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Oncolytic Vesicular Stomatitis Virus as a Treatment for Neuroendocrine Tumors

Reese W. Randle, MD¹, Scott A. Northrup, BS¹, S. Joseph Sirintrapun, MD², Douglas S. Lyles, PhD³, and John H. Stewart IV, MD¹

¹Department of General Surgery, Wake Forest School of Medicine, Winston-Salem NC

²Department of Pathology, Wake Forest School of Medicine, Winston-Salem NC

³Department of Biochemistry, Wake Forest School of Medicine, Winston-Salem NC

Abstract

Background—Therapeutic goals for neuroendocrine tumors (NETs) not amenable to surgical cure are limited to relieving symptoms and slowing progression. However, many cancers acquire defective antiviral responses as they undergo unregulated proliferation. Therefore, we explored the abilities of recombinant wild-type vesicular stomatitis virus (rwV-VSV) and an attenuated Matrix protein mutant (M51R-VSV) to exploit defective antiviral pathways in NETs.

Methods—Viral infectivity and lethality were evaluated in a panel of human NET cell lines H727, UMC-11, and CNDT2.5. We evaluated γ -interferon pathways in these cells to define the acquired defect. Murine xenografts were treated with a single intratumoral injection of M51RVSV to study viral efficacy in vivo.

Results—VSV infected >99% of cells within 24 hours and killed >95% within 72 hours. NET cells did not produce significant amounts of γ -interferon after infection, but exogenous γ -interferon protected cells from oncolysis. Treatment with M51R-VSV resulted in significantly suppressed tumor growth compared with mock-infected xenografts for H727 (87±72% vs. 2197±335%, p<0.001), UMC-11 (12±59% vs. 1471±324%, p<0.001), and CNDT2.5 (81±121% vs. 1576±349%, p=0.001).

Conclusions—VSV infects and kills human NETs by exploiting their inability to produce a type I antiviral response. Therefore, M51R-VSV is an excellent candidate for the treatment of advanced NETs.

Background

Neuroendocrine tumors (NETs), otherwise known as carcinoids, arise from enterochromaffin cells located throughout the body, but are most commonly found in the gastrointestinal tract and tracheobronchial tree.^{1,2} While both historical and modern

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Correspondence and reprint requests should be addressed to: John H. Stewart, IV, MD Department of Surgery Wake Forest School of Medicine Medical Center Boulevard Winston-Salem, North Carolina, 27157 Phone: 336-716-0545 Fax: 336-716-6637 jhstewart@wfbmc.edu.

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classification schemes focus on differences in origination, NETs share similar histology, pathology, behavior, and treatment regardless of their location.¹⁻⁵

Although generally perceived to be uncommon, the incidence of NETs is increasing, and regional or distant metastatic disease is present in nearly half of all cases at diagnosis.^{1,2} Cure is only achieved with surgery, yet surgery alone is insufficient to cure many of these patients.^{2,6-8} Unfortunately for patients with NETs not amenable to surgical cure, therapeutic goals are limited to controlling symptoms and slowing disease progression.^{7,9} However, many malignant cells acquire defects in antiviral pathways as homeostatic mechanisms are overcome by those favoring unregulated growth and proliferation, and these defects have become the focus of targeted oncolytic viral therapy.^{10,11}

Type I interferon (IFN) is one of the main components of a normal cell's non-specific, innate antiviral response. Cells with intact antiviral pathways both produce IFN in response to infection and are responsive to its downstream effects that include arresting proliferation, blunting angiogenesis, and promoting apoptosis.

Vesicular stomatitis virus (VSV) is an oncolytic virus unique in its exquisite sensitivity to the effects of IFN. Thus, it selectively targets cells with defects in either IFN production or IFN responsiveness, and has demonstrated ability to infect and kill many malignant cell lines while leaving normal cells intact.¹⁰⁻¹⁴ M51R, a variant of VSV, contains a mutation which renders its Matrix (M) protein incapable of suppressing the host genome while allowing retention of its functions in viral assembly.¹⁵ Theoretically, this mutation broadens the therapeutic window by allowing normal cells to mount an uninhibited antiviral response. Furthermore, administration in mice results in no discernible toxicity.¹⁶

We aimed to evaluate the oncolytic effects of rwt-VSV and M51R-VSV in a panel of human NET cell lines representing the most common primary sites and identify potential mechanisms of susceptibility. To this end, we performed a series of *in vitro* experiments to evaluate viral infectivity, lethality, and interplay with the innate antiviral response of NETs. Furthermore, we studied viral efficacy *in vivo* utilizing a murine model of human NET xenografts treated with a single intratumoral injection of M51R-VSV.

Materials and Methods

Cell Culture and Viruses

H727 human bronchus carcinoid and UMC-11 human lung carcinoid cell lines were obtained from American Type Culture Collection, and CNDT2.5 human ileal carcinoid cell line was a kind gift from Dr. Lee Ellis, M.D. Anderson Cancer Center, Houston, TX. Although some investigators question the authenticity of CNDT2.5, it has displayed many features of carcinoid cells including functional somatostatin receptors and neurosecretory granules and is in use as a model for midgut carcinoid around the world.^{17,18} Culture media consisted of RPMI 1640 supplemented with 10% fetal bovine serum, L-glutamine, and penicillin and streptomycin (H727 and UMC-11) or DMEM with supplements according to Van Buren et al (CNDT2.5).¹⁸ Cells were grown in an incubator at 37°C with 5% CO₂. All cell lines were screened for animal pathogens by RADIL, Columbia, Missouri and tested negative. Viruses and viral stocks were prepared as previously described.¹⁹

Viral Infectivity

In order to determine the ability of M51R virus to infect NETs, cell lines were plated at 450,000 cells per well in 6-well plates and inoculated with M51R-VSV encoded with green fluorescent protein (GFP) at various multiplicities of infection (MOIs). At specified time points, cells were removed from the plates and fixed in 2% paraformaldehyde. Intact cells

were analyzed for the presence of GFP expression with an Accuri™ C6 flow cytometer (Becton Dickinson, Franklin Lakes, NJ).

Viral Lethality

To evaluate viral lethality each cell line was plated on a 96 well plate with 3,000 cells per well and subsequently infected with rwt-VSV or M51R-VSV at various MOIs. Using 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)2H-tetrazolium (MTS) assays (CellTiter 96 Aqueous One Solution Cell Proliferation Assay; Promega, Madison, Wisconsin) we measured viable cells at 24, 48, and 72 hours post-infection.

β-IFN Production

H727, UMC-11, and CNDT2.5 were plated in 60 mm dishes with 3 ml of media and infected with an MOI of 5 plaque-forming units (pfu)/ cell. 150 μL media was drawn off each well at specified time points in order to quantify the amount of β-IFN produced by the NET cells. IFN level was determined with enzyme-linked immunosorbent assay (VeriKine Human Interferon-Beta ELISA Kit; PBL InterferonSource, Piscataway, New Jersey).

Impact of Exogenous β-IFN

All cell lines were plated on 96-well plates with 3,000 cells per well and pretreated for 8 hours with increasing concentrations of human β-IFN. Cells were then mock-infected or infected with rwt-VSV or M51R-VSV at an MOI of 5 pfu/ cell. MTS assays determined cell viability after 24, 48, and 72hrs.

Xenografts

Female, 4-6 week old athymic nude mice were obtained from Charles River Laboratories. Subcutaneous xenografts were created in the right flanks of thirty mice per cell line using 5×10^6 cells suspended in 0.1 ml of phosphate buffered saline. Once mice developed palpable, growing tumors, half were injected once with 0.05 ml media and the other half with media containing 3×10^8 pfu M51R-VSV. Two mice in each group were euthanized on day 2 post-treatment, and their tumors were harvested for histological examination and immunohistochemical analysis. The remaining mice were observed three times weekly for 4 weeks with weights collected to ensure good health and tumors measured to follow response to treatment. Previously described physiologic signs of viral toxicity were also monitored.¹⁶ Tumor volume was calculated with the formula, $\text{volume} = (\text{width}^2 \times \text{length})/2$. All animal studies were approved by the Institutional Animal Care and Use Committee of Wake Forest School of Medicine.

Immunohistochemistry

Tumor sections were stained with Hematoxylin and Eosin (H&E) for histological examination. Immunohistochemical staining was performed utilizing capillary action technology to identify infected cells within the tumors. Briefly, 5 μm sections were incubated with a 1:100 dilution of rabbit antibodies against VSV envelope glycoprotein (G-protein) which covers the viral envelope and binds to target cells at infection (Fitzgerald Industries International, Sudbury Road, MA 01720). This was followed by incubation with biotinylated goat-antirabbit and subsequently Streptavidin alkaline phosphatase (BioGenex, 4600 Norris Canyon Road, San Ramon, CA 94583). Bound antigen-antibody complex was stained red using Vector Red substrate kit I (Vector Laboratories Inc., 30 Ingold Road, Burlingame, CA 94010). Lastly, sections were counterstained with Hematoxylin.

Statistical Analysis

Analysis of variance was used to compare means between experimental groups. Percent change between M51R treated xenografts and mock-treated xenografts were compared utilizing repeated measures analysis of variance at individual time points. P values less than or equal to 0.01 were considered statistically significant.

Results

Viral Infectivity in NET Cells

Using flow cytometry gated on intact cells, GFP expression served as a marker for both successful infection and viral protein synthesis. MOIs were determined using VSV-susceptible baby hamster kidney cells as a standard. Theoretically, an MOI of 0.1 pfu/ cell correlates with the potential to infect 10% of susceptible cells while an MOI of 10 pfu/ cell correlates with the potential to infect 10 times the number of cells present. Inoculation with an MOI of 0.1 pfu/ cell represents a multiple-cycle infection where viral replication and spread throughout the culture is necessary to infect more than 10% of cells while MOIs 1 represent single-cycle infections where all cells may potentially be infected with the inoculating dose of virus.

The percentage of GFP expressing cells increased in both a time and MOI dependent manner; however, by 24 hours post-inoculation, GFP was identified in greater than 99% of cells across all NET cell lines and at all MOIs tested (Figure 1). The fact that 99% of cells eventually expressed GFP after inoculation at an MOI of 0.1 pfu/ cell indicated that active viral progeny were released from the initially infected cells and able to spread throughout the culture. Furthermore, there was no difference in the percentage of infected cells after 24 hours regardless of MOI suggesting that multiple cycle infection was both rapid and effective.

Viral Lethality in NET Cells

MTS assay allowed us to determine NET cell survival after infection with either rwt-VSV or M51R-VSV. Results were plotted as a percentage of mock-infected controls (Figure 2). All cell lines tested were immensely sensitive to both viruses. Moreover, there was little difference in survival between cells exposed to the wild type versus the mutated form of VSV, indicating that suppression of the host genome by intact viral M protein played little role in the sensitivity of NET cells to VSV. Both viruses were able to kill greater than 95% of cells across all cell lines and at all MOIs ($p < 0.001$ for all cell lines at each MOI). At 72 hours post-inoculation, there was no significant difference in cell viability between MOIs across all cell lines.

Mechanism of NET Susceptibility to VSV

NET cells did not produce detectable amounts of IFN after being challenged with either rwt-VSV or M51R-VSV highlighting a defect in their ability to mount an antiviral response. Interestingly, when cells were pretreated with increasing concentrations of human α -IFN and subsequently inoculated with either rwt-VSV or M51R-VSV, IFN conferred protection against viral lethality in a dose dependent manner (Figure 3). Less IFN generally afforded more protection against M51R-VSV than rwt-VSV indicating that when exogenous IFN is present, host genome suppression by intact M protein does play a role in the virulence of VSV. As expected, IFN also decreased viability of NET cells when compared to controls, yet IFN alone was unable to reproduce the decrease in viability observed with virus in the absence of exogenous IFN. Furthermore, the concentration of IFN necessary to completely protect NET cells from the oncolytic effects of VSV (range, 100 u/ml to 1,000 u/ml IFN) was much higher than levels attainable in humans.²⁰ Collectively, these results demonstrated

that NET cells are susceptible to VSV due at least in part to a defect in their ability to initiate a type I IFN mediated antiviral response.

Xenograft Response to Treatment with M51R-VSV

Within 2 weeks from injection of NET cells, greater than 90% of mice had formed palpable, growing tumors. Tumors treated with a single intratumoral injection of M51R-VSV experienced drastically blunted growth when compared to the mock-treated cohort mirroring the *in vitro* data (Figure 4). This difference became significant 5 days after treatment and increased to the conclusion of the study in H727 ($87 \pm 72\%$ vs. $2197 \pm 335\%$, $p < 0.001$) and UMC-11 ($13 \pm 59\%$ vs. $1471 \pm 324\%$, $p < 0.001$) xenografts. The difference between M51R-VSV treated and mock-treated CNDT2.5 xenografts reached significance at day 10 and also remained significant through day 28 ($81 \pm 121\%$ vs. $1576 \pm 349\%$, $p = 0.001$). Interestingly, 3 out of 13 (23%) H727, 6 of 11 (55%) UMC-11, and 2 of 11 (18%) CNDT2.5 xenografts treated with M51R were undetectable at the conclusion of the study. No mice displayed toxicity secondary to infection with M51R-VSV.

Tissue Staining

All treated xenografts stained strongly for viral G-protein 2 days after treatment with M51R-VSV indicating successful infection and spread throughout the tumors (Figure 5). H&E staining of treated xenografts harvested at the conclusion of the study revealed large areas of necrosis and fibrosis with occasional rests of residual tumor cells in contrast to mock-treated tumors (Figure 6).

Discussion

A significant portion of patients with NETs will present with advanced disease and little hope for a cure. Due to the relatively slow growth of NETs, traditional chemotherapies are ineffective. Somatostatin analogs can transiently slow disease progression but are generally used to control the symptoms of carcinoid syndrome.⁹ IFN can affect tumor response in a minority of patients but is associated with higher toxicity than somatostatin analogs.²¹ The paucity of effective treatment for NETs that escape surgical cure drives the need for new approaches to this disease.

Our results demonstrate that VSV effectively infects and kills representative NET cell lines from the lungs and small bowel. For NETs, one mechanism of susceptibility lies in the inability to produce IFN in response to infection, thereby allowing viral propagation and cell death. Similarly to NET cells, sensitive colorectal cancer cell lines were defective in their ability to produce IFN rendering them susceptible to VSV.¹³ Other investigators have shown VSV sensitivity in pancreatic cancer cells due combined defects in IFN production and responsiveness.¹⁴ Together, these findings support the theory that VSV sensitivity is derived from acquired defects in IFN signaling.^{10;11}

Compared to many chemotherapeutic agents that rely on rapid tumor cell replication, VSV replicates in the cytoplasm independently of the cell cycle and thus is appropriately suited for tumors with slow growth kinetics.²² Certainly our data confirm that VSV is able to replicate rapidly within cells and spread throughout cultures in such a manner that low doses of virus yield similar results to high doses *in vitro*. Furthermore, M51R-VSV is both safe and effective *in vivo* following a single administration and is therefore an exciting potential candidate for the treatment of human NETs.

One of the limitations of the current study, however, is that murine IFN produced in response to viral infection offers no protection to human NET xenografts as there is no IFN cross reactivity between species. It is reasonable to expect that in humans, normal cells

would produce IFN that could potentially protect adjacent NETs from oncolysis. However, our *in vitro* data suggest that the concentration of human IFN required to completely protect NET cells exceeds the levels found in normal human sera.²⁰ As VSV approaches clinical trials, it seems logical that in the case of NETs, VSV would be more efficacious alone than combined with IFN. On the other hand, administering VSV as a combined therapy with IFN could broaden the therapeutic window by further protecting normal cells from viral effects. Needless to say, accurately predicting the interplay between IFN and VSV applied to a NET in a human is beyond the scope of this study and likely only to be determined with clinical trials.

Another obstacle left unaddressed by this study is delivery of VSV to the NET. Intratumoral injection used in our *in vivo* model is hardly realistic in humans. VSV has been applied to hepatocellular carcinoma and metastatic colorectal carcinoma in rats using hepatic artery infusion with promising results.^{23;24} Hepatic artery infusion could potentially target metastatic disease from primary gastroenteropancreatic NETs. Another possibility would be to genetically incorporate a somatostatin analog onto the viral envelope as has been done to target adenoviruses to carcinoid tumors.²⁵ This adjustment would theoretically make M51R-VSV even more selective for carcinoid cells and possibly facilitate systemic administration.

In conclusion, VSV infects and kills human NETs by exploiting an inability of NETs to produce an IFN mediated antiviral response. M51R-VSV is both safe and effective *in vivo* and is therefore an excellent candidate for the treatment of advanced NETs. Furthermore, the findings described herein provide a fundamental framework for future clinical trials using oncolytic VSV as a treatment for NETs.

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Abbreviations

NET	Neuroendocrine tumor
IFN	Interferon
VSV	Vesicular stomatitis virus
M51R	Matrix protein mutant
rwt	Recombinant wild-type
GFP	Green fluorescent protein
MOI	Multiplicity of infection
MTS	3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)2H-tetrazolium
PFU	Plaque forming units
H&E	Hematoxylin and Eosin
G-protein	VSV envelope glycoprotein

Reference List

- (1). Modlin IM, Lye KD, Kidd M. A 5-decade analysis of 13,715 carcinoid tumors. *Cancer*. 2003; 97:934–959.
- (2). Yao JC, Hassan M, Phan A, et al. One hundred years after "carcinoid": epidemiology of and prognostic factors for neuroendocrine tumors in 35,825 cases in the United States. *J Clin Oncol*. 2008; 26:3063–3072. [PubMed: 18565894]
- (3). Klimstra DS, Modlin IR, Coppola D, Lloyd RV, Suster S. The pathologic classification of neuroendocrine tumors: a review of nomenclature, grading, and staging systems. *Pancreas*. 2010; 39:707–712. [PubMed: 20664470]
- (4). Oberg K. Carcinoid tumors: molecular genetics, tumor biology, and update of diagnosis and treatment. *Curr Opin Oncol*. 2002; 14:38–45. [PubMed: 11790979]
- (5). Williams ED, Sandler M. The classification of carcinoid tumours. *Lancet*. 1963; 1:238–239. [PubMed: 14000847]
- (6). Norton JA. Surgical management of carcinoid tumors: role of debulking and surgery for patients with advanced disease. *Digestion*. 1994; 55(Suppl 3):98–103.
- (7). Oberg KE. The management of neuroendocrine tumours: current and future medical therapy options. *Clin Oncol (R Coll Radiol)*. 2012; 24:282–293. [PubMed: 21907552]
- (8). Pasieka JL. Carcinoid tumors. *Surg Clin North Am*. 2009; 89:1123–1137. [PubMed: 19836488]
- (9). Paulson AS, Bergsland EK. Systemic therapy for advanced carcinoid tumors: where do we go from here? *J Natl Compr Canc Netw*. 2012; 10:785–793. [PubMed: 22679120]
- (10). Stojdl DF, Lichty B, Knowles S, et al. Exploiting tumor-specific defects in the interferon pathway with a previously unknown oncolytic virus. *Nat Med*. 2000; 6:821–825. [PubMed: 10888934]
- (11). Balachandran S, Barber GN. Vesicular stomatitis virus (VSV) therapy of tumors. *IUBMB Life*. 2000; 50:135–138. [PubMed: 11185959]
- (12). Blackham AU, Northrup SA, Willingham M, D'Agostino RB Jr, Lyles DS, Stewart JH. Variation in susceptibility of human malignant melanomas to oncolytic vesicular stomatitis virus. *Surgery*. 2013; 153:333–343.
- (13). Stewart JH, Ahmed M, Northrup SA, Willingham M, Lyles DS. Vesicular stomatitis virus as a treatment for colorectal cancer. *Cancer Gene Ther*. 2011; 18:837–849. [PubMed: 21886191]
- (14). Murphy AM, Besmer DM, Moerdyk-Schauwecker M, et al. Vesicular stomatitis virus as an oncolytic agent against pancreatic ductal adenocarcinoma. *J Virol*. 2012; 86:3073–3087. [PubMed: 22238308]
- (15). Ahmed M, Lyles DS. Identification of a consensus mutation in M protein of vesicular stomatitis virus from persistently infected cells that affects inhibition of host-directed gene expression. *Virology*. 1997; 237:378–388. [PubMed: 9356348]
- (16). Ahmed M, Marino TR, Puckett S, Kock ND, Lyles DS. Immune response in the absence of neurovirulence in mice infected with m protein mutant vesicular stomatitis virus. *J Virol*. 2008; 82:9273–9277. [PubMed: 18614644]
- (17). Ellis LM, Samuel S, Sceusi E. Varying opinions on the authenticity of a human midgut carcinoid cell line--letter. *Clin Cancer Res*. 2010; 16:5365–5366. [PubMed: 20959409]
- (18). Van Buren G, Rashid A, Yang AD, et al. The development and characterization of a human midgut carcinoid cell line. *Clin Cancer Res*. 2007; 13:4704–4712. [PubMed: 17699847]
- (19). Kopecky SA, Willingham MC, Lyles DS. Matrix protein and another viral component contribute to induction of apoptosis in cells infected with vesicular stomatitis virus. *J Virol*. 2001; 75:12169–12181. [PubMed: 11711608]
- (20). Ghyka G, Savi I. Interferon level in human normal sera. *Med Interne*. 1987; 25:43–46. [PubMed: 3035701]
- (21). Schnirer II, Yao JC, Ajani JA. Carcinoid--a comprehensive review. *Acta Oncol*. 2003; 42:672–692.
- (22). Leja J, Dzojic H, Gustafson E, Oberg K, Giandomenico V, Essand M. A novel chromogranin-A promoter-driven oncolytic adenovirus for midgut carcinoid therapy. *Clin Cancer Res*. 2007; 13:2455–2462. [PubMed: 17438105]

- (23). Shinozaki K, Ebert O, Kournioti C, Tai YS, Woo SL. Oncolysis of multifocal hepatocellular carcinoma in the rat liver by hepatic artery infusion of vesicular stomatitis virus. *Mol Ther.* 2004; 9:368–376. [PubMed: 15006603]
- (24). Shinozaki K, Ebert O, Woo SL. Treatment of multi-focal colorectal carcinoma metastatic to the liver of immune-competent and syngeneic rats by hepatic artery infusion of oncolytic vesicular stomatitis virus. *Int J Cancer.* 2005; 114:659–664. [PubMed: 15609320]
- (25). Leja J, Yu D, Nilsson B, et al. Oncolytic adenovirus modified with somatostatin motifs for selective infection of neuroendocrine tumor cells. *Gene Ther.* 2011; 18:1052–1062. [PubMed: 21490682]

Figure 1.

Viral Infectivity- GFP encoded M51R-VSV was able to infect all human NET cell lines in both a time and MOI dependent manner. Results are plotted as a percentage of gated cells expressing GFP and expressed as mean $\pm$ standard error of 3 independent experiments.

Figure 2.

Viral Lethality- MTS assays revealed that both rwt-VSV and M51R-VSV effectively killed NET cell lines regardless of inoculating dose ($p < 0.001$ at 48 and 72 hours for all cell lines at each MOI tested). Results are plotted as a percentage of mock-infected controls and expressed as mean $\pm$ standard error of three independent experiments run in quadruplicate.

Figure 3.

Effects of Exogenous γ -IFN- Using MTS assays, we determined that the presence of human γ -IFN protected NET cells against the oncolytic effects of both rwt-VSV and M51R-VSV in a dose dependent manner. Results are plotted on a logarithmic scale of IFN concentrations as mean percentage of mock-infected controls not exposed to IFN $\pm$ standard error and represent 3 independent experiments run in quadruplicate.

Figure 4.

M51R-VSV Efficacy in Xenografts- Human NETs grown as murine xenografts and treated with intratumoral injection of M51R-VSV revealed significantly suppressed tumor growth when compared to mock-treated xenografts. Data points represent mean percent change in tumor volume $\pm$ standard error.

Figure 5. Immunohistochemistry - H727, UMC-11, and CNDT 2.5 xenografts harvested 2 days following mock-treatment or treatment with M51R-VSV as indicated. Viral G-protein (stained red) was present throughout M51R-VSV treated tumors indicating efficient viral spread *in vivo*. Images were taken at 50x magnification.

Figure 6. Histology- H&E stains of H727, UMC-11, and CNDT 2.5 xenografts harvested 28 days following mock-treatment or treatment with M51R-VSV as indicated. Treatment with M51R-VSV resulted in marked tumor necrosis (N) and fibrosis (F) leaving small nests of residual tumor (RT). In contrast, large viable tumors with areas of central necrosis (CN) remained following mock treatment. Images were taken at 80x magnification.