential response between NV1023 and NV1042. (d) Timing of efficacy measurement: In the implanted tumor models, efficacy measurements were conducted within a period of 3 to 4 weeks after treatment as compared with 6 to 12 weeks after treatment in the spontaneous tumor model. Such variations in response to the same virus depending on the type of tumor model highlight the importance of evaluating oncolytic viruses in more than one model while emphasizing the need to use models such as the TRAMP mice that are most representative of in situ prostate cancer development and progression.

We believe that this is the first report of treating a spontaneous cancer model using systemically administered oncolytic HSVs. We recently reported the intraneoplastic use of oncolytic HSVs in the C3(1)/T-Ag model, which develops mammary tumors spontaneously (42). Although a direct comparison of NV1023 and NV1042 was not conducted, NV1042 significantly delayed mammary tumor progression as compared with mock. Data from both these studies substantiate the utility of NV1042 against spontaneous tumor models, whether administered intraneoplastically or i.v. An IL-12–expressing vector may have some advantages over a noncytokine vector: (a) IL-12 binds to receptors on T cells and natural killer (NK) cells, which enhances their proliferation and cytotoxicity, driving a T helper 1 response. It is a central immunoregulator acting as a cross-bridge between innate and adaptive immunity (43) so that when expressed at the site of tumor antigen production, it can boost both arms of the immune response. (b) An IL-12–expressing oncolytic virus, which can both cause tumor destruction and deliver an immune-enhancing cytokine in the vicinity of tumor destruction, would be highly beneficial as compared with the direct administration of cytokines into the tumor without sufficient tumor antigens or "danger signals." (c) IL-12 also has antiangiogenic properties (44).

Results from multiple studies previously conducted both by us and other investigators to identify the mode of action of NV0142 have also shown consistently without exception that the virus acts through immune and antiangiogenic mechanisms. T cells, specifically CD8+ cells, are essential to the antitumor immune response of the NV0142 virus (17, 23, 38, 45). The role of NK cells seems to vary with the tumor model used; in the metastatic prostate TRAMP-C2 lung tumor model, NK cell activity was observed in mice treated with NV1042 but not NV1023 or virus buffer, whereas in a colorectal micrometastatic model, NK cell depletion did not
interfere with NV1042 efficacy (23, 46). Finally, IL-12 expression by NV1042 virus leads to substantial antiangiogenic activity as shown by the decreased vascularity in prostate and head and neck squamous cell carcinoma models (17, 47).

The current study of systemic HSV treatment in an aggressive spontaneously developing prostate cancer model advances the validity of using oncolytic HSV therapy for prostate cancer patients, especially those with metastatic disease who are severely limited in their treatment options. Importantly, in our study, treatment was initiated not just after primary tumors had developed but also after metastases were apparent, similar to the situation in clinical practice. Given that human cancer cells (including prostate cancer) are more susceptible to HSV oncolysis than mouse cells (ref. 14 versus ref. 17), the TRAMP mouse model serves as a stringent test for efficacy, and it might be expected that the results noted in this animal model could indicate even further efficacy when tested in patients. We have shown that systemically administered oncolytic HSV, in particular the IL-12–expressing NV1042 virus, was effective not only against the primary tumor but also against metastatic tumors independent of their location. These desirable therapeutic features of NV1042 render it a highly valuable agent either as a primary treatment option or as an adjuvant following surgery to eliminate micrometastases.

Acknowledgments

Received 2/19/2007; revised 8/3/2007; accepted 8/14/2007.

Grant support: NIH grants R01CA102139-01A1 (R.L. Martuza) and Department of Defense grants DAMD17-98-1-8490 and W81XWH-04-1-0254 (R.L. Martuza) and W81XWH-05-1-0367 (S. Varghese). The real-time PCR core was supported by NIH grant P30 NS045776. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

We thank Meredith Begin from Dana-Farber/Harvard Cancer Core for assistance with the statistical analyses, and Sara Varghese and Dimitri Varghese for help with the breeding, Dana Xu and Thanh Thao Huynh for aid in histology, and Luba Zagachin for help in real-time PCR. R.L. Martuza and S.D. Rabkin are consultants to MediGene AG, which provided us with the viruses tested in this study.

References

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