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Oncolytic Vaccinia Virotherapy for Endometrial Cancer

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Abstract

Objective—Oncolytic virotherapy is a promising modality in endometrial cancer (EC) therapy. In this study, we compared the efficacy of the Copenhagen and Wyeth strains of oncolytic vaccinia virus (VV) incorporating the human thyroidal sodium iodide symporter (hNIS) as a reporter gene (VVNIS-C and VVNIS-W) in EC.

Methods—Infectivity of VVNIS-C and VVNIS-W in type I (HEC1A, Ishikawa, KLE, RL95-2, and AN3 CA) and type II (ARK-1, ARK-2, and SPEC-2) human EC cell lines was evaluated. Athymic mice with ARK-2 or AN3 CA xenografts were treated with one intravenous dose of VVNIS-C or VVNIS-W. Tumor regression and *in vivo* infectivity were monitored via NIS expression using SPECT-CT imaging.

Results—All EC cell lines except KLE were susceptible to infection and killing by VVNIS-C and VVNIS-W *in vitro*. VVNIS-C had higher infectivity and oncolytic activity than VVNIS-W in all cell lines, most notably in AN3 CA. Intravenous VVNIS-C was more effective at controlling AN3 CA xenograft growth than VVNIS-W, while both VVNIS-C and VVNIS-W ceased tumor growth and induced tumor regression in 100% of mice bearing ARK-2 xenografts.

Conclusion—Overall, VVNIS-C has more potent oncolytic viral activity than VVSIN-W in EC. VV appears to be most active in type II EC. Novel therapies are needed for the highly lethal type II EC histologies and further development of a VV clinical trial in type II EC is warranted.

Introduction

Oncolytic virotherapy is an emerging treatment modality that uses replication-competent viruses to specifically destroy cancers. The potential of oncolytic virotherapy using herpes

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Conflict of Interest

Drs Russell, Wang, Bell and Peng, and Mayo Clinic have a financial interest in this research.

simplex virus, measles virus, vesicular stomatitis virus, vaccinia virus, adenovirus, retrovirus, poliovirus has been demonstrated by numerous preclinical and clinical studies [1]. Oncolytic viruses are genetically engineered so that they are attenuated, armed and/or retargeted [1]. Poxvirus, particularly vaccinia virus (VV), is a promising oncolytic virus due to its well-characterized safety profile with its extensive use in the World Health Organization's smallpox eradication campaign during the 1960s [2]. Vaccinia virus has a large genome which can be easily genetically modified and can accommodate inserts exceeding 25 kb using homologous recombination [3]. Also, even though VV is known to infect a wide range of cells, it replicates and propagates productively only in cancer cells [4]. Therefore, placing a reporter or therapeutic gene under a late promoter has the advantage of ensuring high levels of transgene predominantly in the cancer cells.

There are a number of preclinical and clinical studies using the Western Reserve, Lister and Wyeth strains of VV in cancer [1, 5, 6]. A number of VV strains differing in pathogenicity and host range exist mainly due to the evolution of the virus in different parts of the world during smallpox vaccination history [7]. The New York City Board of Health (NYCBH) strain was originally used for smallpox vaccination in the United States. The Wyeth strain has been used extensively as vaccines in clinical trials. The Western Reserve (WR) is a particularly virulent strain derived from Wyeth after passage in laboratory mice. Other strains, such as Copenhagen, Lister, IHD-W, and IHD-J, and several attenuated strains, such as the modified Vaccinia Ankara, are also frequently used for various applications [7]. Several different full length VV genomes have been used for rescue of oncolytic viruses including WR [8], Wyeth[9], Copenhagen[10], and Lister [11].

The human sodium iodide symporter (hNIS) is a membrane-bound glycoprotein present on the basolateral surface of thyroid follicular cells which concentrate iodine required for synthesis of thyroid hormones. NIS expression in thyroid tissue is exploited clinically when radioiodine I^{123} is used in imaging for thyroid disorders and I^{131} is used in radiotherapy for thyroid cancers. Ectopic expression of NIS in a cell also enables it to concentrate iodine from its surroundings. Several oncolytic viruses, such as measles virus (MV), vesicular stomatitis virus (VSV), adenovirus (Ad), and herpes simplex virus (HSV) have been engineered to express the NIS gene to enable noninvasive, real-time monitoring of the pharmacokinetics of viral replication in tumors using I^{123} or I^{125} with SPECT/CT imaging or to combine with I^{131} radiotherapy to increase the efficacy of tumor cell killing [12–14]. MV expressing hNIS (MV-NIS) is being evaluated in several phase I clinical trials in patients with ovarian cancer (intraperitoneal administration), multiple myeloma (intravenous), squamous cell carcinoma of the head and neck (intratumor) and mesothelioma (intrapleural) [12, 15, 16].

Endometrial cancer (EC) is the most common gynecologic malignancy in the United States [17] and optimal systemic treatment for advanced stage and recurrent disease remains undefined. Systemic chemotherapy with or without radiation [18, 19], hormone therapy [20], and most recently, biologic agents such as lapatinib [21] and bevacizumab [22] have been and continue to be investigated in metastatic and recurrent EC. Unfortunately, the response rates for most systemic therapies investigated in metastatic EC remain in the single-digit percentages and overall survival continues to decline in this patient population [23, 24]. Novel systemic therapies are desperately needed.

Oncolytic virotherapy appears to be a promising systemic therapy for EC. While there have only been a few women with metastatic EC who have been treated on oncolytic virotherapy clinical trials [25], the potency of more novel oncolytic viruses in EC is encouraging. We have previously shown that both oncolytic MV and VSV treatment result in effective tumor control in 100% of mice bearing EC xenografts [26]. Here we compare *in vitro* oncolysis

and preclinical efficacy in EC of two different strains of oncolytic VV expressing the hNIS transgene. Expression of hNIS and I^{125} uptake were studied in xenografts treated with either the Copenhagen (VVNIS-C) or Wyeth (VVNIS-W) strains of VV in order to track the sites of viral infection and better understand the mechanism of VV oncolysis.

Materials and Methods

Cells, plasmids, and viruses

The human type I EC cell lines HEC1A, Ishikawa, KLE, RL95-2, and AN3 CA and type II cell lines ARK-1, ARK-2, and SPEC-2 cells were used. KLE and RL95-2 were cultured in Dulbecco Modified Eagle Medium (DMEM) and Ham F-12 Nutrient Mixture (DMEM/F12; Mediatech, Herndon, Virginia) supplemented with 10% FBS (Life Technologies, Grand Island, New York). AN3 CA and Ishikawa were cultured in 10% FBS DMEM (Mediatech). HEC1A, ARK-1, ARK-2, and SPEC-2 were maintained in 10% FBS RPMI-1640 (Mediatech). The monkey kidney (Vero; CCL-81), human osteosarcoma (U2OS), and human cervical cancer (HeLa) cells were purchased from American Type Tissue Collection (ATCC). Vero cells were maintained in 5%FBS DMEM. Both U2OS and HeLa were maintained in 10%FBS DMEM.

Human *NIS* gene (NM_000453.2) was synthetized by GeneART (Regensburg, Germany). Synthetized *hNIS* gene was cloned into pSEL-eGFP (a gift from Dr. David Bartlett, University of Pittsburgh) [27] by replacing the xanthine-guanine phosphoribosyl transferase (*gpt*) gene. Oligos for a late promoter sequence (pSL4) with HindIII and XhoI restriction enzyme sites (AAGCTTACAAAAAAAACTTCTCCAAATAGACTCGAG) were ordered from Invitrogen (Life Technology, Grand Island, NY), annealed and replaced the p7.5 promoter in pSEL-eGFP to form pSC65-eGFP-pSL4-hNIS.

To make the recombinant VVNIS-C and VVNIS-W, U2OS cells were infected with either wild Wyeth strain or Copenhagan strains of vaccinia viruses at a multiplicity of infection (MOI) of 0.01 and then transfected with pSC65-eGFP-pSL4-hNIS using Lipofectamine 2000 (Invitrogen). Cells were incubated at 37°C for 4 hours, the medium was replaced, and then cultured for two more days. Viruses were released from the cell debris via 3 freeze-and-thaw cycles. Harvested viruses were used to infect freshly prepared U2OS cells overnight. GFP-positive cells were sorted using flow cytometry and purified by selecting 3 more rounds of GFP-positive plaques on U2OS cells with CMC overlay [3]. As a final step of plaque purification, viruses from the selected plaque were filter into a U2OS plate through a 0.65 μM filter (Sartorius Stedim, Goettingen, Germany) and agarose overlay was applied instead of CMC overlay. The resultant viruses, VVNIS-C and VVNIS-W, were further amplified in HeLa cells for in vitro and in vivo experiments.

Virus Infection, Cell Viability, and Progeny Production

For the virus killing and cell viability assays, cells in 96 well plates were exposed to either the VVNIS-W or VVNIS-C at specified multiplicity of infection (MOI; 0, 0.001, 0.01, 0.1, 1, and 10). Cell viability was assessed at 72 hours post infection using the (3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium (MTS) cell proliferation assay according to manufacturer's instructions (Promega, Madison, WI). Photos were taken using a Nikon Eclipse TE300 microscope mounted with a Nikon U2 digital camera prior to the MTS assay. The median effective concentration (EC_{50}) values of the viruses in each cell line were calculated using PRISM analysis software (GraphPad, La Jolla, CA).

For the viral progeny propagation assays, cells (2×10^5/well) were seeded in 12 well plates, infected with viruses (MOI 0.02) and incubated at 37°C. Two hours later, the virus inoculum was removed and replaced with growth media. Cells were harvested at 48 hours post virus infection, and viral titers were determined by $TCID_{50}$ assay on HeLa cells.

Flow Cytometry

Cells (6×10^5/well) in 6 well plates were infected with virus, VVNIS-W or VVNIS-C, at MOI 0.1 in 1 mL serum-free OPTI-MEM. After 2 hours, the virus medium was removed, replaced with 1 mL growth medium and incubated at 37°C. Cells were collected at 24 hours post infection with VV, washed with PBS, and fixed in 500uL 4% Paraformaldehyde for 90 minutes. The cells were washed and re-suspended in 500 uL PBS. The percent of GFP positive cell was measured by flow cytometry (BD BioSciences, FACScan) and the data was analyzed using FlowJo software (Tree Star, Ashland, OR).

I^{125} uptake in vitro

Cells (2×10^5/well) in 12 well plates were infected with viruses (MOI 0.1) and incubated at 37°C. 24 hours after infection, cells were washed with Hank's Balanced Salt Solution (HBSS; VWR, Radnor, PA) and replaced with HBSS supplemented with 10mM of HEPES. ^{125}I (500,000cpm) was then added into each well with or without $KClO_4$ to inhibit NIS mediated uptake of ^{125}I and incubated at 37°C. 2 hours after incubation, cells were washed with iced HEPES-HBSS 3 times and replaced with 1M NaOH (500ul/well). The NaOH solution was then transferred to polypropylene tubes 10 minutes later and the radioactivity was quantitated using Isodata-10 gamma counter (ICN Biomedicals, Costa Mesa, CA).

Animal Experiments

All animal experiments were approved by and performed according to the guidelines of the Mayo Clinic Institutional Animal Care and Use Committee. 4- to 5-week-old female athymic mice were purchased from Harlan (Indianapolis, Indiana). To study the effect of different viruses on tumor growth and animal survival, mice were implanted subcutaneously in the right flank with 2×10^6 ARK-2 or AN3 CA cells. When tumors reached 0.3 to 0.5 cm in diameter, 100 μL of viruses or PBS (vehicle control) was injected intravenously through the tail vein (10^6 $TCID_{50}$/mouse). Tumors were measured twice to three times per week, and mice were euthanized when tumor mass reached 10% of the mouse's body weight, became ulcerated or interfered with a mouse's ability to reach food or water.

During the efficacy study, NIS gene expression was also monitored using a SPECT-CT imaging machine (U-SPECT-II, MI Labs, Netherlands) at day 7, 14, 21 post treatment. At the day of imaging, two mice from each group were injected with 300 uCi of I^{125} intraperitoneally 1hour before imaging and then imaged for 20–25 minutes. The DICOM image files were blinded and analyzed by a nuclear imaging service provider (Imanis Life Sciences, Rochester, MN).

Statistical Analysis

The tumor size was compared between the three treatment groups with a mixed-effects model with a random effect for mouse and fixed effects for time and treatment, including a quadratic effect for time as well as the time-by-treatment interaction. All analyses were performed using JMP version 9 software (2010 SAS Institute).

Results

EC cell viability after VVNIS infection in vitro

A panel of 8 human endometrial cancer cell lines was used to compare the potency of VVNIS-C and VVNIS-W *in vitro* at various MOIs. Most of the EC cell lines, except for KLE, were susceptible to infection and killing by both VVNIS-C and VVNIS-W. Type I EC cell lines were less efficiently killed by both VVNIS-C and VVNIS-W when compared to the Type II EC cell lines (Figure 1a and Table 1). By 72 hours, almost 100% of Type II EC cells were killed by both VVNIS at MOI 0.1 or 1, while a MOI of 1 or 10 was required to achieve 100% killing of Type I EC cell lines (Figure 1a). KLE was highly resistant infection by VVNIS-C and VVNIS-W. iOnly 25% of KLE cells were killed at an MOI of 10. Both VVNIS-W and VVNIS-C had comparable potency in all cell lines tested, except in AN3 CA cells where VVNIS-C was substantially more potent (Figure 1a and Table 1). The concentration of VVNIS-C required to kill 50% of AN3 CA cells (EC_{50}) was about 80-fold lower than VVNIS-W (Table 1).

Viral titers calculated from the TCID50 assay on HeLa cells 48 hours after infection with VVNIS showed that most of the EC cell lines supported both VVNIS-C and VVNIS-W replication (Figure 1b). KLE and SPEC2 showed the lowest output of progeny virus, while higher titers were observed in HEC1A, Ishikawa, ARK-1, and ARK-2. VVNIS-C infection resulted in at least 100-fold higher viral titers than VVNIS-W in all 8 human EC cell lines, suggesting that the Copenhagen strain is more potent in its ability to propagate and spread in the human EC cells than the Wyeth strain.

Early infectivity and spreading of VVNIS in EC in vitro

The infectivity of VVNIS-C and VVNIS-W in EC cell lines was determined by measuring the percentage of GFP positive cells at early time points after infection but before significant cell death occurred. Overall, EC cell lines were more susceptible to infection by VVNIS-C than VVNIS-W (Figure 2a). The most impressive difference was seen in AN3 CA cells where 85.2% of cells were infected by VVNIS-C compared to only 6.87% by VVNIS-W. Type II EC cell lines were also highly susceptible to infection by VVNIS-C and VVNIS-W where 67.1% (ARK-1) and 78.6% (ARK-2) of cells were GFP positive at 24 hours after VVNIS-W infection. KLE cells were resistant to infection by both VVNIS-C and VVNIS-W with only 4.6% (VVNIS-W) and 16.6% (VVNIS-C) of cells being GFP positive 24 hours post infection (Figure 2a).

The ability of the virus to spread within the culture was monitored by GFP fluorescence at low and high MOIs (Figure 2b). By 48 hours, there were abundant GFP-positive cells in the VVNIS-W and VVNIS-C infected cultures despite the low MOI of 0.001 (Figure 2b). For cell lines that were highly susceptible to VVNISs infection (e.g. ARK-1 and ARK-2), the GFP signals decreased due to an increase in cell death with increase in MOI. For cell lines that were more resistant to virus infection (e.g. AN3 CA with VVNIS-W or KLE for both viruses), the GFP signals increased with the increase in MOI (Figure 2b).

I^{125} uptake in EC cell lines infected with VVNISs in vitro

Prior to *in vivo* experiments to determine the oncolytic activity of VVNIS in EC xenografts, we evaluated the ability of VVNIS-infected cells to concentrate radioiodine in an I^{125} uptake assay, with and without the addition of potassium perchlorate (a competitive inhibitor of NIS-mediated iodine uptake), in type I (AN3 CA) and type II (ARK-2) cell lines. There was significant I^{125} uptake in both VVNIS-W and VVNIS-C infected ARK-2 cells at 50–100 fold above that of uninfected cells (Figure3a and 3c). The I^{125} uptake was specifically due to NIS as potassium perchlorate effectively prevented I^{125} uptake in these infected cells. In

contrast, I^{125} uptake in VVNIS infected AN3 CA cells were lower compared to ARK- 2. AN3 CA cells were able to concentrate I^{125} at 10–40 fold above background (Figure3b and 3c). Due to the higher infectivity of VVNIS-C in AN3 CA cells, there was correspondingly higher I^{125} uptake in the VVNIS-C infected culture compared to VVNIS-W infected culture.

Intravenous VVNIS causes oncolysis in both type I and type II EC xenografts

We next compared the oncolytic activity of VVNIS-C and VVNIS-W in ANC 3A (type I EC) and ARK-2 (type II EC) xenografts. When subcutaneous tumor xenografts reached 0.3 to 0.5 cm in diameter, mice were administered one intravenous dose of 10^6 $TCID_{50}$ of VVNIS-C or VVNIS-W. A cohort of mice was used for SPECT-CT imaging to track NIS expression in the tumors at day 7, 14, and 21 post-treatment. Figure 4 shows the SPECT-CT images and quantitation of the amount of isotope uptake in tumors of mice at day 14 post-treatment. There was I^{125} uptake in the thyroid and stomach which are sites of endogenous NIS expression. In addition, strong I^{125} signals were seen in the tumors of all 8 imaged mice (Figure 4a, b). The I^{125} uptake signals gradually increased as the virus spread in the enlarging tumors and decreased with decreasing tumor size as the tumors responded to the VV therapy (Figure 4c). I^{125} uptake was detected in most of the tumors even until day 21 post treatment, suggesting ongoing viral replication in the tumors grown in these immunocompromised mice (Figure 4c).

For both xenograft models, tumors in the saline control group continued to grow over time, and mice were euthanized because of tumor burden or tumor ulceration (AN3 CA, Figure 5a; ARK-2, Figure 5e). For the AN3 CA xenograft model, tumors in VVNIS-W treated mice continued to grow over time (Figure 5b) and the effect of time on tumor growth in the VVNIS-W treatment group was not significantly different from the saline control (*p* value= 0.8401, Figure 5d). In contrast, VVNIS-C treatment attenuated AN3 CA tumor progression (Figure 5c). While there were no complete regressions observed in AN3 CA xenografts treated with VVNIS-C, there was significantly slower growth in those treated with VVNIS-C compared to saline (*p* value < 0.0001, Figure 5d). For mice bearing ARK-2 xenografts (Figure 5e–5h), therapy with both VVNIS-W (Figure 5f) and VVNIS-C (Figure 5g) effectively halted tumor progression and induced tumor regression in 100% of mice. In fact, 2 of the 10 VVNIS treated mice (1 from VVNIS-W, 1 from VVNIS-C) had complete tumor regression. ARK-2 xenografts treated with either VVNIS-C or VVNIS-W had impressive responses compared to xenografts in the saline cohort (both *p* value< 0.0001, Figure 5h).

Discussion

Despite contemporary systemic therapies, the persistently high mortality rate secondary to metastatic and recurrent EC drives the imperative to develop novel therapeutics for this cancer. We have previously shown that VSV as well as the Edmonston strain of MV have potent oncolytic activity against EC both *in vitro* and *in vivo* making systemic oncolytic virotherapy an attractive new therapy to study in EC [26]. Prior to finalizing a candidate virus for clinical trial development, we investigated the antitumor activity of VV in the same panel of EC cell lines. Two new recombinant VVs (Copenhagen and Wyeth strains) encoding the human NIS gene were generated to enable noninvasive monitoring of the pharmacokinetics of virus replication. Expression of NIS in the tumors also allowed us to reliably correlate tumor response with real-time virus replication and gene expression in the target lesions. Overall, VVNIS-C appears to have superior oncolytic activity compared to VVNIS-W in EC. Additionally, both type I and type II ECs are susceptible to VV infection and oncolysis. But most notable is the impressive oncolytic activity of VV against type II EC which is particularly recalcitrant to present-day adjuvant therapies.

While type II EC accounts for only 20% of all ECs diagnosed, type II histologies are highly lethal with a median overall survival of only 2 years among stage IV cases treated with curative intent multimodal therapy [28, 29]. Type II EC clinically behaves more aggressively than type I EC with a higher propensity to present at an advanced stage. Type II EC is also particularly refractory to systemic chemotherapy [28] and novel effective systemic agents are vital in the efforts to improve survival from this aggressive form of EC. Both VV and VSV [26] appear to have potent oncolytic activity against type II EC. While MV has activity against EC [26], population-wide MV vaccination precludes systemic MV administration as antibody neutralization of the virus occurs rapidly after intravenous administration [30].

One of the major advantages of VVs over other oncolytic viruses is their potential ability to spread systemically though the blood [8, 9, 27]. VV produces several distinct antigenic forms of viral particle, including an enveloped virus form (EEV) which is capable of evading recognition by complement and neutralizing antibodies by shrouding itself in a host cell-derived envelope that contains several host complement control proteins and few exposed viral proteins [31, 32]. Therefore, the systemic delivery of VVs and their spread between tumors may be highly efficient [33] making it an attractive agent for the treatment of widely metastatic or multi-site recurrent EC.

Interestingly, vaccine strains of VV have already been shown to inherently target cancer [33]. The replication and spreadof VV is associated with activation of the epidermal growth factor receptor (EGFR)-Ras signaling pathway in cancer cells [34]. Thus, in addition to deregulation in cell cycle control and immune evasion that makes cancer cells more susceptible to virus infection, activation of EGFR-Ras pathway in most human cancers suggests that VV could be particularly suited as a selective oncolytic agent [35]. To further enhance the cancer selectivity of VV, a range of viral gene deletions that reduce the ability of the virus to productively replicate in most normal cells have been introduced [36, 37]. In both VVNIS-C and VVNIS-W, the vaccinia encoded thymidine kinase gene (*TK*) was deleted and replaced by the *hNIS* gene to further enhance the safety and specificity profiles of the VV [27]. Deletion of the vaccinia encoded *TK* gene results in dependence of the virus on cellular thymidine kinase which is constitutively expressed at high levels in most cancers but only transiently expressed during the S phase of the cell cycle in proliferating normal cells [36]. Despite deletion of *TK* in VVNIS-C and VVNIS-W there was toxicity associated with VV virotherapy in our immunocompromised mice, especially for the Copenhagen strain. VV infection of normal tissues resulted in weight loss and pox formation on the tails and feet in some of the VV treated athymic mice. This suggests that further attenuation of the VV might be required, especially if the viruses will be administered to immunocompromised cancer patients.

Alternatively, we also found that among the EC cell lines tested, one cell line (KLE) was particularly resistant to VV infection. Interestingly, this same cell line has previously been shown to be resistant to MV and VSV infection [26]. Some tumor cells are intrinsically resistant to VSV due to constitutive induction or expression of high levels of antiviral interferon (IFN) responsive genes such as *OAS* and *MXA* [38]. However, VV encodes multiple genes that can antagonize the host cell antiviral responses [39] and further exploration of the mechanism of resistance to MV, VSV, and VV infection should contribute to improving the therapeutic index of these viruses.

The strains of VV used in different areas of the world during the smallpox eradication program vary in their characteristics, pathogenicity and host range, probably due to variations in the expression or functionality of different virulence genes between strains [7, 33]. Clinical trials where the oncolytic VV from the Wyeth strain, JX594, engineered to

express GM-CSF, was administered intratumorally to patients with nonresectable hepatocellular carcinoma led to objective responses in three of ten evaluable patients [1, 37]. Oncolytic VVs from other strains, such as WR strain (vvDD-CDSR, also called JX-929) and Lister strain (GL-ONC1), are also being evaluated in clinical trials against solid tumors, squamous cell carcinoma of the head and neck (SCCHN), and peritoneal carcinomatosis [1, 8, 40], a common presentation of advanced stage type II EC.

In summary, both strains of VV have oncolytic activity against most EC cell lines, although the Copenhagen strain appeared to be more potent with higher rates of infectivity and substantially higher progeny production (10–10^6 fold) in infected cells compared to the Wyeth strain. *In vivo*, the Copenhagen strain was effective at controlling tumor growth in both type I and type II EC. Given these findings, we believe that the Copenhagen strain should be pursued further for Phase I testing although the formation of pox lesions in the immunocompromised mice is concerting. To attenuate the virus, we plan to plaque purify the virus further to identify an isolate that has high oncolytic activity but does not cause lesions in the immunocompromised mice before initiating a clinical trial of VVNIS-C in type II EC.

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The human type I cell lines were provided by William Cliby, MD, Mayo Clinic, and the type II cell lines were provided by Sean Dowdy, MD, Mayo Clinic.

Abbreviations

DMEM	Dulbecco Modified Eagle Medium
EC	endometrial cancer
VV	vaccinia virus
EEV	enveloped virus from
VVNIS-W	Wyeth strain of VV expressing the thyroidal sodium iodide symporter
VVNIS-C	Copenhangen strain of VV expressing the thyroidal sodium iodide symporter
WR	Western Reserve strain of VV
GFP	green fluorescent protein
IFN	interferon
hNIS	human sodium iodide symporter
MV	measles virus
MV-NIS	Edmonston strain MV expressing the thyroidal sodium iodide symporter
OV	oncolytic virus
IV	intravenous
MOI	multiplicity of infection
$TCID_{50}$	50% tissue culture infective dose
VSV	vesicular stomatitis virus

HSV	herpes simplex virus
Ad	adenovirus
EGFR	epidermal growth factor receptor
TK	thymidine kinase
SCCHN	squamous cell carcinoma of the head and neck
EC_{50}	half maximal effective concentration

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Highlight

- Both Type I and Type II endometrial cancer (EC) cell lines and xenografts undergo oncolysis when exposed to vaccinia virus (VV).
- Copenhagen strain of VV is more potent in EC oncolysis than Wyeth strain.
- A VV clinical trial in type II EC is warranted.

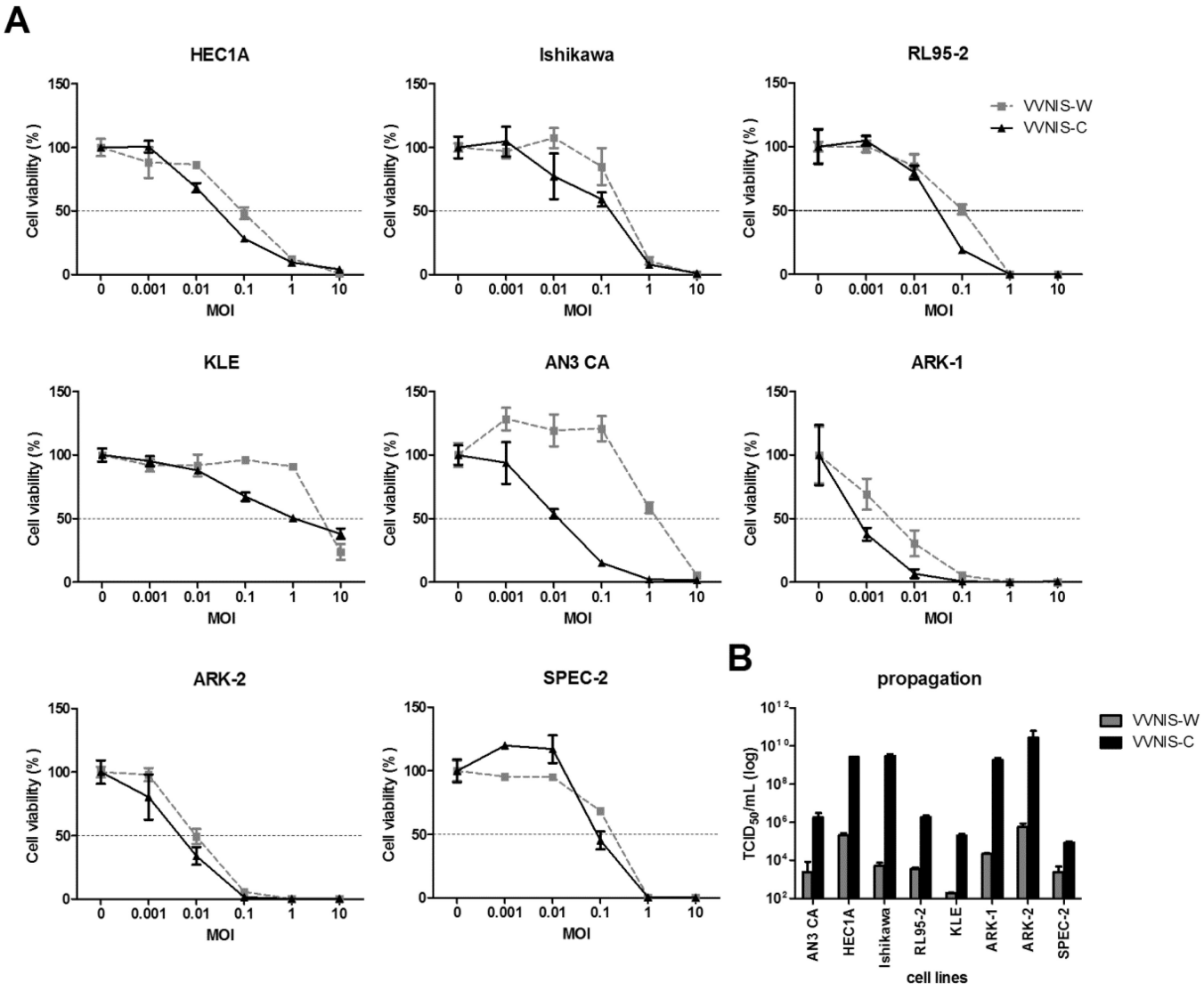


Figure 1.

Virus induced cell killing and viral progeny propagation in human EC cell lines. A, Cell viability of Type I EC cell lines, AN3 CA, Ishikawa, HEC1A, KLE, and RL95-2, and Type II EC cell lines, ARK-1, ARK-2, and SPEC-2, at 72 hours post VV infection. B, Cells were infected with VV at low MOI (0.02) to enable multiple-cycles of infection. The amount of viral progeny was determined by $TCID_{50}$ assay 48 hours later.

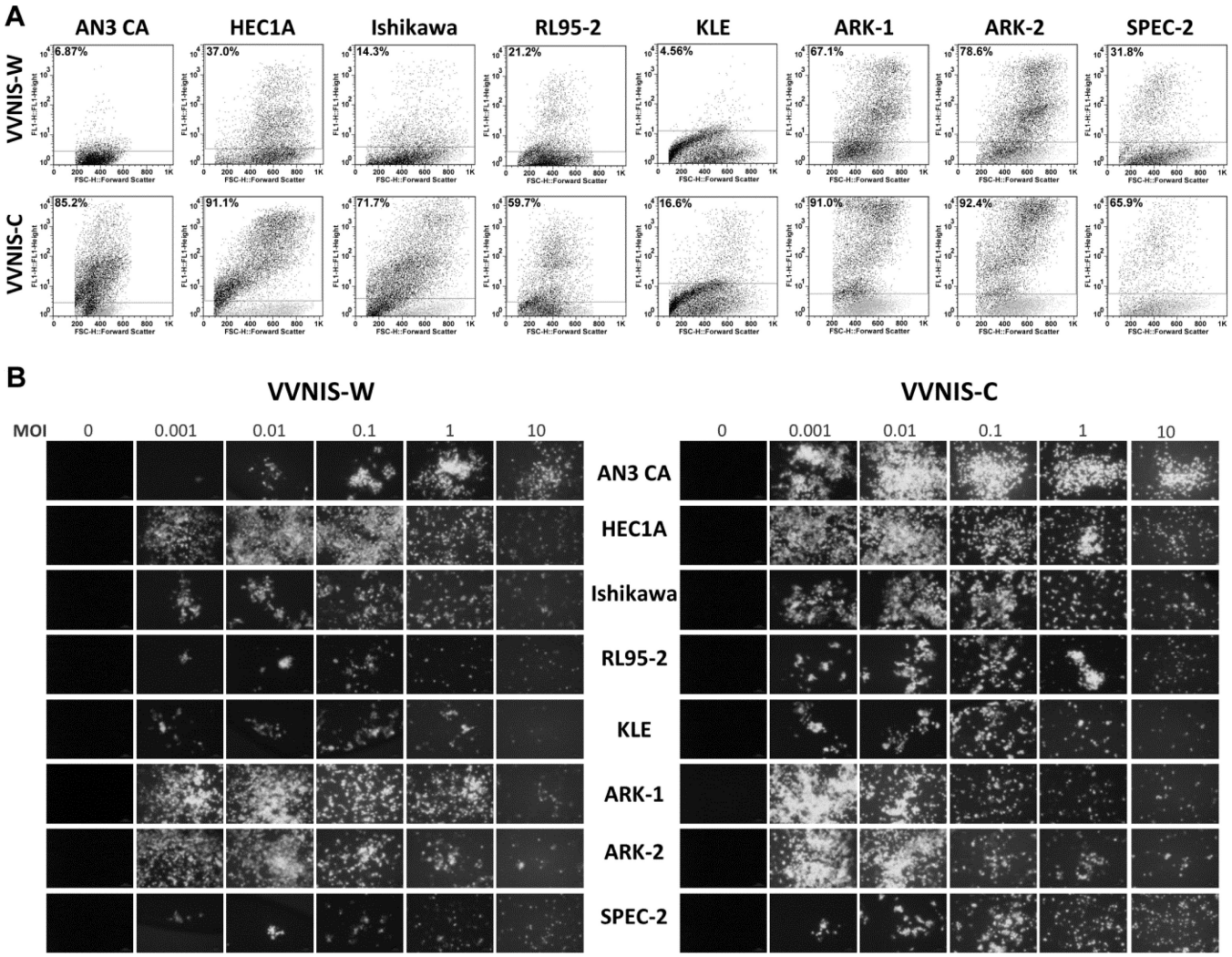


Figure 2.

Infectivity and spread of VVNIS in human EC cell lines. A, Cells were infected with viruses at MOI 0.1 and the percent of GFP positive cells were analyzed by flow cytometry 24 hours after infection. Numbers within each panel indicate the percentage of GFP positive cells. B, Photographs of infected EC cells at 72 hours after infection at various multiplicity of infection.

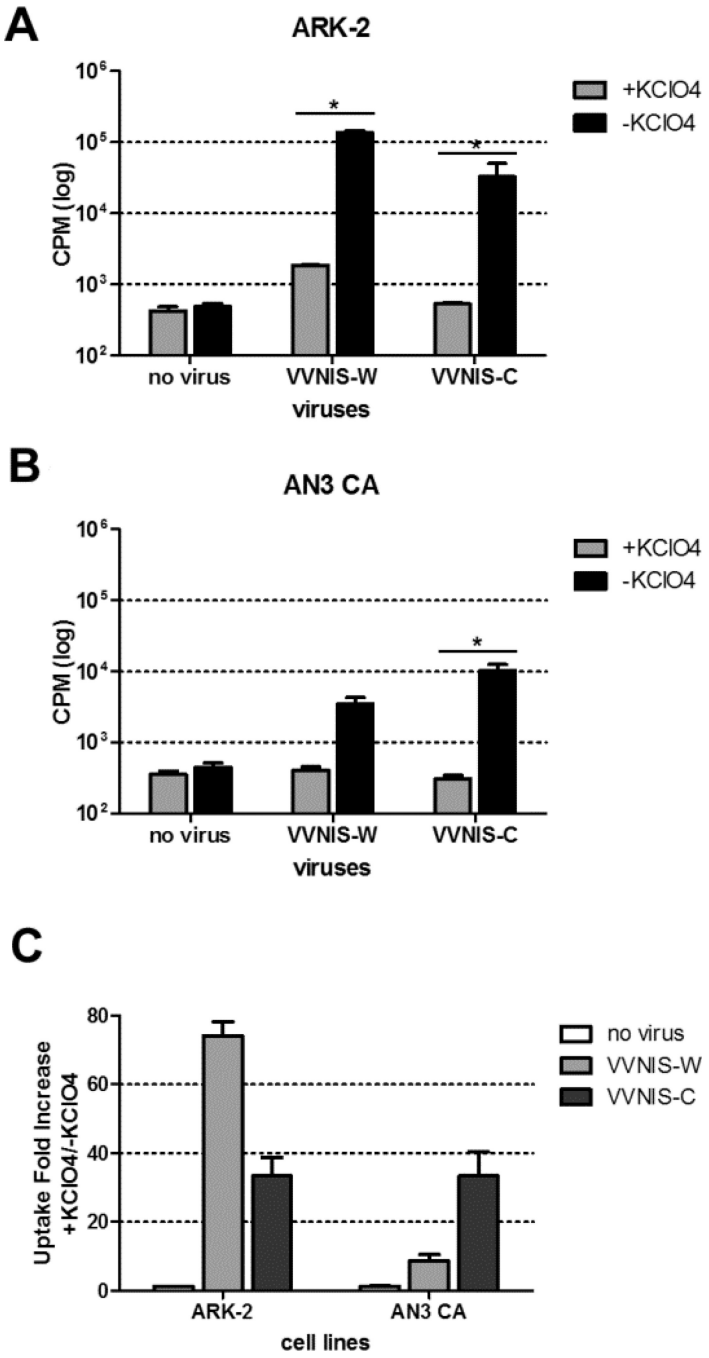
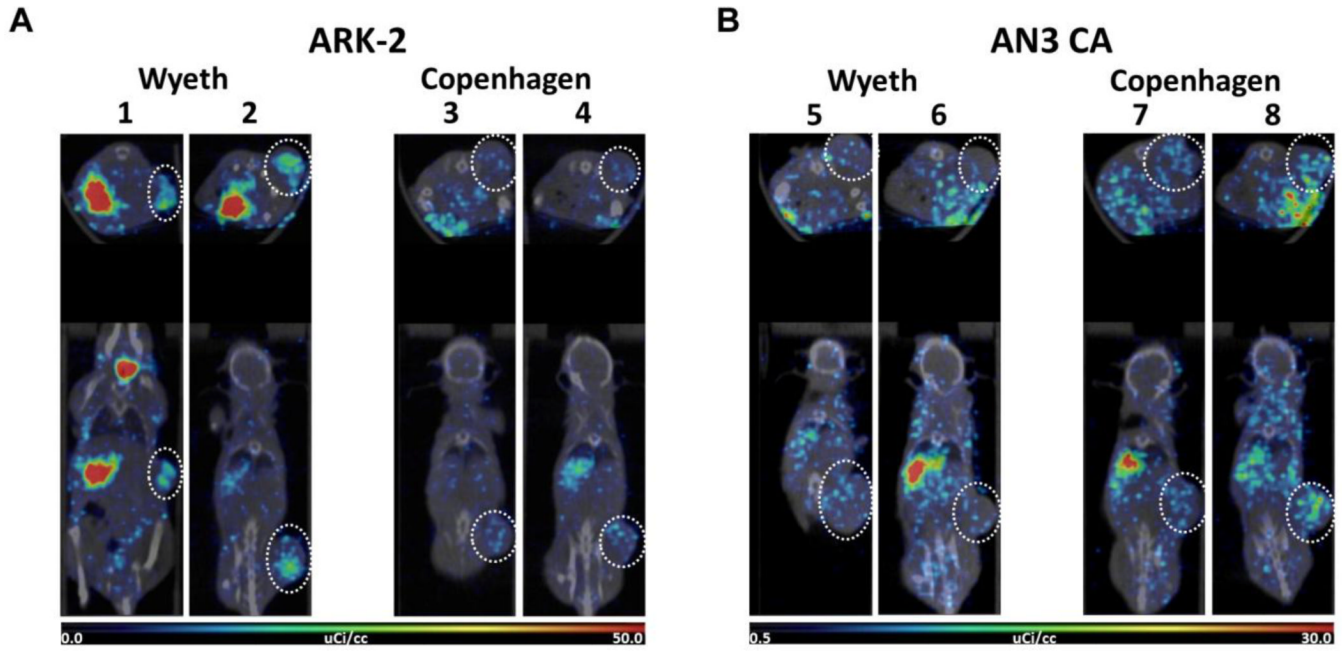


Figure 3.

Assay to measure NIS activity in infected EC cells. (A) ARK-2 and (B) AN3 CA cells were mock infected (no virus) or infected with viruses at MOI 0.1 and uptake of I^{125} (counts per minute CPM) was analyzed in the presence or absence of $KClO_4$ at 24 hours after infection. C. Fold increase in I^{125} uptake in VVNIS infected ARK-2 and AN3 CA were calculated by dividing the uptake amount in the absence of $KClO_4$ by the uptake amount in the presence of $KClO_4$. * $P<0.05$ (Unpaired student t test).



C

			Day 7		Day 14		Day 21	
		Mice ID	Tumor uptake (uCi/ROI)	Tumor volume (cm^3)	Tumor uptake (uCi/ROI)	Tumor volume (cm^3)	Tumor uptake (uCi/ROI)	Tumor volume (cm^3)
ARK-2	W	1	0.2983	0.1633	1.9991	0.2432	0.2143	0.0512
		2	0.3070	0.2062	2.9083	0.4643	8.5948	0.6898
	C	3	0.5650	0.3712	1.1275	0.4035	0.2199	0.1384
		4	0.4733	0.1859	1.0929	0.2770	1.0746	0.2189
AN3 CA	W	5	0.7015	0.3033	1.6464	0.7524	2.7006	1.4067
		6	0.8461	0.2248	0.8960	0.4790	3.6490	1.0532
	C	7	3.5754	0.9071	1.7308	0.7250	2.2555	0.7740
		8	2.5082	0.6666	2.9471	0.5435	2.7555	0.7250

Figure 4.

Noninvasive SPECT-CT monitoring of the pharmacokinetics of VVNIS infection in EC xenografts. Mice with subcutaneous EC xenografts were injected intravenously with VVNIS-C or VVNIS-W. On the day of imaging, each mouse was receiving 300 μCi I^{125}. A and B, SPECT/CT fused images of mice bearing ARK-2 (A) or AN3 CA (B) xenografts on day 14 post virus infection. Location of tumor on each mouse is circled. C, Tumor I^{125} uptake (uCi/ROI) and tumor volume (cm^3) in each mouse on day 7, 14, 21 post virus infection.

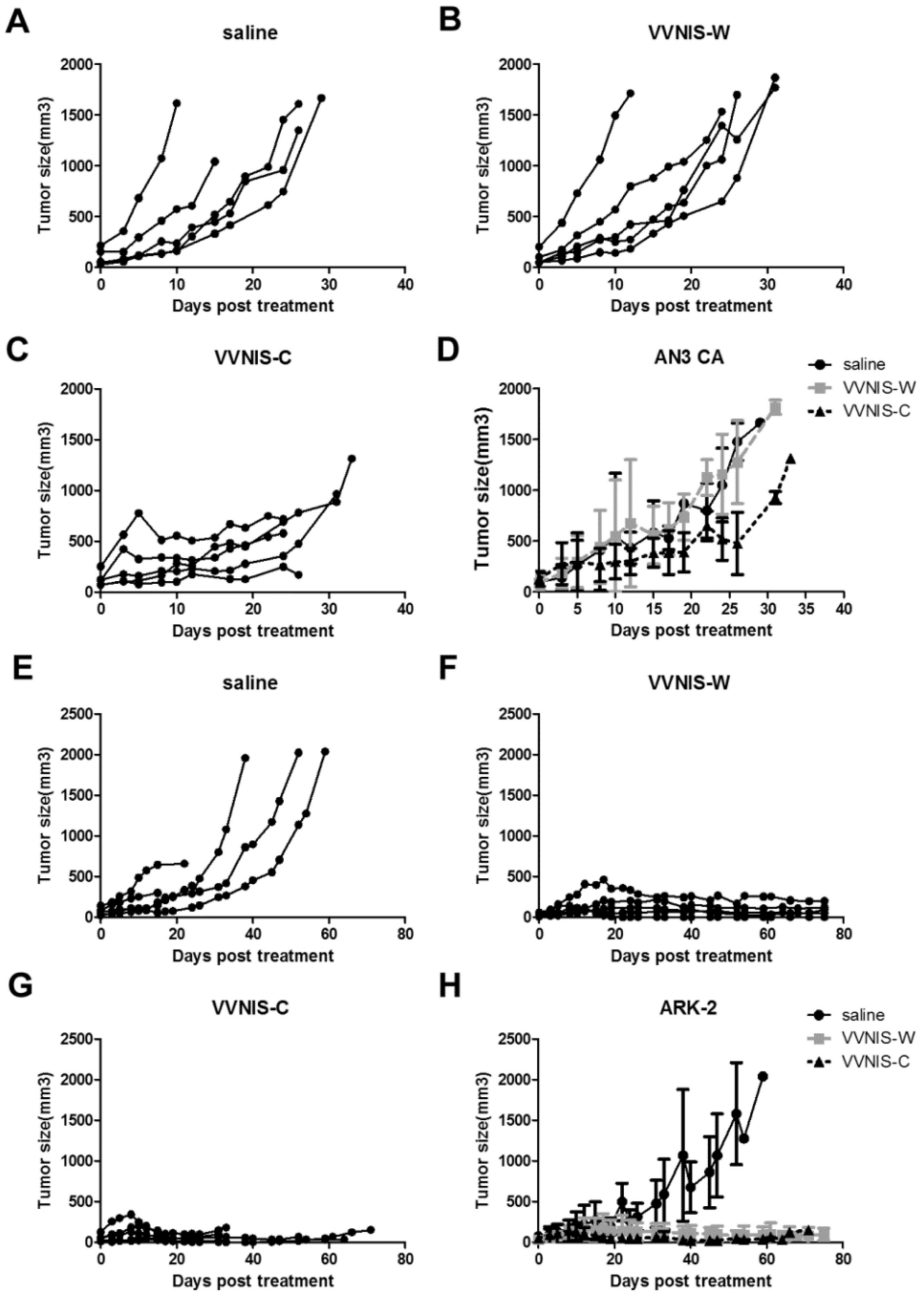


Figure 5.

Tumor response curves post intravenous administration of VVNIS-C or VVNIS-W in mice with subcutaneous (A–D) AN3 CA or (E–H) ARK-2 xenografts. Mice were randomly assigned to a single IV injection of saline, VVNIS-C or VVNIS-W (10^6 $TCID_{50}$). A–C and E–G, Individual tumor volumes in each mouse. D and H, Average tumor volumes of each study group.

Table 1

EC_{50} values of VVNIS-W and VVNIS-C in EC cells 72 hours after infection

	VVNIS-W	VVNIS-C
AN3 CA	0.98	0.012
HEC1A	0.11	0.023
Ishikawa	0.196	0.12
RL95-2	0.1	0.025
ARK-1	0.002	0.00034
ARK-2	0.0099	0.0050
SPEC2	0.18	0.09