Characterization and evaluation of a new oncolytic Vaccinia Virus strain LIVP6.1.1 for canine cancer therapy

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Virotherapy on the basis of oncolytic vaccinia virus (VACV) strains is one novel approach for canine cancer therapy. In this study we described for the first time the characterization and the use of new VACV strain LIVP6.1.1 as an oncolytic agent against canine cancer in a panel of four canine cancer cell lines including: soft tissue sarcoma (STS-1A), melanoma (CHAS), osteosarcoma (D-17) and prostate carcinoma (DT08/40). Cell culture data demonstrated that LIVP6.1.1 efficiently infected and destroyed all four tested canine cancer cell lines. In two different xenograft models on the basis of the canine soft tissue sarcoma STS-1A and the prostate carcinoma DT08/40 cell lines, a systemic administration of the LIVP6.1.1 virus was found to be safe and led to anti-tumor and immunological effects resulting in the significant reduction of tumor growth in comparison to untreated control mice.

In summary, the pre-clinical evaluation has demonstrated the efficacy of LIVP6.1.1 for canine cancer therapy. Furthermore, a clinical trial with canine cancer patients has already been started.

Keywords: cancer, canine cancer therapy, oncolytic virus, oncolysis, canine xenografts

Abbreviations: ATCC, American Type Culture Collection; DMEM, Dulbecco’s modified Eagle’s medium; FBS, fetal bovine serum; hpvi, hours post-virus-infection; IACUC, Institutional Animal Care and Use Committee; LIVP, a Lister strain of vaccinia virus; MDSCs, myeloid-derived suppressor cells; MOI, multiplicity of infection; ORF, open reading frame; OV, oncolytic virus; pfu, plaque-forming unit; tk, thymidine kinase; VACV, vaccinia virus; XTT, 2,3-bis[2-methoxy-4-nitro-5-sulfophenyl]-2H-tetrazolium-5-carboxanilide inner salt

Introduction

Cancer is the most common cause of natural death in dogs and endemic in both developed and developing countries (http://www.wfaertheuere.org/). Incidence of cancer ranges from 1 to 2% in the canine population and currently accounts for about half of the deaths in dogs older than 10 years.1-2 The major treatment options for cancer in dogs include surgery, radiation therapy, chemotherapy, hyperthermia and photodynamic therapy. Despite progress in the diagnosis and treatment of advanced canine cancer, overall treatment outcome has not substantially improved in the past. Therefore, the development of new therapies for advanced canine cancer is a high priority. One of the most promising novel cancer therapies is oncolytic virotherapy. This method is based on the capacity of oncolytic viruses (OVs) to preferentially infect and lyse cancer cells without causing excessive damage to surrounding normal tissue. Several oncolytic viruses including various human and canine adenoviruses,3-5 canine distemper virus4 and vaccinia virus strains, namely GLV-1h68, LIVP1.1.17-9 and GLV-1h10910 have been successfully tested for canine cancer therapy: in preclinical settings (for review, see refs. 11 and 12). However, in contrast to human studies, the clinical trials with oncolytic viruses for canine cancer patients are just at the beginning.

In this study, we have analyzed the oncolytic potential of a new vaccinia virus strain LIVP6.1.1 against different canine cancer cells in cell culture and analyzed the therapeutic effect against canine soft tissue sarcoma and prostate carcinoma in xenograft models. LIVP6.1.1 was isolated from a wild type stock of Lister strain of vaccinia virus (Lister strain, Institute of Viral Preparations) and represents a “native” virus (no genetic manipulations were conducted). The sequence analysis revealed
These results indicated that the LIVP6.1.1 virus infection led to efficient killing of all four canine cancer cell lines in these experimental settings.

Efficiency of LIVP6.1.1-replication in STSA-1 and DT08/40 canine cancer cells. The oncolytic potential of OVs is dependent on their ability to efficiently infect and replicate in cancer cells. In order to test the efficiency of virus replication, STSA-1 and DT08/40 cells were infected with either LIVP6.1.1 or GLV-1h68 at an MOI of 0.1. In these experimental setting GLV-1h68 was used as a control. Standard plaque assays were performed for all samples to determine the viral titers at different time points during the course of infection (Fig. 2A and B). Efficient LIVP6.1.1 viral replication (> 100-fold titer increase at 48 or 96 hpi) was observed in both cell lines. The maximum viral titers were determined for LIVP6.1.1 (5.34 × 10^6 pfu/well) and for GLV-1h68 (2.98 × 10^6 pfu/well) in STSA-1 at 48 hpi (Fig. 2A). Interestingly, the highest virus titers in virus-infected DT08/40 cells were identified for LIVP6.1.1 (8.24 × 10^6 pfu/well) and for GLV-1h68 (1.53 × 10^6 pfu/well) at 96 hpi (Fig. 2B). These data demonstrated that the virus replication efficiency is dependent on the infection time point and tumor types. Under these experimental conditions, LIVP6.1.1 can replicate more efficiently than GLV-1h68 in both cancer cell lines.

Oncolytic effect of a single systemic application of LIVP6.1.1 on STSA-1 and DT08/40 canine cancer cells. The oncolytic potential of OVs is dependent on their ability to efficiently infect and replicate in cancer cells. In order to test the efficiency of virus replication, STSA-1 and DT08/40 cells were infected with either LIVP6.1.1 or GLV-1h68 at an MOI of 0.1. In these experimental setting GLV-1h68 was used as a control. Standard plaque assays were performed for all samples to determine the viral titers at different time points during the course of infection (Fig. 2A and B). Efficient LIVP6.1.1 viral replication (> 100-fold titer increase at 48 or 96 hpi) was observed in both cell lines. The maximum viral titers were determined for LIVP6.1.1 (5.34 × 10^6 pfu/well) and for GLV-1h68 (2.98 × 10^6 pfu/well) in STSA-1 at 48 hpi (Fig. 2A). Interestingly, the highest virus titers in virus-infected DT08/40 cells were identified for LIVP6.1.1 (8.24 × 10^6 pfu/well) and for GLV-1h68 (1.53 × 10^6 pfu/well) at 96 hpi (Fig. 2B). These data demonstrated that the virus replication efficiency is dependent on the infection time point and tumor types. Under these experimental conditions, LIVP6.1.1 can replicate more efficiently than GLV-1h68 in both cancer cell lines.

**Results and Discussion**

Analysis of the oncolytic potential of LIVP6.1.1 virus against different canine cancer cell lines. The oncolytic effect of LIVP6.1.1 against a panel of four different canine cancer cell lines including soft tissue sarcoma STSA-1, melanoma CHAS, osteosarcoma D17 and prostate carcinoma DT08/40 cells, was examined. For this purpose, the cells were seeded three days prior to infection in 24-well plates and then were infected with LIVP6.1.1 at multiplicities of infection (MOIs) of 1.0 and 0.1. The cell viability was analyzed at 24, 48 and 72 h post-virus-infection (hpvi) by XTT-assays (Fig. 1). At MOI of 1.0, the LIVP6.1.1 virus was highly cytotoxic to three cell lines (STSA-1, D17 and CHAS), resulting in at last 83% cytotoxicity over 3 d. One day later similar cytotoxicity was also observed for LIVP6.1.1-infected DT08/40 cells.

These results indicated that the LIVP6.1.1 virus infection led to efficient killing of all four canine cancer cell lines in these experimental settings.

Efficiency of LIVP6.1.1-replication in STSA-1 and DT08/40 canine cancer cells. The oncolytic potential of OVs is dependent on their ability to efficiently infect and replicate in cancer cells. In order to test the efficiency of virus replication, STSA-1 and DT08/40 cells were infected with either LIVP6.1.1 or GLV-1h68 at an MOI of 0.1. In these experimental setting GLV-1h68 was used as a control. Standard plaque assays were performed for all samples to determine the viral titers at different time points during the course of infection (Fig. 2A and B). Efficient LIVP6.1.1 viral replication (> 100-fold titer increase at 48 or 96 hpi) was observed in both cell lines. The maximum viral titers were determined for LIVP6.1.1 (5.34 × 10^6 pfu/well) and for GLV-1h68 (2.98 × 10^6 pfu/well) in STSA-1 at 48 hpi (Fig. 2A). Interestingly, the highest virus titers in virus-infected DT08/40 cells were identified for LIVP6.1.1 (8.24 × 10^6 pfu/well) and for GLV-1h68 (1.53 × 10^6 pfu/well) at 96 hpi (Fig. 2B). These data demonstrated that the virus replication efficiency is dependent on the infection time point and tumor types. Under these experimental conditions, LIVP6.1.1 can replicate more efficiently than GLV-1h68 in both cancer cell lines.

Oncolytic effect of a single systemic application of LIVP6.1.1 on STSA-1 and DT08/40 cell xenografts. In this...
study the therapeutic effect of LIVP6.1.1 on the progression of soft tissue sarcoma STSA-1 subcutaneous xenografts was evaluated in vivo by measuring tumor volumes at various time points. Tumors were generated by implanting 1 × 10^6 STSA-1 canine soft tissue sarcoma cells subcutaneously into the right hind leg of 6- to 8-week-old female nude mice (NCI/Hsd/Athymic Nude-Foxn1nu). Five weeks post implantation, all mice developed tumors with volumes of 600 to 1000 mm^3. We have chosen mice with larger tumors for virus injection since this late stage of the tumor development is more representative and interesting for the clinical praxis. Animals were separated into two groups (n = 6 per group) and were injected either with a single dose of LIVP6.1.1 (5 × 10^6 pfu) or PBS intravenously into the lateral tail vein. As shown in Figure 3A, the virus treatment led to a significant decrease in STSA-1 tumor growth in all virus-treated mice compared with PBS control mice. Due to excessive tumor burden (> 3000 mm^3), all animals in the control PBS group were euthanized after 14 dpi.

The therapeutic effect of LIVP6.1.1 was also evaluated on the progression of the slow growing canine prostate carcinoma DT08/40 tumors in nude mice by measuring the tumor volume at various time points. Data demonstrated again that a single injection with LIVP6.1.1 vaccinia virus led to significant inhibition of the tumor growth (p < 0.05) of all virus-treated mice compared with the control PBS animals on 35, 42 and 49 dpi (Fig. 3B).

Finally, the toxicity of the LIVP6.1.1 virus was determined by monitoring the relative weight change of mice over time (Fig. 3C and D). All LIVP6.1.1 treated mice showed relatively stable mean weight over the course of studies. There were no signs of virus-mediated toxicity.

In summary, therapy with the vaccinia strain LIVP6.1.1 demonstrated anti-tumor activity in canine soft tissue sarcoma and canine prostate xenograft models. Therefore we propose that vaccinia virus strain LIVP6.1.1 may be useful for the treatment of these cancer types in dogs.

Biodistribution and persistence of LIVP6.1.1 in STSA-1 tumor-bearing nude mice. At late time points after virus treatment, we analyzed the virus distribution and persistence in LIVP6.1.1-treated STSA-1 xenografted mice. Table 1 summarizes the virus distribution data at 35 dpi. In all virus-treated mice the highest viral titers were identified in primary tumors. In addition, low copies of LIVP6.1.1 virus particles were also detected in liver, lung, spleen and kidney of the treated animals (Table 1). The later presence of LIVP6.1.1 in these organs could be due to leakiness of blood vessels in solid tumors, circulating virus-infected tumor cells or cell particles may end up in healthy tissues such as the lung, liver, spleen and kidney. However, LIVP6.1.1 seems to be tumor-selective, as we found about 10^9 to 10^10-fold more virus particles in solid tumors compared with healthy tissues of the treated animals.

The virus biodistribution in the primary STSA-1 tumors was also analyzed by immunohistochemical staining at 55 dpi (Fig. 4). The data revealed that the tumors in all treated mice were completely infected with vaccinia virus, which led to oncolyis and destruction of tumor tissues.

Increased presence of immune cells in tumors of STSA-1 xenografted mice after LIVP6.1.1 treatment. To investigate the immunological aspect of oncolytic therapy, we have analyzed the effect of virus infection on host immune cells in tumors of STSA-1-tumor-bearing mice. Our flow cytometry data demonstrated a significant increase of Gr-1^highCD11b^+ (MDSCs, monocytes), F4/80^+CD45^+ (macrophages) and HMCII^+CD45^+ cells in the LIVP6.1.1 infected tumors compared with PBS-treated tumors (Table 2). In addition, we used the Gr-1^highCD11b^+ cells as markers for monitoring viral infection on a systemic level. For this purpose, a parallel flow cytometric analysis of Gr-1^highCD11b^+ cells in the peripheral blood was performed. There was no significant difference in the number of Gr-1^highCD11b^+ cells between virus and PBS-treated STSA-1 xenografted mice (Table 2). These data suggest that the changes in granulocytic MDSCs were not systemic, but rather due to a change in recruitment and/or persistence within virus treated tumors.

On the basis of these data, we assume that the strong anti-tumor effect of LIVP6.1.1 in STSA-1 tumor bearing mice could be at least in part dependent on the increased number of these host immune cells in the tumor bed. Moreover, we and others have reported that virotherapy induces massive tumoral infiltration of...
In conclusion, we propose that the LIVP6.1.1 vaccinia virus strain may be useful for the treatment of canine cancer patients. First clinical trial with this virus has already started.

Materials and Methods

Ethics statement. All animal experiments were performed in accordance with protocols approved by the Institutional Animal Care and Use Committee (IACUC) of Explora Biolabs (protocol number: EB11-025) and/or the government of Unterfranken, Germany (permit number: 55.2-2531.01-17/08 and 55.2-2531.01-24/12).

Cell culture. African green monkey kidney fibroblasts (CV-1) were obtained from the American Type Culture Collection (ATCC). STSA-1 cells were derived from a canine patient with a low grade II soft tissue sarcoma.15 DT08/40 was diagnosed as canine prostate carcinoma. 16 The canine melanoma CHAS cell line was provided by Dr Ogilvie (Angel Care Cancer Center, Carlsbad, USA), and canine osteosarcoma D17 was obtained from ATCC. Cells were cultured in DMEM supplemented with antibiotic-solution (100 U/ml penicillin G, 100 units/ml streptomycin) and 10% fetal bovine serum (FBS; Invitrogen GmbH) for CV-1.

The virus titers were determined by standard plaque assays on CV-1 cells using aliquots of the homogenized organs and were displayed as mean pfu/gram of organ or tissue. For each organ, two aliquots of 0.1 ml were measured in triplicates. nd, not detected (detection LIMIT > 10 pfu/organ).

Table 1. Biodistribution of LIVP6.1.1 in virus-treated STSA-1 xenografted mice at 35 d post virus injection (d.p.i.)

| PFU per gram (g) | STSA-1 xenografted mice treated with 5 × 10^6 pfu LIVP6.1.1 |
|----------------|---------------------------------------------------------|
| Mouse No |
| 301 | 302 | 304 |
| Tumor | 2.75 × 10^7 | 3.58 × 10^7 | 3.76 × 10^7 |
| Lung | 1.16 × 10^2 | 4.40 × 10^2 | 1.42 × 10^2 |
| Liver | 4.00 × 10^1 | 5.33 × 10^1 | 1.06 × 10^2 |
| Spleen | 7.00 × 10^1 | 3.33 × 10^1 | 1.25 × 10^2 |
| Kidney | 2.50 × 10^2 | 1.46 × 10^2 | 3.06 × 10^2 |

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Table 1. Biodistribution of LIVP6.1.1 in virus-treated STSA-1 xenografted mice at 35 d post virus injection (d.p.i.)

The virus titers were determined by standard plaque assays on CV-1 cells using aliquots of the homogenized organs and were displayed as mean pfu/gram of organ or tissue. For each organ, two aliquots of 0.1 ml were measured in triplicates. nd, not detected (detection LIMIT > 10 pfu/organ).
Vaccinia virus strain LIVP6.1.1 was derived from LIVP (Lister strain, Institute of Viral Preparations, Moscow, Russia). The sequence analysis of LIVP6.1.1 revealed the presence of different mutations in several genes (Chen et al., manuscript in preparation). In addition, LIVP6.1.1 demonstrated different plaque morphology in comparison to GLV-1h68 in CV-1 cells. GLV-1h68 is an oncolytic virus strain designed to colonize and destroy cancer cells without harming healthy tissues or organs.17

Cell viability assay. 1 × 10^5 cells per well were seeded in 96-well plates (Nunc). After 24 h in culture, cells were infected with vaccinia virus strains using multiplicities of infection (MOI) of 0.1 and 1.0. The cells were incubated at 37°C for 1 h, then the infection medium was removed and subsequently the cells were incubated in fresh growth medium.

The amount of viable cells after infection was measured using two 3-h-[3H]-thymidine incorporation assay (Cell Proliferation Kit II, Roche Diagnostics), according to the manufacturer’s protocol, at 24, 48, or 72 h after virus infection. Quantification of cell viability was performed in an ELISA plate reader (Tecan Sunrise, Tecan Trading AG) at 490 nm with a reference wavelength of 690 nm. The relative number of viable cells was expressed as percent cell viability. Uninfected cells were used as a reference and were considered 100% viable.

Viral replication. For the viral replication assay, different canine cancer cells were infected with LIVP6.1.1 or GLV-1h68 at an MOI of 0.1. After one hour of incubation at 37°C with gentle agitation every 20 min, the infection medium was removed and replaced by a fresh growth medium. After 1, 12, 24, 48, 72 h, the cells and supernatants were harvested. Following three freeze-thaw cycles, serial dilutions of the supernatants and lysates were titrated by standard plaque assays on CV-1 cells. All samples were measured in triplicate.

Vaccinia virus-mediated therapy of STSA-1 and DT08/40 xenografts. Tumors were generated by implanting either 1 × 10^6 canine tissue sarcoma STSA-1 cells or 5 × 10^6 canine prostate DT08/40 cells subcutaneously into the right hind leg of 6– to 8-week-old female nude mice [Har-Athymic Nude-Foxn1^nu; Harlan]. Tumor growth was monitored weekly in two dimensions using a digital caliper. Tumor volume was calculated as [(length × width^2)/2]. When tumor volume reached approximately 600–1000 mm^3 (STSA-1) and 200–400 mm^3 (DT08/40), groups of mice (n = 6) were infected either with 5 × 10^5 pfu of LIVP6.1.1 virus or PBS (control) into the tail vein intravenously (i.v.). The significance of the results was calculated by Student’s t-test. Results are displayed as means ± standard deviation (SD). P values of < 0.05 were considered significant.

Figure 4. Immunohistochemical staining of infected and uninfected STSA-1 xenograft tumors at 35 dpo. Tumor-bearing mice were either mock treated (PBS) or infected with LIVP6.1.1. Tumor sections were labeled with anti-vaccinia virus antibodies (red) and nuclei were stained with Hoechst (blue). Scale bars, 2.5 μm.
Table 2. Presence of immune cells in tumors and peripheral blood of STS-A xenografted mice 7 days after LIVP6.1.1 or PBS treatments

| Immune Cells in (%) | PBS 6 days | P-value in comparison | LIVP6.1.1 6 days | LIVP6.1.1 6 days vs PBS 6 days |
|---------------------|------------|-----------------------|----------------|-----------------------------|
| MHCII+CD45+         | 0.66% ± 0.23% | 2.67% ± 0.35% | ** (p = 0.002) | nt | nt |
| F4/80+CD45+         | 0.77% ± 0.40% | 4.61% ± 0.25% | ** (p = 0.001) | nt | nt |
| CD11b+Gr-1+         | 0.2% ± 0.08% | 3% ± 0.69% | * (p = 0.018) | nt | nt |
| CD11b+Gr-1mhi        | 0.27% ± 0.08% | 2.49% ± 0.70% | * (p = 0.00) | 27.39% ± 7.67% | 25.49% ± 3.96% |

$mAb$ (RB6–8C5) has long been used to stain MDSCs and allows the distinction of at least two subtypes of granulocytic (Gr-1+CD11b+) and monocytic cells (Gr-1+CD11bH) [18]. Stained cells were subsequently analyzed, using Accuri C6 Cytometer and FACS analysis software CFlow Version 1.0.227.4 (Accuri Cytometers, Inc.).

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References

1. Mello DF, Rouli TM, Malegno C, Cappi M, Cofildi U, Capote C, et al. Cancer incidence in pet dogs from the Tumor Registry of the University of Veterinary Medicine, J Vet Intern Med 2006; 20:754-60; PMID:16965425; http://dx.doi.org/10.1111/j.1939-1676.2006.00334.x.

2. Kalten BJ, Moreau AL, Glucksman ET. Epidemiologic studies of tick-borne diseases in pets. Epidemiol Rev 1988; 20:26-37; PMID:3519189; http://dx.doi.org/10.1017/S0377891000060723.

3. Tsimmou VS, Lou IR, Bokouma N, Smith RE, Segal CE, Carrat D. Positive replication of human adenovirus type 5 in dogs. J Virol 2006; 81:1308-11; PMID:16633570; http://dx.doi.org/10.1128/JVI.81.3.1308-11.2006.

4. Smith RE, Carrat D, Tsimmou VS, Karageorgos AC, Baker HS, Cox N, et al. Administration of a conditionally replicating oncolytic canine adenovirus in normal dogs. Cancer Biol Ther 2006; 6:389-99; PMID:16435350; http://dx.doi.org/10.4161/cbt.6.3.10441.

5. Le DB, Lin AR, Agrafe JM, Tsimmou VS, Wu R, Wang M, et al. Inducible enhancement for additional transient expression of canine oestrogen receptors. Gene Ther 2006; 13:389-95; PMID:16872351; http://dx.doi.org/10.1038/sj.gt.3302874.

6. Swayne ML, Cher MS, Boat ML, Yang P, Caratone R, Veres W, et al. In vitro canine distemper virus infection of canine lymphoid cells: a potential in oncologic therapy for lymphoma. J Canine Res 2005; 5:177-82; PMID:16713068; http://dx.doi.org/10.1177/1532050X05024-3994.

7. Gansch Herbst M, Bratke K, Donat U, Hess M, et al. Regression of canine transmissible venereal tumor with oncolytic vaccinia virus GLV-1h68. J Biomed Biotechnol 2010; 2010:489759; PMID:20379368; http://dx.doi.org/10.1155/2010/489759.

8. Gansch Herbst M, Bratke K, Donat U, Hess M, et al. Regression of canine transmissible venereal tumor with oncolytic vaccinia virus GLV-1h68 showing on ant- v Ziegler (Chloramphenicol Pseudomonas aeruginosa) end cell microenvironment. J Infect Dis 2012; 205:1235-48; PMID:22794577; http://dx.doi.org/10.1093/infdis/jir446.

9. Arrabi M, Nair M, Morgan DM. Oncolytic gene therapy for canine cancers: current status and future prospects for canine patients. J Transl Med 2010; 10:123; http://dx.doi.org/10.1186/1479-5876-10-123.

10. Gilbert M, Berman M, Sobolev M, Rubin D, Cinnabar MJ, Chen Y, et al. Phenytoin deficiency augments the efficacy of oncolytic vaccinia virus against primary and metastatic tumours in mice. Br J Cancer 2011; 105:1512-21; PMID:21989183; http://dx.doi.org/10.1038/bjc.2011.429.

11. Klein RE, McFadden KD, Jones K, Czop JA, Ali SM, et al. Targeted immunotherapy during oncolytic virus therapy can improve survival in a mouse model. J Cancer Res 2007;15:1060-9; PMID:17759951; http://dx.doi.org/10.3922/jcompare.2007.0360225.

12. Gansch Herbst M, Basile M, Basile E, et al. Regression of canine transmissible venereal tumor with oncolytic vaccinia virus GLV-1h68. J Biomed Biotechnol 2010; 2010:489759; PMID:20379368; http://dx.doi.org/10.1155/2010/489759.

13. Beloja S, Basile M, Basile E, et al. Regression of canine transmissible venereal tumor with oncolytic vaccinia virus GLV-1h68. J Biomed Biotechnol 2010; 2010:489759; PMID:20379368; http://dx.doi.org/10.1155/2010/489759.

14. Basile S, Basile M, Basile E, et al. Regression of canine transmissible venereal tumor with oncolytic vaccinia virus GLV-1h68. J Biomed Biotechnol 2010; 2010:489759; PMID:20379368; http://dx.doi.org/10.1155/2010/489759.

15. Gansch Herbst M, Basile M, Basiel E, et al. Regression of canine transmissible venereal tumor with oncolytic vaccinia virus GLV-1h68. J Biomed Biotechnol 2010; 2010:489759; PMID:20379368; http://dx.doi.org/10.1155/2010/489759.

16. Reimann-Berg N, Wirthwein A, Memas Ebciul, Elbes, N, Gansch Herbst M, Basile M, et al. Regression of canine transmissible venereal tumor with oncolytic vaccinia virus GLV-1h68. J Biomed Biotechnol 2010; 2010:489759; PMID:20379368; http://dx.doi.org/10.1155/2010/489759.

17. Zhang Q, Yu WA, Yang E, Chen N, Danner RL, Mannix PS, et al. Regression of solid human breast tumor in nude mice with an intravenously injected herpesvirus oncolytic vaccinia virus. Cancer Res 2007; 67:4038-46; PMID:17942938; http://dx.doi.org/10.1158/0008-5472.CAN-06-4609.

18. Gansch Herbst M, Basile M, Basile E, et al. Regression of canine transmissible venereal tumor with oncolytic vaccinia virus GLV-1h68. J Biomed Biotechnol 2010; 2010:489759; PMID:20379368; http://dx.doi.org/10.1155/2010/489759.

19. Gansch Herbst M, Basile M, Basile E, et al. Regression of canine transmissible venereal tumor with oncolytic vaccinia virus GLV-1h68. J Biomed Biotechnol 2010; 2010:489759; PMID:20379368; http://dx.doi.org/10.1155/2010/489759.