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TITLE: Systemic Oncolytic Cytokine HSV Therapy of Prostate Cancer

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Systemic Oncolytic Cytokine HSV Therapy of Prostate Cancer

We have made substantial progress toward the goals outlined in our grant application. In particular, we demonstrate the efficacy of systemically administered oncolytic viruses for the treatment prostate cancer in the transgenic TRAMP mouse model. We show that while intravenous administration of the NV1023 parental virus at 12 weeks of age (presence of prostate adenocarcinoma) resulted in reduced tumor burden, the effects by the IL-12 expressing NV1042 virus were substantially more robust in reducing the frequency of primary tumor growth. Moreover, injection of this cytokine producing virus at 18 weeks of age (presence of metastatic disease) was more effective than NV1023 in reducing the occurrence of lung metastasis. Finally, persistence of tumor infectivity by NV1042 was established by detecting the $\text{LacZ}$-expressing NV1042 virus in the prostate at least 1 week after the final virus infection and also by identifying viral DNA by real-time PCR in both primary and metastatic tumors, but not in liver or blood at 2 weeks after final virus injection.
TABLE OF CONTENTS

Cover..................................................................................................................1

SF 298..............................................................................................................2

Introduction.....................................................................................................4

Body..................................................................................................................4
  Figure 1.........................................................................................................6
  Figure 2.........................................................................................................7
  Figure 3.........................................................................................................9
  Figure 4.......................................................................................................10
  Table 1.......................................................................................................12

Key Research Accomplishments.................................................................11

Reportable Outcomes....................................................................................11

Conclusions....................................................................................................11

References.....................................................................................................11

Appendices.....................................................................................................11
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. Accumulating evidence suggests that oncolytic HSV mutants are potentially useful agents for the treatment of prostate cancer. To-date, however, all efficacy studies with HSV vectors for prostate cancer has utilized implanted tumor models, which are artificial systems with respect to their milieu and lympho-vascular supply. While 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. The genetically engineered TRAMP mouse model develops prostate cancer spontaneously is currently the most representative model to conduct efficacy studies. We hypothesized that the systemic efficacy of NV1023, NV1042 and NV1034 viruses can be more appropriately assessed in TRAMP and that cytokines expressed by these viruses will lead to significant improvement in their effectiveness for prostate cancer. The following report summarizes our most recent work on the efficacy of HSV administration on transgenic TRAMP mice.

BODY

AIM 1. Determine the maximum tolerated dose and toxicology of NV1023, NV1042 and NV1034 in TRAMP transgenic mice.

Recently, we reported that the NV1042-expressing IL-12 virus was superior to NV1034-GM-CSF in killing TRAMP-C2 and Pr14-2 tumors in syngenic mouse models of prostate cancer (1). Thus, in the following report the NV1042 virus and its non-cytokine parent virus NV1023 were evaluated in the transgenic TRAMP prostate cancer model. It should be noted that studies are planned to address whether the combination NV1042 and NV1034 are a more efficacious form viro-therapy than NV1042 alone (Aim 2). Preliminary results using TRAMP-C2 subcutaneous tumors suggest that this could be the case (unpublished results; S.Varghese).

Since TRAMP mice were bred in-house, it was essential to first determine the time-line of prostate cancer and metastasis development prior to using them in viral therapy studies (Figure
1). TRAMP mice on the FVB/N background display low grade PIN by 8 weeks of age (Fig 1B), which progressed to high grade PIN (adenoma) by 10 weeks (Fig 1C), prostate adenocarcinoma by 12 weeks (Fig 1D), and metastasis in the periaortiс lymph nodes (PA-LN) and lung by 18 weeks of age (Fig 1E,F). For comparison, histology of normal prostate from a non-transgenic littermate is shown in Figure 1A. Based on the above data, systemic treatment was initiated at 12 weeks (primary prostate carcinoma) or 18 weeks (metastasis) and mice were subsequently sacrificed at 24 weeks of age.

Figure 1. Time-line of prostate cancer and metastasis in TRAMP mice.

Both NV1023 and NV1042 harbor the *E.coli* Lac Z gene, which acts as a reporter to track the bio-distribution these viruses following systemic administration. TRAMP mice treated at 12 weeks with four doses of $2 \times 10^7$ pfu (see Aim 2 for the rationale of the injection schedule) of intravenous NV1042 were sacrificed 1, 3, and 7 days after the last treatment, and various tissues (prostate, lung, liver, brain) removed for X-gal staining. Results as illustrated in Figure 2 show that one day following the final virus injection significant amounts of staining was observed
in the prostate as compared to a few isolated cells in the liver and lung and none in brain (brain data not shown). By day 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 post-treatment in the cancerous prostates but not in the normal organs.

Figure 2. Virus bio-distribution in NV1042 treated mice.

Furthermore, the bio-distribution 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 (Table 1). DNA was isolated from organs (prostate/seminal vesicles, peri-aortic lymph nodes, lung, liver, brain, and blood) harvested on days 11, 13, and 24. Results shown in Table 1 illustrate that viral DNA was detected until day 24 in those organs that harbor primary and metastatic cancers (prostate, peri-aortic lymph nodes, and lungs). In contrast, while many viral copies were detected in the liver and blood on day 1, the level decreased to non-detectable (negative) by
day 24, suggesting clearance of virally infected cells or degradation of viral DNA. No viral DNA was detected in the brains of any animal on any day tested.

**AIM 2. Assess the efficacy of intravenously delivered NV1023, NV1042 and NV1034 in inhibiting the progression of in situ prostate cancer in TRAMP mice.**

We will also publish this year data demonstrating that systemically administered NV1042 is effective against pre-established metastatic TRAMP-C2 lung tumors (2). Based on the success of this injection regimen in established TRAMP-C2 lung tumors, we have chosen to implement a similar treatment strategy for our transgenic TRAMP mice studies. Starting at 12 weeks of age, intravenously delivered NV1023 and NV1042 was administered at $2 \times 10^7$ PFU every third day for a total four injections. These mice were carefully monitored for mortality and morbidity after each injection and thereafter twice weekly until they reach 24 weeks of age and subsequently did not yield significant changes in body weight (data not shown). This treatment regimen proved to extremely efficacious in controlling both prostate cancer progression (Figure 3) and metastasis (Aim 3; Figure 4) in our transgenic TRAMP mouse model.

Distribution of prostate/seminal vesicle tumor weights (Fig. 3b) illustrates that Mock-treated mice harbored tumors with a mean weight of 10.17g, NV1023 with 3.98g ($P=0.026$ vs. Mock; Mann-Whitney test) and NV1042 with 2.79g ($P=0.003$ vs. Mock; Mann-Whitney test). In this experiment, 2 of 9 mice from both the Mock and NV1023 treated groups died within 2 days of the 24 week sacrifice and 1 mouse from the NV1042 group died one week after treatment (at ~14 weeks of age). Histological 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 confirmed the presence of poorly differentiated carcinoma in 8 out of 9 (89%) Mock mice as compared to 6 out of 9 (67%) in NV1023 and 2 out of 8 (25%) in NV1042 treated mice ($P=0.015$ vs. Mock, Fisher’s Exact test). Representative H&E stained prostates, based on the most frequently observed histological stage, from various treatment groups are illustrated in Fig 3c-e. The largest tumors were highly necrotic (seen as pink areas in Fig 3c) with islands of tumor cells closely apposed to blood vessels within the necrotic areas.
Figure 3. Efficacy of systemic oncolytic HSV therapy on primary prostate cancer.  
(A) Photograph of representative prostate and seminal vesicles excised en bloc from various treatment groups illustrating prostate tumors.  
(B) Distribution of weights of prostate/seminal vesicle from Mock (n=7), NV1023 (n=7), and NV1042 (n=8). Mean is denoted by the line and mean weight ± SEM for each group are as follows: Mock = 10.17± 1.68g; NV1023 = 3.98± 1.39g (P = 0.026 vs. Mock; Mann-Whitney test); NV1042 = 2.79± 0.91g (P = 0.0037 vs. Mock; Mann-Whitney test). Mice dying prior to week 24 were not included in the analysis.  
(C-E) H&E staining of prostates from Mock (C) showing larger areas of necrosis, NV1023 (D), and NV1042 (E) treated mice.

**Aim 3.** Evaluate the efficacy of NV1023, NV1042 and NV1034 for the systemic treatment of metastatic prostate cancer in TRAMP mice.
In order 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. A significant reduction in frequency of metastasis to the PA-LN was observed with NV1023 ($P=0.04$ vs. Mock, Fisher’s Exact test) and NV1042 ($P=0.013$ vs. 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 1 out of 14 (7%) mice exhibiting carcinoma ($P=0.014$ vs. Mock, Fisher’s Exact test) as compared to 5 out of 9 (56%) Mock and 5 out of 14 (36%) NV1023 treated mice.

Figure 4. Efficacy of systemic oncolytic HSV therapy on metastasis.
(A) Photograph of representative prostate and seminal vesicles excised en bloc from various treatment groups illustrating prostate tumors. (B) Distribution of weights of prostate/seminal vesicle from each treatment group. Mean is denoted by the line in each group; mean weight ± SEM for each group are as follows: Mock = 12.25± 2.3g; NV1023 = 6.54± 1.20g ($P = 0.04$ vs. Mock; Mann-Whitney test); NV1023 = 3.66± 0.72g ($P = 0.002$ vs. Mock; Mann-Whitney test).
KEY RESEARCH ACCOMPLISHMENTS

- Demonstration that an oncolytic HSV expressing the IL-12 cytokine is effective after intravenous administration on both primary prostate cancer and peri-aortic lymph node metastasis in a transgenic mouse model of prostate cancer.

REPORTABLE OUTCOMES

none

CONCLUSIONS

We have demonstrated that systemically administered oncolytic HSVs, in particular the IL-12 expressing NV1042 virus, was not only effective against the primary prostate tumors but also 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.

REFERENCES


APPENDICES

None
Table 1: Real time PCR for HSV gB sequences in various tissues from TRAMP mice treated at 18 weeks with oncolytic HSVs. 2x10^7 pfu of NV1042 was administered systemically on days 0, 3, 7, and 10 in TRAMP mice at 18 weeks of age and various tissues were harvested on day 11, 13, and 24 for absolute quantification of gB viral sequences using Taqman real time PCR. M denotes mice; numbers depict mean viral copies ± S.D. for the entire organ, NQ is not quantifiable (1-15 copies), ND is No DNA obtained including GAPDH, Neg. is Negative.

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