In Vivo Estimation of Oncolytic Virus Populations within Tumors

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Abstract

The use of replication-competent viruses as oncolytic agents is rapidly expanding, with several oncolytic viruses approved for cancer therapy. As responses to therapy are highly variable, understanding the dynamics of therapy is critical for optimal application of virotherapy in practice. Although mathematical models have been developed to understand the dynamics of tumor virotherapy, a scarcity of in vivo data has made difficult parametrization of these models. To tackle this problem, we studied the in vitro and in vivo spread of two oncolytic measles viruses that induce expression of the sodium iodide symporter (NIS) in cells. NIS expression enabled infected cells to concentrate radioactive isotopes that could be reproducibly and quantitatively imaged using SPECT/CT. We observed a strong linear relationship in vitro between infectious virus particles, viral N and NIS gene expression, and radioactive isotope uptake. In vivo radioisotope uptake was highly correlated with viral N and NIS gene expression. Similar expression patterns between viral N and NIS gene expression in vitro and in vivo implied that the oncolytic virus behaved similarly in both scenarios. Significant titers of viable virus were consistently isolated from tumors explanted from mice that had been injected with oncolytic measles viruses. We observed a weaker but positive in vivo relationship between radioisotope uptake and the viable virus titer recovered from tumors; this was likely due to anisotropies in the viral distribution in vivo. These data suggest that methods that enable quantitation of in vivo anisotropies are required for continuing development of oncolytic virotherapy.

Significance: These findings address a fundamental gap in our knowledge of oncolytic virotherapy by presenting technology that gives insight into the behavior of oncolytic viruses in vivo. Cancer Res. 78(20); 5992–6000. ©2018 AACR.

Introduction

The introduction of oncolytic viruses for cancer therapy has provided a novel avenue of therapy that is showing increasingly promising results (1–3). Oncolytic viruses have been engineered to selectively infect and spread within the tumor cell population, leading to cancer cell death due to a variety of mechanisms including direct cell lysis, cell-to-cell fusion, expression of therapeutic genes or enzymes that leads to the activation of toxic molecules, and stimulation of the immune system with break-through of immune tolerance (4–8). The first proof of principle demonstrating successful therapy of a disseminated malignancy (multiple myeloma) with a single, systemic injection of an engineered oncolytic measles virus (MV-NIS) was recently reported (9). Oncolytic viruses are already approved for the therapy of head and neck cancer and malignant melanoma (10), and other indications likely will soon follow (11). Successful tumor therapy with oncolytic viruses is based on the premise that the virus gains access to the tumor and then selectively proliferates within the tumor cell population, leading to its destruction (12). However, the outcomes in animal models and human studies have been variable. Oncolytic viruses that reliably eliminate tumor cell line populations in vitro give variable results in vivo when the same cells are used to generate tumor xenografts (13, 14). It is critical to understand the dynamics of the virus within the tumor if we want to make sense of the outcome of therapy and optimize the use of these novel therapeutic agents. Mathematical models of tumor virotherapy have provided important insights into the dynamics of tumor therapy with viruses (15–21). However, given the scarcity of in vivo data available, approaches that enable the in vivo quantitation of the tumor and/or virus populations would be highly beneficial to allow for proper parameterization of such models. In this regard, strategies that enable repeated and noninvasive monitoring of the biodistribution of the oncolytic virus and reliable estimates of the in vivo virus and infected cell populations are critical for successful translation of these novel therapeutics. MV-NIS is a unique oncolytic virus in this respect as expression of the sodium iodide symporter (NIS) by infected tumor cells may enable visualization of the in vivo biodistribution of the oncolytic virus, and allow for a quantitative determination of virus population dynamics within the tumor (9, 22, 23). In this work, we report our results on inferring the oncolytic virus population within a tumor in vitro and in vivo using molecular imaging techniques. Although our approach works well both in vitro and in vivo, additional complexities in vivo likely require a combination of imaging techniques to enable an accurate estimation of virus populations.
**Materials and Methods**

**Cell lines**

All cell lines were obtained from the ATCC and maintained in their respective media at 37°C in an environment with 5% carbon dioxide. Vero cells (ATCC® CRL-1586) were grown in DMEM (Gibco) with 5% FBS. The human pancreatic adenocarcinoma cell line BxPC3 (ATCC® CRL-1667) was cultured in RPMI with 10% FBS. The ATCC guarantees the authenticity of the cell lines. The cell lines are kept in culture after thawing for a maximum of 2 months (10 passages) and after that a new vial is thawed to maintain a low passage. The cells are routinely monitored for mycoplasma infection by PCR (IDEXX BioResearch) and were consistently negative. Prior to injection in animals, all cells were washed in sterile cold PBS and suspended at a concentration of 1 x 10^6 cells/100 μL.

**Viruses**

The oncolytic virus MV-NIS has been described previously (22, 24). It is a recombinant virus based on the Edmonton vaccine strain of measles virus (MV-Edm) with the gene for the human sodium iodide symporter (NIS) inserted downstream of the viral hemagglutinin gene.

MV-eGFP-NIS was generated by digestion of plasmids p(+) MV-eGFP and p(+) MV-NIS with NotI and SacII (New England Biolabs). The smaller fragment from p(+)MV-eGFP (5712 bp) and the larger fragment from p(+)MV-NIS (16125 bp) were isolated by gel electrophoresis and ligated overnight at room temperature. The recombinant plasmid was amplified in *E. coli* (Top 10) and used to rescue the recombinant virus by transfection in 293 (Top 10) and used to rescue the recombinant virus by transfection in Vero cells at an MOI of 0.03 and cell-associated virus was harvested in Opti-MEM (Gibco) when 80% of the cells were infected. The cell-associated virus was released by freeze-thawing of the cell preparation three times in liquid nitrogen followed by centrifugation to precipitate cellular debris. The virus-containing supernatant was harvested and the viral titer was determined by serial logarithmic dilutions of the virus-containing supernatant that were used to infect Vero cells in a 96-well plate. The 50% tissue culture infective dose (TCID₅₀/mL) was determined 4 days later using the method of Spearman and Karber (26–28). Virus stocks were maintained at –80°C until used for *in vitro* or *in vivo* experiments.

**In vitro iodide uptake studies**

*In vitro* iodide uptake studies were performed as described previously (22, 29). Cell lines were infected with MV-NIS, MV-198A-NIS or MV-Edm (control) at an MOI of 0.03, 0.1, 1 and 3 and iodide uptake determined at specific time intervals by washing the infected cells with Hank’s balanced salt solution (HBSS) supplemented with HEPES (10 mmol/L), pH 7.3, and cold potassium iodide (100 μmol/L). In half of the wells, the specific NIS inhibitor potassium perchlorate (100 μmol/L) was added and, in all wells, Na⁺/K⁺ was added (activity of 1 x 10⁵ cpm/0.1 mL) and incubated for 45 minutes at 37°C. Subsequently, the cells were washed twice with ice-cold HBSS and the retained activity measured in a gamma counter (Isodata 2010 Gamma Counter, ICN Biomedicals, Inc.). Uninfected cells served as controls. All experiments were performed in triplicate and set up simultaneously. At each specific time point, triplicate samples were selected for the *in vitro* iodide uptake studies as well as viral titer and gene expression measurements.

**qRT-PCR**

RNA was extracted from control (uninfected) or infected cell lines (5 x 10⁶) using the RNeasy kit (Qiagen) as recommended by the manufacturer. RNA from tumor samples harvested from animals at the time of autopsy was isolated using the same approach although the tumor samples were immediately placed in RNAlater (Qiagen) and stored according to the manufacturer's recommendations prior to RNA isolation. Five-hundred nanograms of RNA was used for reverse transcription (RT) reaction using random hexamers and SuperScript III reverse transcriptase (Invitrogen) in a 30-μL reaction. Five microliters of the resulting cDNA was used for real-time PCR using TaqMan gene expression assay for NIS or Measles N according to the manufacturer's instructions.

For the generation of RNA for standard curve, a plasmid encoding the measles virus N gene, under control of a T7 promoter, pTM1-MV-N, was obtained from Dr. R. Cattaneo (Mayo Clinic, Rochester, MN). This plasmid was linearized by digestion with Xhol (New England Biolabs) in CutSmart Buffer for 90 minutes at 37°C in 20-μL reaction volume. Forty microliters of 100% ethanol was added to the reaction and it was placed at –20°C for a minimum of 20 minutes. The DNA was centrifuged at 14,000 rpm for 15 minutes. The supernatant was removed and the pelleted DNA was resuspended in RNase-free water. *In vitro* transcription was performed using the linearized pTM1-MV-N as a template according to the protocol for Life Technologies MEGAscript T7 *in vitro* transcription Kit (AM133). DNA was removed by addition of DNase Turbo. The generated RNA was purified using a MEGAClear kit (Life Technologies AM1908) and quantified using a Nanodrop and aliquoted to avoid multiple freeze thaw cycles. All samples were stored at –20°C and used to generate a standard curve.

qRT-PCR was performed as described previously (23) using the Roche Master Hydrolysis Probes kit (catalog no. 4991885001). Primers to detect NIS were as follows: forward primer 5’-GCCGCCGGATGTGGTGG-3’ and reverse primer 5’-CCAGTG-GGAGTGCTCTT CAGGAC-3’ and probe labeled 5’/5’-FAM/TGGGCAG-3’/5’-AGAAGCCAGGGAGAGCTACAGA-3’. The reaction was run on Roche LightCycler 480 II Real Time PCR Instrument in 96-well plates.
Results

Measles virus infection and NIS expression in vitro

Initially, we wanted to determine the relationship between oncolytic MV infection, expression of virus-encoded proteins, and iodide uptake in vitro. BxPC3 cells were infected at several multiplicities of infection (MOI of 0.03, 0.1, 1, and 3) with MV-NIS or MV-198A-NIS. Starting 24 hours after infection, iodide uptake studies were performed as described in Materials and Methods and continued daily. In parallel, cells were lysed and cell-associated virus was released by freeze-thawing of the cells in liquid nitrogen three times and virus titers were determined. Similar cell conditions were used to isolate total cellular RNA to determine virus encoded gene expression using qRT-PCR. All experimental conditions for NIS-mediated isotope uptake, viral titer, and RNA extraction (to quantitate NIS and N gene expression) were in triplicate at each time point. We determined by qRT-PCR that BxPC3 cells do not express endogenous NIS (3.15 x 10^–1 copies/ng RNA). In contrast, cells infected (MOI = 1.0) with recombinant MV that include the NIS gene expressed the reporter gene (1.17 x 10^10 copies/ng for MV-NIS and 2.19 x 10^7/ng for MV-198A-NIS, respectively, at 48 hours postinfection, P < 0.0015 for all comparisons). As can be seen from Fig. 1A, iodote uptake peaked at 48 hours after infection in the case of MV-NIS, but the peak was not reached at 120 hours with MV-198A-NIS (n = 3 per time point per condition). The peak at 48 hours for MV-NIS also coincided with the highest levels of expression for the measles virus “N” and “NIS” (Fig. 1B) gene expression (data for MV-NIS shown). The correlation between in vitro isotope uptake (μCi) and MV-mediated NIS gene expression as measured by qRT-PCR was excellent—Spearman ρ (ρ = 0.979; 95% confidence interval CI: 0.89 – 0.99; R^2 = 0.96; P < 0.0001; Fig. 1C). A high correlation between isotope uptake (activity) and viable virus as measured by TCID_{50}/ml was also observed (Fig. 1D).

We used this time-dependent data to correlate the levels of virus encoded “N” and “NIS” genes with the titer of infectious virus (TCID_{50}/ml). As can be seen from Fig. 2A, the levels of expression of both genes correlated well with the virus titer (R^2 = 0.93 and R^2 = 0.99 for “N” and “NIS,” respectively). The coefficient for “N” gene copy number as a function of virus titer (TCID_{50}/ml) was higher (0.096% 95% CI, 0.0826–0.109) compared with “NIS” (0.076% 95% CI, 0.0713–0.0802). This is expected given that the gene encoding “N” is upstream of “NIS” in the genome and therefore is transcribed at a higher level than the NIS gene (33, 34). We also correlated NIS and N gene expression by qRT-PCR at all specific time points in vitro (Fig. 2B) and established that there is a linear relationship between the two (correlation coefficient, ρ = 0.96; R^2 = 0.93; 95% CI: 0.91 – 0.99; P < 0.0001) and this relationship is independent of the MOI used to initiate infection. In addition, the linear relationship between NIS and N gene expression, as expected, showed that the level of NIS gene expression is lower than N (slope: 0.739 = 0.048; 95% CI: 0.638 – 0.841; P < 0.0001). This is in keeping with the above reported relationship between viral gene expression and titer and also compatible with the known biology of the virus (33, 34).

In vivo imaging and iodide uptake studies

A key component for reliable estimation of in vivo viral titer using molecular imaging is the need for objective and reproducible analysis of the imaging data. To address this issue, we implanted BxPC3 tumors in nude mice (see Materials and Methods) and when the tumors reached approximately 0.5 cm in diameter, the mice were injected with MV-NIS or MV-198A-NIS and imaged serially using SPECT/CT. The mice were euthanized immediately after imaging, the tumors excised and weighed and the intratumoral isotope activity determined (See Materials and Methods). The activity injected in each mouse across all days of the experiment was determined and found not to be significantly

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different across groups (median 314 μCi or 11.6 MBq, range: 10.73–12.65 MBq; P > 0.11 across all time points). The median activity retained in the syringe after injection was 3.2% or 10.06 mCi (0.37 MBq: range 0.13–0.78 MBq), across all experiments. Two independent investigators evaluated the in vivo imaging studies to determine the activity within the tumor using the region of interest (ROI) analysis approach after correction for background activity outside and inside each mouse. The data were individually correlated with the activity measured using the radiation counter. As can be seen from Fig. 2C, there was a linear relationship between the measured activity using imaging and the actual activity found in the excised tumor (r = 0.89; R² = 0.98; 95% CI: 0.81 – 0.96). As expected, the activity estimated from imaging was consistently less than the actual activity likely due to attenuation and losses due to collimation. The two independent investigators also agreed on the estimate of the intratumoral isotope activity (r1 = 0.82; R² = 0.97; 95% CI: 0.72 – 0.92 and r2 = 0.88; R² = 0.98; 95% CI: 0.77 – 0.98; P = 0.677; paired Mann–Whitney test; Fig. 2D).

In vivo imaging showed hardly any background activity in tumors not injected with an NIS-expressing virus (Fig. 3A, i) compared with mice injected with MV-NIS or MV-I98A-NIS (Fig. 3A, ii and iii). The patchy distribution of the infected foci is also evident.

We measured the isotope activity in the excised tumors and normalized it to their mass (activity/gram, μCi/g). The results were compared using the Mann–Whitney test. The median activity in the control tumors, which were not injected with the virus and therefore do not express NIS, was 0.04 μCi/g (0.01 – 0.05; Fig. 3B). This did not vary with the mass of the tumor as a function of time. In contrast, we observed variable intratumoral isotope uptake due to viral infection and NIS expression with different kinetics for the two viruses (Fig. 3C). The median activity in tumors infected with MV-NIS was 1.72 μCi/g (0.92–7.17; 0.636 MBq/g, range: 0.34–2.65 MBq/g) and 3.93 μCi/g (1.03–6.22; 1.45 MBq/g, range: 0.381–2.3 MBq/g) for mice injected with MV-I98A-NIS (Fig. 3D). Therefore, the tumors that were infected with the recombinant viruses expressed NIS and concentrated the isotope at a minimum of 7.7-fold higher compared with background. The difference between controls and infected tumors was highly statistically significant (P = 0.0009) for both viruses. This provides evidence that a radionuclide signal above background is a reliable indicator of MV infection and virus-mediated NIS expression within the tumor cell population.

We quantitated the level of isotope uptake in the tumor as a function of time after MV-NIS or MV-I98A-NIS injection. As can be seen, from Fig. 3C, isotope uptake was different for tumors infected with different viruses. MV-NIS spread rapidly and by inducing cell death, led to a rapid loss of isotope signal. In
contrast, NIS expression due to MV-I98A-NIS increased with time, suggesting spread of the infection with higher levels of NIS expression and a larger population of the virus and virus-infected cells within the tumor. As expected, tumor cells infected with MV-I98A-NIS generally had higher levels of isotope uptake, due to higher levels of NIS expression (Fig. 3D). Moreover, because the cells infected with MV-I98A-NIS remain viable for longer (25), this also likely enhances isotope uptake and retention within the tumor ($\rho_1 = 11.5, R^2 = 0.79$; $\rho_2 = 14.4, R^2 = 0.94$ for MV-NIS and MV-I98A-NIS, respectively). Therefore, radionuclide imaging is sensitive enough to detect subtle differences in the behavior of two viruses based on the same platform (MV) but with different abilities to spread from cell to cell (25) and different rates of cell killing.

**In vivo correlations**

We performed qRT-PCR for MV encoded NIS and N genes on RNA isolated from the excised tumors after in vivo imaging. N and NIS gene expression levels mirrored intratumoral activity (Fig. 4A). Moreover, at most time points the levels of expression of N were higher than NIS. We found that the correlation coefficient between NIS and N in vivo was similar to the in vitro observations with $\rho = 0.93$; $R^2 = 0.86$; 95% CI : 0.64 – 0.99; $P = 0.0009$ for MV-NIS and $\rho = 0.96$; 95% CI : 0.77 – 0.99; $R^2 = 0.92$; $P = 0.0002$ for MV-I98A-NIS. Linear fitting of NIS versus N results in a slope of 0.68 ± 0.11; 95% CI : 0.41 – 0.95; $R^2 = 0.86$; $P = 0.0009$ (Fig. 4B). The difference in the slopes of the correlations in vitro and in vivo is approximately 5% and not statistically significant ($P = 0.06$). This result suggests that viral gene expression kinetics and virus replication dynamics in vivo are similar to what was observed in vitro.

We also compared the intratumoral radioisotope activity to MV-related viral NIS and N gene expression (Fig. 4C and D). Again, high-level correlations between intratumoral radioisotope activity and viral NIS and N gene expression was found ($\rho_{NIS} = 0.9733$; 95% CI : 0.87 – 0.99; $R^2 = 0.95$; $P < 0.0001$; $\rho_{N} = 0.94$; 95% CI : 0.65 – 0.99; $R^2 = 0.89$; $P = 0.0015$). Our results therefore suggest a linear correlation between in vivo isotope uptake and viral-mediated NIS and N gene expression. In addition, we showed that both in vitro and in vivo, viral N and NIS gene expression levels correlate well with each other with a similar ratio. The levels of both genes also correlated well with the titer of viable virus in vitro. Therefore, assuming that the relationship between viral N or NIS gene expression and TCID<sub>50</sub>/mL also holds in vivo, we can use the in vivo quantitation of isotope uptake to estimate the virus population in vivo.

Finally, although our studies were not aimed to evaluate for tumor growth control, Fig. 5A shows that both oncolytic viruses...
slowed down the rate of growth of the pancreatic adenocarcinoma tumor xenografts. Except for the early time points, the size of the treated tumors was smaller than controls (two-way ANOVA $P < 0.002$ for both MV-NIS and MV-I98A-NIS compared with controls).

**In vivo isotope uptake and virus population**

Although viral gene expression such as 'N' and 'NIS' are surrogates for the presence of the virus in the tumor, we wanted to determine whether there is a linear relationship between NIS-mediated isotope uptake and the virus population within the tumors. Therefore, after pancreatic tumor xenografts were established in nude mice, they were injected with MV-NIS and after pertechnetate injection (see Materials and Methods), the mice were imaged using SPECT/CT and immediately afterwards, they were euthanized and the tumors extracted. Isotope activity in the tumors was measured immediately after using a radiation counter. Each tumor was divided into two parts and weighed. One half of the sample was used to determine viral N and NIS gene expression and the other half was used to isolate virus, which was then titrated by infection on Vero cells (see Materials and Methods).

Oncolytic virus was detectable in all mice injected with the oncolytic viruses but in none of the controls. The median titer of MV-NIS was $2.6 \times 10^4$ TCID$_{50}$/gram ($8.3 \times 10^3$–$9.2 \times 10^5$) of tumor versus no titer in the controls ($P = 0.0009$, ANOVA). Similarly, we could not detect the N and NIS gene in control tumors, but we detected high levels of virus-specific RNA expression in mice injected with the oncolytic viruses: $N_C = 1.5$ versus $N_{MV} = 76.9$; $P = 0.001$; $NIS_C = 4.2$ versus $NIS_{MV} = 50.3$; $P = 0.0078$. The higher levels of N gene expression compared with NIS is again consistent with the known biology of the virus and our prior results. We also confirmed a strong positive correlation between the intratumoral isotope activity with MV-mediated N and NIS gene expression ($r_N = 0.68$, $P = 0.007$; $r_{NIS} = 0.75$, $P = 0.003$). We subsequently correlated the intratumoral isotope concentration based on imaging with the virus titer isolated from the explanted tumors (Fig. 5B). When we take all the mice studied ($n = 42$), we found a positive correlation between isotope uptake and viable virus titer: $\rho = 0.4545$ (range: $0.1747$–$0.6665$); $P = 0.0025$. If the analysis was restricted to mice with intratumoral activity $> 1 \mu$Ci/g, ($n = 31$; to exclude a possible threshold effect), we found a similar correlation ($\rho = 0.425$; range: $0.083$–$0.6774$; $P = 0.0172$). Another analysis that excluded the high outliers...
(>10 µCi/g) with a sample size of n = 37 gave us similar results (r = 0.4662; range: 0.1674 – 0.6866; P = 0.0036). Finally, an analysis restricted to mice with an intratumoral isotope concentration >1 µCi/g and <10 µCi/g (n = 26), also led to a similar correlation (r = 0.4475; range: 0.0726 – 0.712; P = 0.0219; Fig. 5B). We note that the range for the correlation coefficient is quite large and likely due to inter sample variability, anisotropic distribution of the virus within the tumor, or the virus levels were below threshold for accurate determination of activity by SPECT/CT.

**Discussion**

Cancer therapy has seen a revolution in the last decade with the development of many novel therapeutics including small molecules, mAbs, and now oncolytic viruses that can selectively infect, replicate, and kill tumor cells. Proof of principle that an oncolytic virus can lead to long-term disease control for disseminated malignancy was also recently reported when one patient with disseminated relapsed and refractory multiple myeloma experienced meaningful tumor cytoreduction after a single dose of MV-NIS and remained in remission for almost 2 years (9). Other viruses have shown similar activity even in immunocompetent animal models of disease (35). However, tumor virotherapy depends on the complex dynamic interactions between the tumor, the virus and the immune system, and understanding these interactions is critical for their optimal use (1, 15–21, 36). Mathematical modeling can greatly assist in these optimization approaches, but an accurate estimation of the virus population as it propagates in time will likely facilitate this strategy, and in particular can identify potential reasons for therapeutic failures and methods to improve the outcome. For this reason, MV-NIS was generated, and in this work, we tested the hypothesis that NIS-mediated isotope uptake in the tumor can be used to estimate the viral load in the tumor population as a function of time.

Our results show that in vitro, MV-mediated N and NIS gene expression are tightly correlated and also correlate well with NIS-mediated isotope uptake and virus titer. This suggested that the oncolytic virus is behaving in the same way as in the in vitro environment. There is isotope uptake within tumors that is above background and this correlates well with the levels of viral N and NIS gene expression.

![Figure 4.](image-url)  
*In vivo* correlations between MV infection, isotope, and gene expression. **A**, qRT-PCT for MV "N" and "NIS" gene expression (data for MV-I98A-NIS shown) illustrate the parallel expression of the two viral genes and how it mirrors isotope uptake (c.f. Fig. 3C). **B**, NIS and N gene expression in vivo were highly correlated. **C** and **D**, similar to the *in vitro* scenario, intratumoral pertechnetate activity was well correlated with viral "NIS" and "N" gene expression.
We could also demonstrate a positive correlation between isotope uptake and the titer of the virus recovered from the tumors although there is considerable variability in the correlation between these two important variables. There are several potential explanations for these observations including (i) a threshold effect relationship between the virus titer in the tumor and the amount of isotope concentrated within the tumor and detectable by SPECT/CT imaging (31), (ii) anisotropies in the distribution of virus within the tumor that interfere with the correlation between isotope uptake and the virus population (32, 36). Although sampling variability could also contribute to this weak correlation, this is unlikely to be a reasonable explanation if enough tumors are studied. It is known that the initial distribution of the virus in the tumor is not uniform (32, 36, 37) and the tumor architecture may interfere with the spread of the oncolytic virus within a tumor (37). Our own preliminary studies in vitro using tumor cells as a monolayer versus a spheroid also show significant differences in the rate of spread of the same virus and the duration of infected cell viability (Dingli and colleagues, unpublished observations). It is possible that the 3-dimensional structure of the in vivo tumor introduces new complexities in the model and dynamics that limit our ability to infer virus population from the in vivo imaging studies.

Despite the fact that the tumors did not regress (this was not an endpoint of the study), we could show that the tumors in mice treated with MV were smaller compared with controls at each time point. Moreover, despite the MV-198A-NIS virus having slower infection kinetics (25), it controlled the tumors equally effectively between these two important variables. There are several potential explanations for these observations including (i) a threshold effect relationship between the virus titer in the tumor and the amount of isotope concentrated within the tumor and detectable by SPECT/CT imaging (31), (ii) anisotropies in the distribution of virus within the tumor that interfere with the correlation between isotope uptake and the virus population (32, 36). Although sampling variability could also contribute to this weak correlation, this is unlikely to be a reasonable explanation if enough tumors are studied. It is known that the initial distribution of the virus in the tumor is not uniform (32, 36, 37) and the tumor architecture may interfere with the spread of the oncolytic virus within a tumor (37). Our own preliminary studies in vitro using tumor cells as a monolayer versus a spheroid also show significant differences in the rate of spread of the same virus and the duration of infected cell viability (Dingli and colleagues, unpublished observations). It is possible that the 3-dimensional structure of the in vivo tumor introduces new complexities in the model and dynamics that limit our ability to infer virus population from the in vivo imaging studies.

Despite the fact that the tumors did not regress (this was not an endpoint of the study), we could show that the tumors in mice treated with MV were smaller compared with controls at each time point. Moreover, despite the MV-198A-NIS virus having slower infection kinetics (25), it controlled the tumors equally effectively in our studies. In addition, because this virus kills infected cells at a slower rate compared with MV-NIS, the infected cells retained the isotope at higher concentrations and this was detectable with SPECT/CT imaging and con

References

Figure 5.
Efficacy of MV on tumor control and in vivo correlations. A, Tumor growth was measured by serial caliper data. Untreated tumor xenografts grew the fastest. MV-NIS did slow down tumor growth, but the effect was maximal for MV-198A-NIS, showing that at least in this model, faster replication may not result in a better outcome. B, A positive correlation (p = 0.4475; P = 0.02) between viable virus isolated from excised tumors and the intratumoral isotope activity determined by imaging data from 26 mice is illustrated.

Disclosure of Potential Conflicts of Interest
No potential conflicts of interest were disclosed.

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Conception and design: D. Dingli, M.-Y. Jung
Development of methodology: D. Dingli, M.-Y. Jung, C.P. Offord
Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): D. Dingli, M.-Y. Jung, M.K. Ennis
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Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): D. Dingli

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