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Measles Edmonston Vaccine Strain Derivatives have Potent Oncolytic Activity against Osteosarcoma

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Abstract

Osteosarcoma is the most common primary bone tumor affecting children and young adults, and development of metastatic disease is associated with poor prognosis. The purpose of this study was to evaluate the antitumor efficacy of virotherapy with engineered measles virus (MV) vaccine strains in the treatment of osteosarcoma. Cell lines derived from pediatric patients with osteosarcoma (HOS, MG63, 143B, KHOS-312H, U2-OS and SJSA1) were examined for MV-GFP and MV-NIS gene expression and cytotoxicity as defined by syncytial formation, cell death, and eradication of cell monolayers: significant antitumor activity was demonstrated. Findings were correlated with *in vivo* efficacy in subcutaneous, orthotopic (tibial bone), and lung metastatic osteosarcoma xenografts treated with the MV derivative MV-NIS via the intratumoral (IT) or intravenous (IV) route. Following treatment, we observed decrease in tumor growth of subcutaneous xenografts ($p=0.0374$) and prolongation of survival in mice with orthotopic ($p<0.0001$) and pulmonary metastatic osteosarcoma tumors ($p=0.0207$). Expression of the NIS transgene in MV-NIS infected tumors allowed for SPECT-CT and PET-CT imaging of virus infected tumors *in vivo*. Our data support the translational potential of MV-based virotherapy approaches in the treatment of recurrent and metastatic osteosarcoma.

Keywords

Measles virus; MV-NIS; oncolytic virus; virotherapy; osteosarcoma

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Conflicts of Interest

The authors report no conflicts of interest.

Introduction

Osteosarcoma is the most common primary bone malignancy effecting children and young adults, and comprises roughly 5% of childhood cancers and 20% of all bone tumors. Classical osteosarcoma usually arises from the medullary cavity of the metaphyseal growth plates of developing long bones, areas of rapid bone growth and turnover in children and adolescents. Histologically, cells are characterized by the production of osteoid matrix, as well as complex chromosomal karyotypes.[1, 2]

Despite advances in combined modality treatment including chemotherapy and surgical techniques, which result in cure in 60%–75% of patients,[3] roughly one-third of young patients recur or develop metastatic disease or metastasis to the lungs. Long term survival for patients with metastatic disease is <20%.[4] There has been little improvement in survival during the last 20 years, and new therapeutic approaches are needed.

Oncolytic measles virus (MV) therapy is a novel therapeutic strategy for treatment of cancer.[5] Interest in this approach was triggered by observation of spontaneous regressions of leukemias and lymphomas in developing countries after infection with wild-type virus dating back to the 1970s.[6, 7] In contrast to wild type virus MV vaccine strains are safe.[8] They have been shown to have therapeutic efficacy in several animal models and have demonstrated early evidence of biologic efficacy in human clinical trials.[9–14] MV is a negative single-stranded RNA virus, which belongs to the family of Paramyxoviridae: the virus has 6 genes encoding 8 proteins. Once inside the host, MV interacts with cellular receptors through its surface H glycoprotein. The cell surface receptors include signaling lymphocyte-activating molecule (SLAM) predominantly found on activated B and T lymphocytes, CD46, an inhibitory complement receptor ubiquitously expressed on all human nucleated cells,[15] and Nectin-4.[16] Of note vaccine strains have been adapted to preferentially enter cells via CD46 as opposed to the SLAM receptor.[17, 18] Furthermore, CD46 and Nectin-4 are overexpressed in tumors,[19, 20] thus conferring tumor specificity. Additional advantages of this oncolytic platform include the fact that MV strains can be engineered and retargeted to different cellular receptors.[21] RNA viruses, including MV-Edm, are potent inducers of an antiviral, interferon mediated, response in normal tissue. Since many tumor cells have defects in interferon signaling, this increases oncolytic viral selectivity for tumors.[22, 23]

We hypothesized that measles virotherapy could represent a novel therapeutic direction in the treatment of refractory osteosarcoma and we tested the potency of engineered MV-strains against osteosarcoma lines and xenografts. In order to monitor viral propagation, a MV Edmonston strain encoding the sodium iodide symporter (NIS) gene was chosen for these experiments. While NIS is normally expressed on follicular thyroid cells, it can also be used as an imaging transgene to monitor viral infection. Expressed NIS co-transports two sodium ions and an iodide ion into cells, with sodium gradients driving cellular uptake.[24] This allows use of gamma camera, single photon emission computed tomography (SPECT), or positron emission tomography (PET) CT for *in vivo* monitoring of viral replication following administration of ^{123}I, 99mTc isotopes or F-18 tetrafluoroborate (F-18 TFB).[24–27].

In this study we demonstrated significant antitumor efficacy of MV derivatives (including MV-NIS and MV-GFP) against osteosarcoma lines and xenografts. Expression of NIS in MV infected cells resulted in effective tumor cell uptake of 99mTc and F-18 TFB for *in vivo* monitoring of virus infection by SPECT and PET-CT. Moreover, we demonstrated that treatment of athymic nude xenografts with MV-NIS resulted in statistically significant decrease in the growth of orthotopic tumors, and conferred a significant survival advantage in mice bearing orthotopic tibial bone or metastatic pulmonary tumors.

Materials

Cell Culture

HOS, MG63, 143B, KHOS-312H, U2OS, and SJSA1 osteosarcoma cell lines were purchased from the American Type Culture Collection (ATCC; Manassas, VA). SJSA1 cells were grown in RPMI and all other cells in DMEM supplemented with 10% FBS and 1× penicillin-streptomycin. Cells were kept at 37°C in a humidified atmosphere of 5% CO_2.

MV Strains

Construction of measles virus expressing green fluorescent protein (MV-GFP) and the sodium iodide symporter (NIS) protein (MV-NIS) has been previously described.[25, 28] The viruses were propagated in Vero cells and titrated as previously described.[26]

Determination of CD46 and Nectin-4 Expression by Flow Cytometry

Cells were grown to confluence in T75 flasks, washed with PBS, and harvested in Versene solution (Gibco). Cells were washed twice with 0.5% BSA-PBS, and incubated with FITC conjugated mouse antihuman CD46 antibody (BD Pharmingen), PE conjugated mouse antihuman Nectin-4 (R&D Systems), or the conjugated isotype control antibody for 1 h on ice. Samples were washed twice with 0.5% BSA-PBS, fixed in 0.5% paraformaldehyde and run on a Becton-Dickinson FACScan Plus cytometer, Flowingsoftware was used for .fcs file analysis.

Cell viability Assays

HOS, MG63, 143B, KHOS-312H, U2OS, and SJSA1 cells (10,000 cells per well) were seeded in a 96-well plate and infected with the indicated virus on the following day at a multiplicity of infection (MOI) of 1 and 0.1, in 50 μl of opti-MEM. On days 1 through 4 after infection, cell viability was measured using the MTS cell proliferation assay (Promega, Madison, WI), following the manufacturer recommendations.

Assessment of Viral Replication in OS Cell Lines

OS lines were plated in six-well plates at a density of 4×10^5 per well. Cells were infected at an MOI of 1, incubated at 32°C in a humidified atmosphere of 5% CO_2, and harvested at 1, 3, and 5 days after infection. The viruses were released by two cycles of freeze/thawing, and the viral titer was determined by end point dilution assay and expressed as 50% tissue culture infectious dose ($TCID_{50}$)/mL on Vero cells.

Western Blot

Cells were collected in RIPA buffer and samples loaded on 7.5% precast polyacrylamide gel (Bio-Rad) for SDS-PAGE. Gel was transferred to polyvinylidene difluoride (PVDF) membrane. Membranes were blocked (5% nonfat milk in Tris-buffered saline–Tween) and incubated with Anti-N protein antibody developed in our laboratory (publication pending, Ianko Iankov) overnight at 4 °C. Rabbit mouse-specific polyvalent immunoglobulin (G, A, M) HRP conjugate (diluted 1:2000 in 5% dry milk in PBS) was used as the secondary antibody (Pierce, Rockford, IL, USA). Anti-human β-actin was used as a control to ensure uniform loading. Antibody binding was visualized by enhanced chemiluminescence (Pierce).

Animal Experiments

143B osteosarcoma cells were transduced with lentivirus expressing firefly luciferase (143B-luc). 1×10^6 cells were then injected subcutaneously into the right flank or into the right tibial bone of 5 week old nude mice, as described elsewhere.[29] For the lung metastasis model, 1×10^6 cells were injected into the tail vein of mice and lung tumors allowed to engraft over 10 days. Mice were considered to have reached the euthanasia endpoint if more than 20% weight loss, tumor exceeding 10% of body weight, or if their tumors developed ulcerations. All experimental protocols were approved by the Mayo Clinic Institutional Animal Care and Use Committee.

In Vivo Imaging

Engraftment in tibial bone was verified 4 days post implantation, and pulmonary engraftment was verified 10 days post implantation on a Xenogen bioluminescence Imaging System. In order to assess viral replication *in vivo*, subcutaneous xenografts were treated intratumorally with MV-NIS every 4 days for two weeks. On day 14 mice were then injected with ^{99}Tc (100 μCi) intraperitoneally, and tumor imaging was performed serially with a high-resolution micro single-photon emission computed tomography (SPECT) system (X-SPECT; Gamma Medica-Ideas, Inc., Northridge, CA).

Dynamic PET imaging was performed in intravenously treated orthotopic xenografts on day 14 of MV-NIS therapy. Following intraperitoneal injection ~0.07 MBq Na [^{18}F]BF_4/g body weight of animal PET scans were acquired for 60 min followed by an X-ray scan using the GENISYS4 PET imaging system (Sofie Biosciences, CA). The images were analyzed for Standardized Uptake Value (SUV) in tumor, stomach, thyroid and bladder using AMIDE image analysis tool. Na [^{18}F]BF_4 was prepared by a modification of Jauregui-Osoro et al.[27] Isotope exchange performed in 0.5M HCl at 130°C for 10 min followed by neutralization on a Dionex OnGuard AG SPE cartridge and fluoride separation on two Waters neutral alumina SPE cartridges. The product was then sterile filtered through a 0.2 micron filter and radiochemical purity was >99% by silica gel TLC (MeOH). The specific activity was 2–5 μCi/μg.

Statistical analyses

Data were analyzed using GraphPad Prism (GraphPad Software, San Diego CA).

Results

MV-GFP and MV-NIS have potent anti-tumor efficacy against osteosarcoma cell lines *in vitro*

We used two trackable oncolytic MV-Edm derivatives in our *in vitro* studies (Figure 1A): MV-GFP expressing the green fluorescence protein and MV-NIS. A panel of six osteosarcoma cell lines were studied, and all of them expressed high levels of the MV receptor, CD46 (Figure 1B). Cell lines were also analyzed for expression of Nectin-4, the epithelial receptor for Measles Virus, and no significant expression was detected (data not shown).

Following infection with MV-GFP at an MOI of 1 there was efficient killing of 4 of 6 osteosarcoma cell line monolayers (U2OS, HOS, KHOS-312H, and SJSA1 cells) within 48–72 hours (Figure 2A). MV-NIS had superior *in vitro* efficacy as compared to MV-GFP, with activity against all six lines, including the MG63 and 143B cells (Figure 2B). Because of the transcriptional gradient during MV replication[30] the different transgene positions within the MV genome (position 1 for MV-GFP vs position 6 for MV-NIS) could explain the difference in cytopathic effect we observed between the two strains.

Though the pulmonary metastatic 143B cell line was intermediately susceptible to MV-GFP oncolysis, infection with MV-GFP led to abundant green fluorescent protein (GFP) expression. Green fluorescence increased over the course of infection, and syncytia grew in size and number ultimately leading to eradication of the monolayer. MV nucleoprotein (N-protein) expression was verified by western blot, and N-protein expression increased over the first 3 days of infection (Fig 3A and 3B). Relative resistance to infection of 143B cells to MV-GFP could be overcome by increasing the multiplicity of infection (not shown). In one step viral growth curves both strains resulted in replication, although higher titers of MV-NIS versus MV-GFP were obtained (Fig 4).

Characterization of 143B-luc flank, orthotopic, and metastatic osteosarcoma xenograft models

To investigate the therapeutic potential of MV-NIS treatment in the recurrent or pulmonary metastatic setting the aggressive and highly metastatic 143B cell line was chosen for xenograft development. 143B cells were transduced by a lentivirus expressing firefly luciferase, to generate 143B-luc cells. We injected 100μL of 143B-luc tumor cells (1×10^6) into the right flank, right tibial bone, or intravenously into the tail vein of 5 week old mice to establish xenografts. Following verification of engraftment mice were then treated either with MV-NIS or heat-inactivated control virus every 4 days for a total of 4 weeks (Figure 5). Subcutaneous and orthotopic tibial tumors could be detected by luciferin bioluminescence within 4 days of implantation. Lung engraftment was similarly verified at 10 days post intravenous implantation (Supplemental Figure).

MV-NIS has potent antitumor activity in mouse xenografts

Flank tumors were generated, and were treated every four days with intratumoral injections of 1×10^6 $TCID_{50}$ MV-GFP, MV-NIS, or heat-inactivated control virus (n=5 per group), and

tumor size was measured with digital calipers. A decrease in tumor growth was observed (Figure 6) with MV-GFP (p=0.0407) and MV-NIS treatment (p=0.0374).

In the orthotopic model, tibial bone tumors grew rapidly in heat-inactivated virus treated animals (n=10) and in some cases tumors were associated with lytic destruction of the underlying tibial bone (Figure 7B and 7C). Intravenous treatment with MV-NIS (n=10) led to a significant decrease in the growth of orthotopic leg tumors, p=0.0014; data shown as mean ± SE (Figure 7A). After two weeks of MV therapy, there was clear evidence of syncytial formation with nuclear coalescence and positive staining for the viral N-protein (Figure 7D) in MV treated tumors. Metastatic tumor foci were often noted post mortem: the majority of metastases were involving the lungs with metastatic involvement of appendicular and axial bony skeleton also being common. Mice with pulmonary tumors generally had to be euthanized due to cachexia, lethargy, hunched posture, and in some instances cord compression and hind limb paralysis secondary to metastatic involvement of the vertebral spine.

We also assessed survival following MV-treatment both in tibial orthotopic and pulmonary metastatic osteosarcoma xenografts. Xenografts treated every 4 days with MV-NIS (n=10) at a dose of 1×10^6 $TCID_{50}$ had a statistically significant prolongation of survival compared to mice treated with heat-inactivated virus (n=10) both in the orthotopic tibial bone model (p<0.0001, Fig 8A) and the pulmonary metastatic model (p=0.0207, Fig 8B). In the orthotopic model, median survival of the treated mice was 60 days compared to 30 days for control animals. In fact, all MV-NIS treated mice with orthotopic tibial xenografts were alive on day 40, as compared to none of the mice in the control group.

MV-NIS infects osteosarcoma xenografts and NIS transgene expression can be monitored *in vivo*

In a separate study in order to assess viral replication *in vivo*, mice were treated with MV-NIS every 4 days for two weeks either directly into flank tumors or intravenously in established orthotopic xenografts. Monitoring of viral infection and replication *in vivo* was then performed using two different imaging modalities. In the subcutaneous flank tumor model MV-NIS treated mice (intratumoral injection) were administered Tc-99m into the peritoneum (IP) on day 14 and then imaged 30 minutes later by CT-SPECT. Intratumoral accumulation of Tc-99m in subcutaneous flank tumors was seen in mice treated with MV-NIS (Figure 9A), but not in animals treated with the inactive virus preparation (Figure 9A, lower panel). Cross-sectional imaging demonstrated MV-NIS replication in tumors: expression of NIS by infected tumor cells resulted in Tc-99m concentration, which we could detect by SPECT imaging.

We next evaluated the ability of intravenous MV-NIS therapy to result in the intratumoral concentration of the radioactive tracer [^{18}F] tetrafluoroborate (F-18 TFB), through NIS mediated transport. PET-CT imaging performed 60 minutes after IP administration F-18 TFB resulted in significant uptake in tibial tumors (Figure 9B) and thereby convincingly demonstrated viral replication and associated NIS gene expression in osteosarcoma tumors following systemic viral administration.

Discussion

Osteosarcoma is an aggressive bone cancer with peak incidence in childhood, adolescence, and early adulthood. While surgical advancements and combination chemotherapy have led to a 65–70% cure rate, no additional improvement in survival has materialized during the last two decades: new therapeutic approaches are urgently needed for this young patient population.

Oncolytic measles virotherapy provides a novel and safe therapeutic strategy for treatment of recurrent and metastatic osteosarcoma. The fact that viruses have adapted over millennia of evolution to efficiently invade cells and overtake the biosynthetic machinery, makes them attractive candidates for the development of novel antitumor approaches. Other groups have used replication-competent oncolytic viruses in the treatment of osteosarcoma mostly focusing on *in vitro* efficacy studies and loco-regional *in vivo* delivery. Oncolytic Semliki forest virus was previously shown to have *in vitro* activity, and intratumoral injections in an orthotopic K7M3 tumor model showed tumor regression and survival benefit. Poliovirus targets the cell surface receptor CD155, and was found to induce apoptosis through induction of caspases 7 and 3 in bone and soft tissue sarcoma cells [31]. Vesicular stomatitis virus delivered by isolated limb perfusion has similarly been shown to suppress osteosarcoma growth in an immune competent rat model of osteosarcoma [29]. Our study represents the first report that demonstrates efficacy of an engineered MV strain in treatment of bone sarcomas, following both intratumoral and systemic administration in challenging to treat orthotopic and lung metastatic models.

In this study, we have demonstrated that MV infects osteosarcoma cell lines and that replication of MV is efficient, with evidence of viral replication to high titers in osteosarcoma cells. Indeed, intravenous delivery of MV-NIS resulted in a decrease in growth of 143B orthotopic tumors and prolongation of survival of tumor bearing mice. We have also shown that SPECT or PET-CT allow for efficient tracking of infected osteosarcoma cells *in vivo*. We showed that intratumoral and intravenous therapy can both be monitored *in vivo*, and that SPECT and PET-CT imaging are sensitive enough to differentiate infected tumor cell uptake. The ability to monitor viral replication and transgene expression *in vivo* also allows for pharmacodynamic measurements of viral distribution and dissemination, and various radioactive substrates can be used to quantify NIS gene expression including perchlorate, tetrafluoroborate, and pertechnetate. Recently reported clinical data support that SPECT-CT can be used to monitor viral replication in patients, for example, myeloma tumor deposits following systemic administration[32]. NIS may also be used as a therapeutic transgene capable of further increasing the oncolytic potency of MV-NIS by allowing the intracellular concentration of radioisotopes, such as the beta particle emitter, ^{131}I as a mediator of radiovirotherapy.[33]

MV has also been shown to directly activate the immune system in immune competent pre-clinical models. The viral hemagglutinin has been shown to interact with and induce toll-like receptor-2 (TLR-2) signaling, and TLR-7 and TLR-9 have also been shown to be involved in viral nucleic acid detection and anti-viral signaling.[34, 35] Similarly, in pre-clinical studies measles virus vaccine-infected tumor cells co-cultured with plasmacytoid dendritic cells

(pDC) induce maturation of dendritic cells into efficient antigen presenting cells, with upregulation of costimulatory molecules CD40 and CD86.[36] The anti-tumor activity of DCs depend on IFN-α mediated autocrine stimulation, and IFN-α can also directly stimulate apoptosis in tumor cells.[37] IFN-α induction by 2-methoxyestradiol in osteosarcoma cells has similarly been shown to have anti-proliferative effects *in vitro*.[38]. MV-Edm derivatives have also been engineered to express immunostimulatory cytokines including GM-CSF (MV-GM-CSF) and IFNβ (MV-IFNβ)[39, 40], both important immune regulators that have been shown to induce antitumor immune responses in several tumor types.[41, 42] Work in our laboratory has also demonstrated that MV transgene expression of the immunomodulatory *Helicobacter pylori* neutrophil-activating protein (NAP) induces a brisk Th1 cytokine response *in vivo*, associated with high levels of TNF-α production as well as prolongation of survival in an aggressive model of lung metastatic breast cancer.[43] These engineered strains could also have excellent applicability in the treatment of osteosarcoma as they could bridge oncolytic virotherapy with sarcoma immunotherapy, and they are currently in preclinical investigation.

MV has an acknowledged record of safety in large-scale immunization campaigns; however, widespread immunization in the Western world can also pose a major challenge to oncolytic measles virotherapy approaches. While delivery challenges are difficult to address in immunocompromised xenograft models, several techniques have been used to augment treatment efficacy. Rapid clearance of virus can take place shortly after the virus is introduced into an immunized host and represents a potential limitation for systemic administration approaches. Strategies for augmenting potency and ensuring safety of virotherapy include viral genetic manipulations, as already described, concurrent chemotherapy, and immunomodulatory use of cyclophosphamide as has been demonstrated for MV-NIS in primate models.[44] Intravenous delivery of high doses of MV in patients lacking anti-MV neutralizing antibodies has been successfully employed against refractory multiple myeloma.[32] However, in the presence of neutralizing titers of anti-measles antibodies infected cell carriers have been used efficaciously to deliver MV to tumor cells;[45] the infected cell carriers can circumvent and increase efficacy in the setting of pre-existing anti-measles humoral immunity. This concept has been demonstrated by our group employing dendritic cells in breast cancer xenografts[11] and mesenchymal stem cell (MSC) carriers in passively immunized mice bearing ovarian xenografts.[46] More recently, human bone marrow-derived mesenchymal stromal cell (BM-MSC) carriers were similarly used in passively immunized mice to efficiently deliver MV in a systemic xenograft model of precursor B-lineage-ALL.[47]

In summary, our results demonstrate that MV-NIS exhibits significant therapeutic effect against human osteosarcoma cell lines *in vitro* and orthotopic and metastatic disease models *in vivo*. MV could provide a new therapeutic addition to multimodality treatment of patients with recurrent or metastatic osteosarcoma, especially given the lack of cross-resistance with existing therapies and continuing improvements in the production of high titer therapeutic viral preparations. Promising emerging data in other tumor types such as ovarian cancer[10] and multiple myeloma[32] further highlight this potential. Based on these encouraging results,

this approach has significant translational potential, and further preclinical and clinical studies are warranted.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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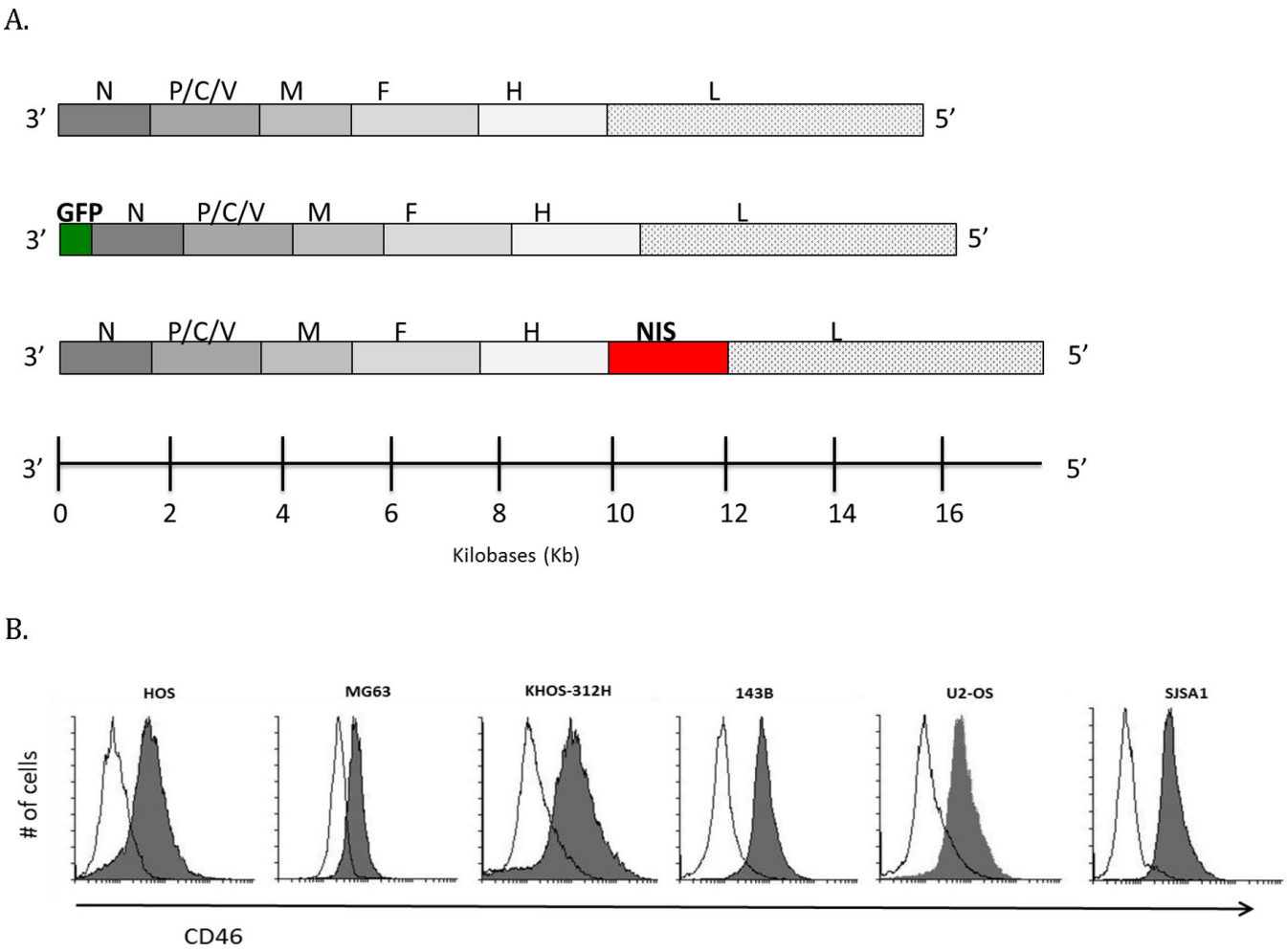
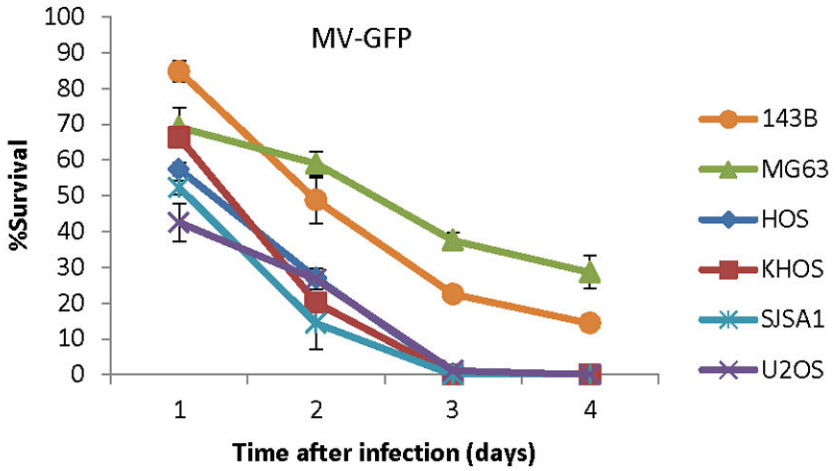


Figure 1.

Virus schema and Measles virus CD46 receptor expression. (A) Genomic organization of MV-Edm derivatives, MV-GFP and MV-NIS. N, nucleoprotein; P, phosphoprotein; M, matrix protein; F, fusion protein; H, hemagglutinin; L, large protein; GFP, green fluorescent protein; NIS, sodium iodide symporter. (B) osteosarcoma cell lines were tested for the MV receptors, CD46 and Nectin-4, by flow cytometry. Solid line histograms represent isotype control staining, while CD46 staining is represented by shaded histograms. CD46 expression was detectable in all cell lines tested.

A.



B.

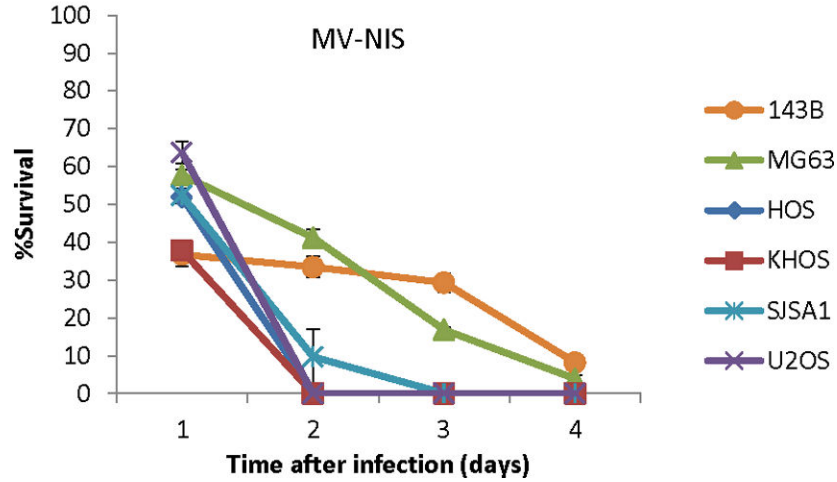


Figure 2.

Osteosarcoma cell lines are susceptible to MV infection. (A) Significant cytopathic effect observed against different osteosarcoma cell lines following MV-GFP infection at an MOI of 1 (n=3 independent experiments). (B) MV-NIS infection at an MOI of 1 similarly led to significant cell death (n=3 independent experiments). The MV-NIS cytopathic effect peaked earlier in the majority of cell lines, likely reflecting the impact of different transgene position on viral replication.

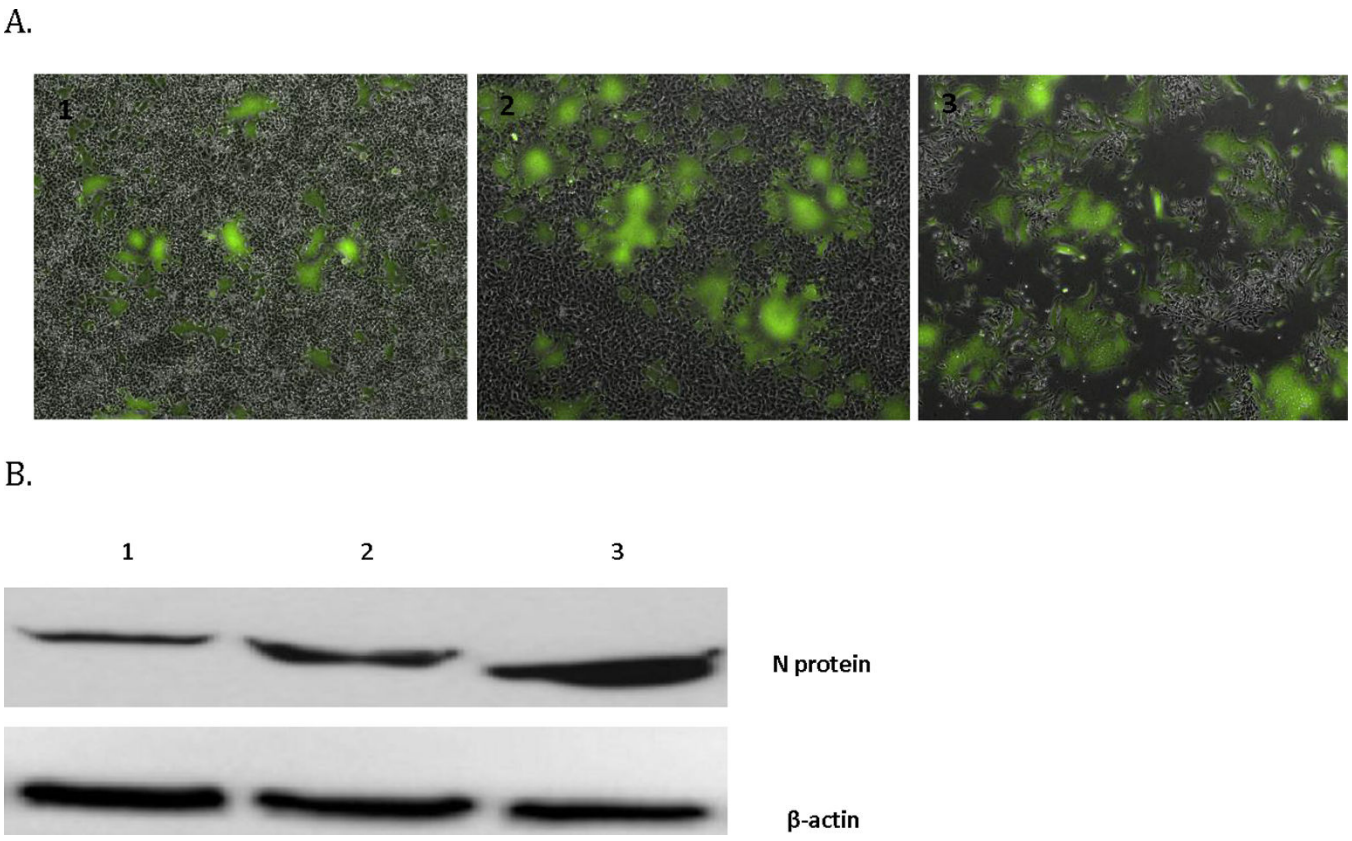


Figure 3.

MV-GFP infection kinetics in the moderately susceptible 143B sarcoma cell line. MV-GFP infection leads to (A) GFP expression is increased over time until monolayer obliteration. Images A1, 2, 3 were taken on days 1, 2, and 3 respectively following infection (4 × magnification). (B) Increased expression of measles N protein during the same time course.

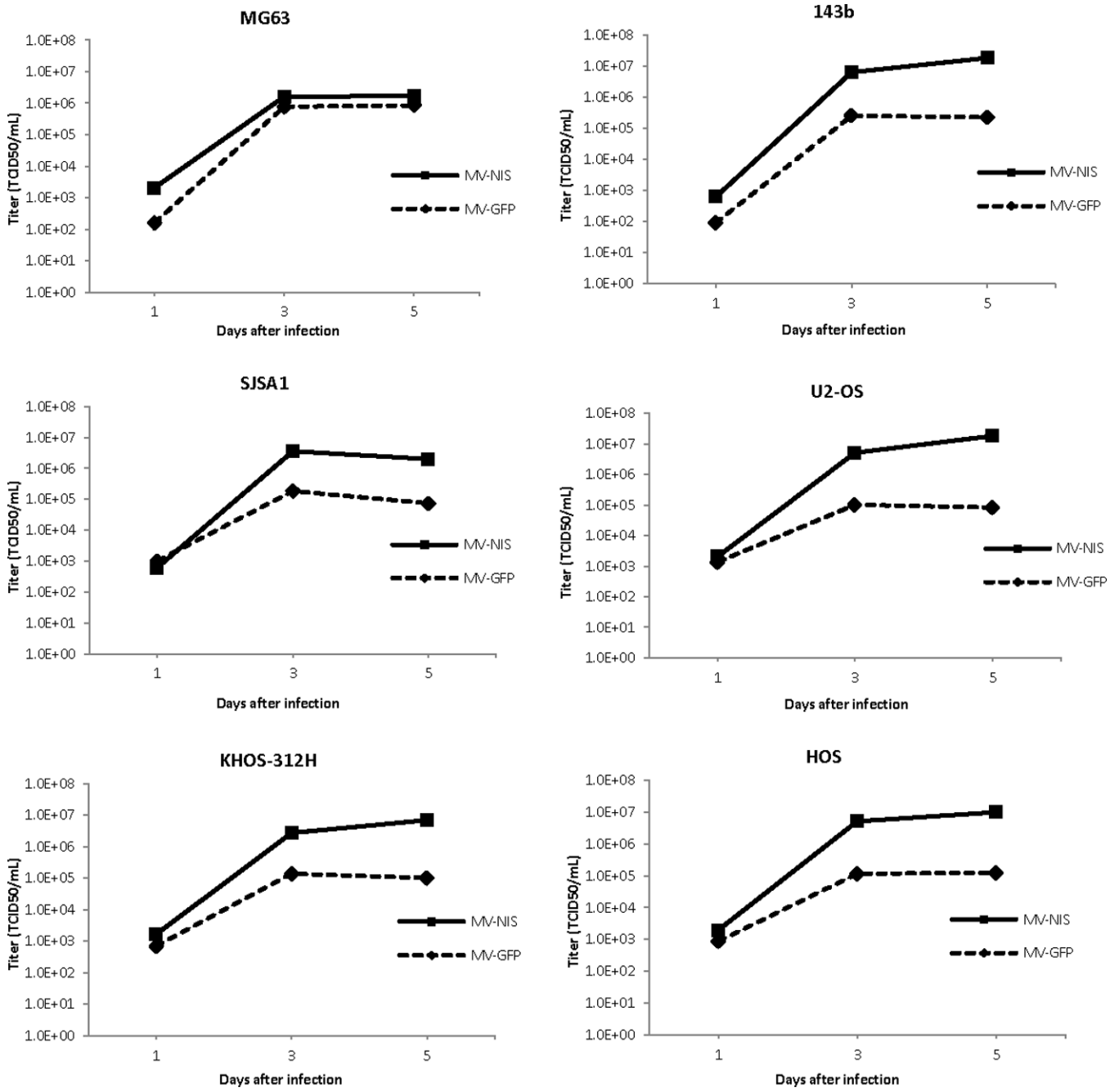


Figure 4.

MV-GFP and MV-NIS replicate efficiently in tumor lines, as demonstrated by one-step viral growth curves. Increased replication of MV-NIS as compared to MV-GFP was seen in the panel of 6 osteosarcoma cell lines.

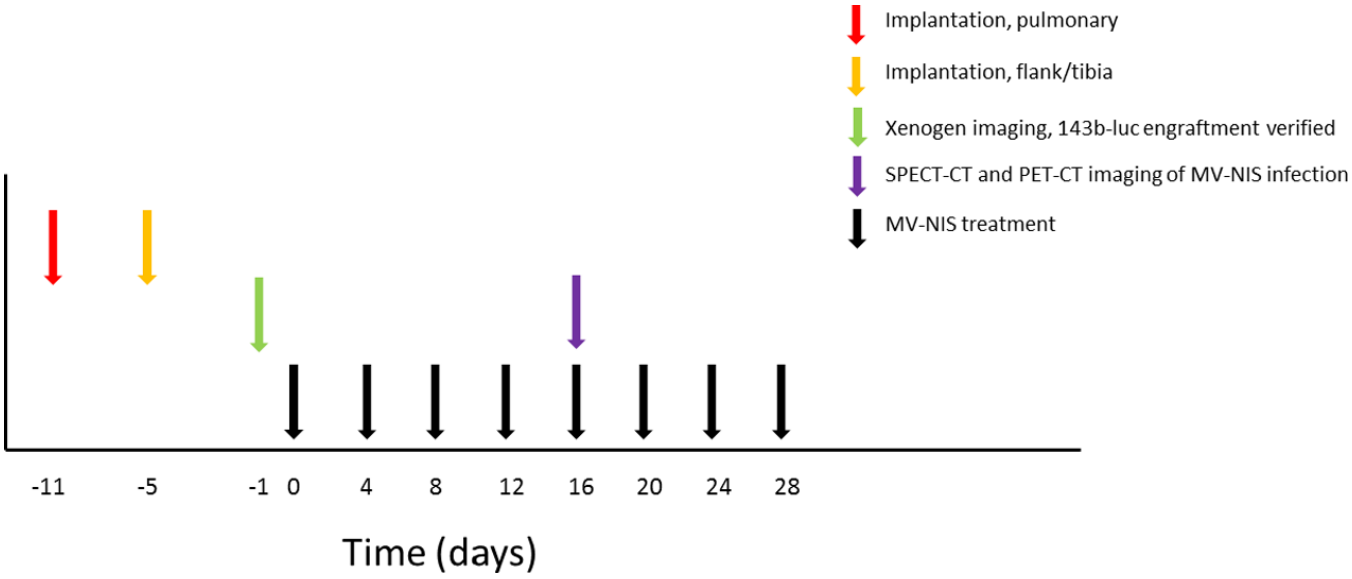


Figure 5.

MV-NIS treatment schema. 143B-luc osteosarcoma cells were implanted into the right flank, right tibial bone, or via tail vein injection (lung metastasis model). On the day prior to the MV-NIS treatment initiation, engraftment was confirmed by Xenogen bioluminescence imaging. Mice were then randomized to receive intravenous MV-NIS treatment or heat-inactivated virus every 4 days for a total of 4 weeks. Two weeks after the initiation of therapy, mice were also imaged by SPECT-CT or PET-CT to monitor *in vivo* viral activity.

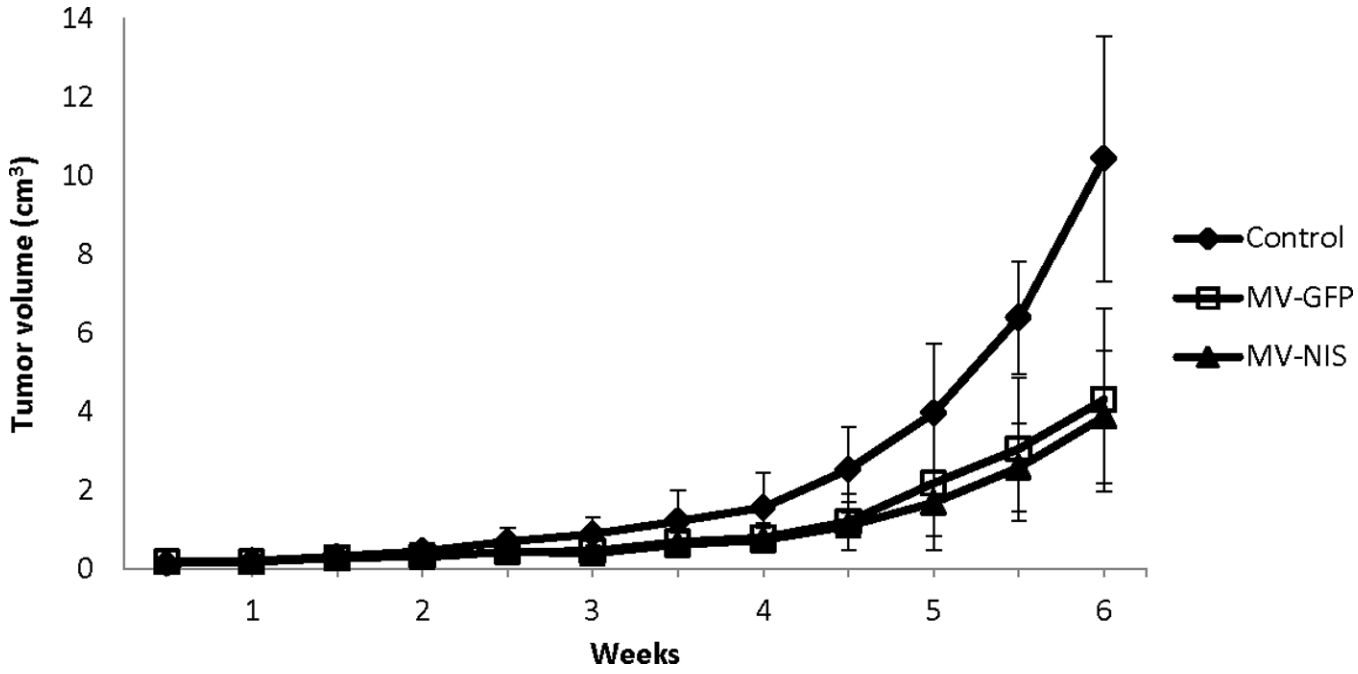


Figure 6.

Flank tumor growth was suppressed by intratumoral MV-NIS therapy. OS flank xenografts were treated every four days with intratumoral injections of 1×10^6 $TCID_{50}$ MV-GFP, MV-NIS, or heat-inactivated control virus (n=5 per group), which led to suppression of tumor growth both in MV-GFP (p=0.0407) and MV-NIS (p=0.0374) treated mice.

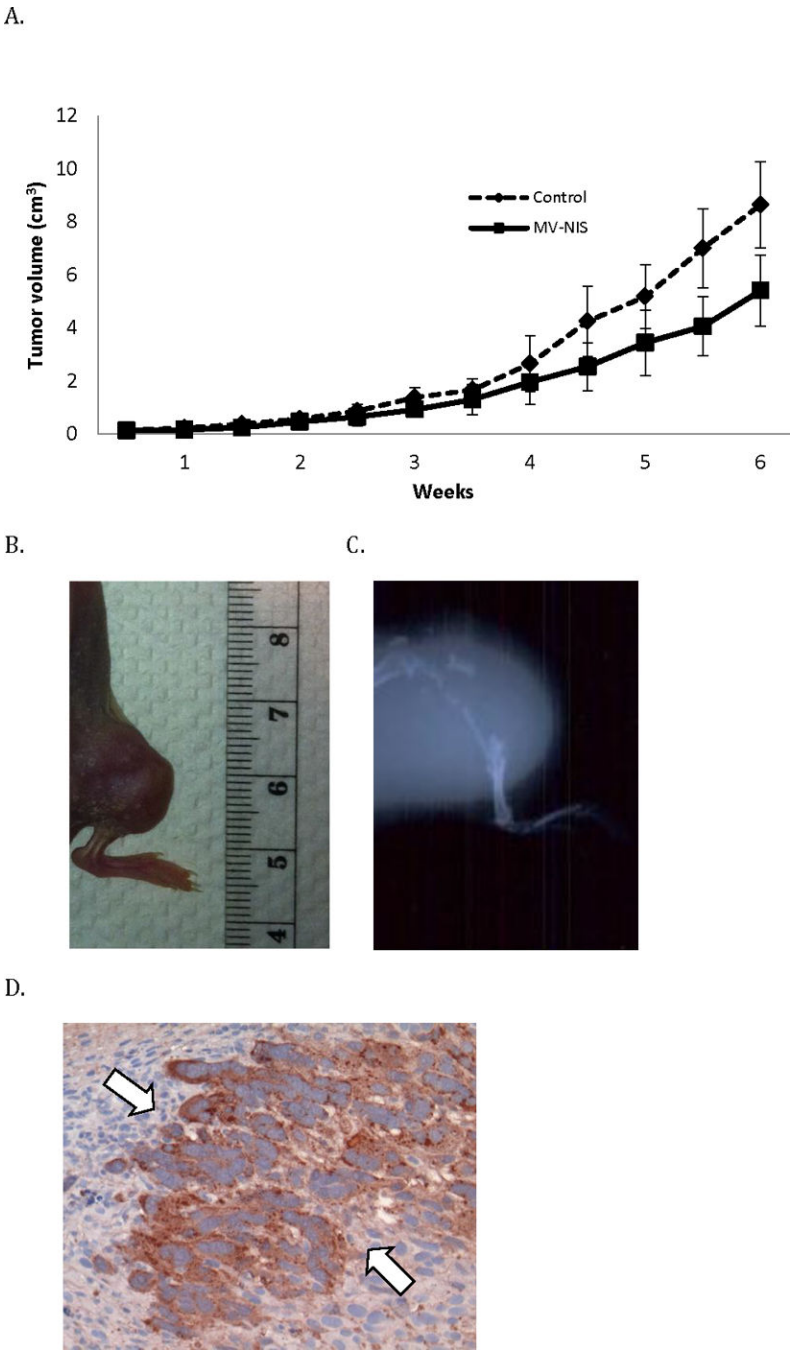


Figure 7.

Systemic MV-NIS therapy shows antitumor activity in orthotopic xenografts. Orthotopic tumors were treated every four days with IV injections of 1×10^6 $TCID_{50}$ MV-NIS or heat-inactivated control virus (n=10 per group). (A) MV-NIS treatment suppressed the the growth of leg tumors compared to controls (p=0.0014), data are shown as mean ± SE, n = 10 per group. A representative photograph of (B) an orthotopic tibial tumor two weeks after implantation and (C) representative radiographic image of an orthotopic leg tumor 6 weeks after engraftment is shown. (D) After two weeks of MV-NIS therapy excised orthotopic

tibial tumors show evidence of syncytia formation and expression of Measles N-protein by immunohistochemistry.

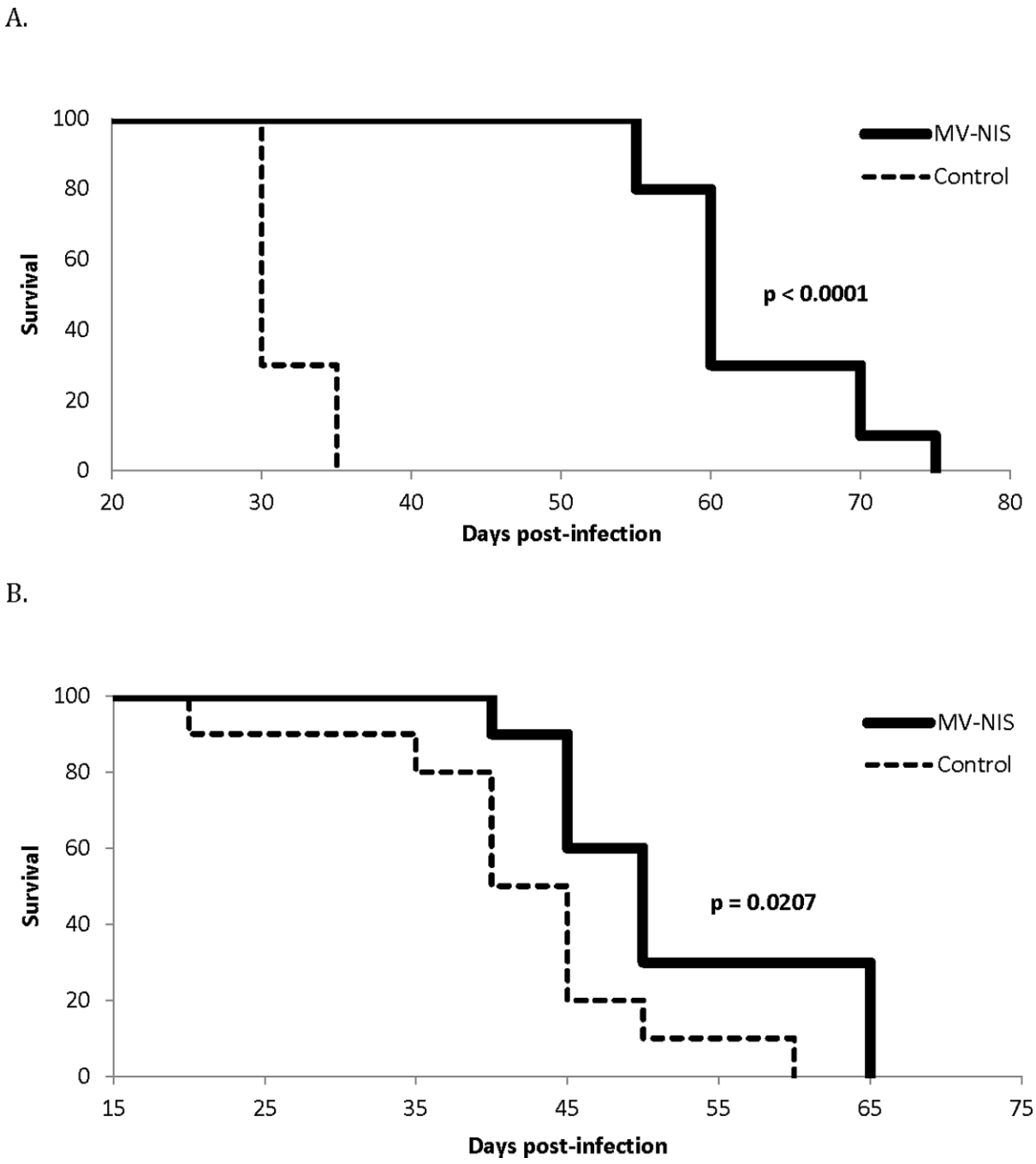


Figure 8.

MV-NIS therapy increases survival in OS xenograft models. MV-NIS treatment was associated with a significant increase in overall survival in (A) the tibial orthotopic ($p<0.0001$) and (B) pulmonary metastatic ($p=0.0207$) models as shown by Kaplan Meier curves comparing control to MV-NIS treatment twice weekly for 4 weeks, n = 10 per treatment group.

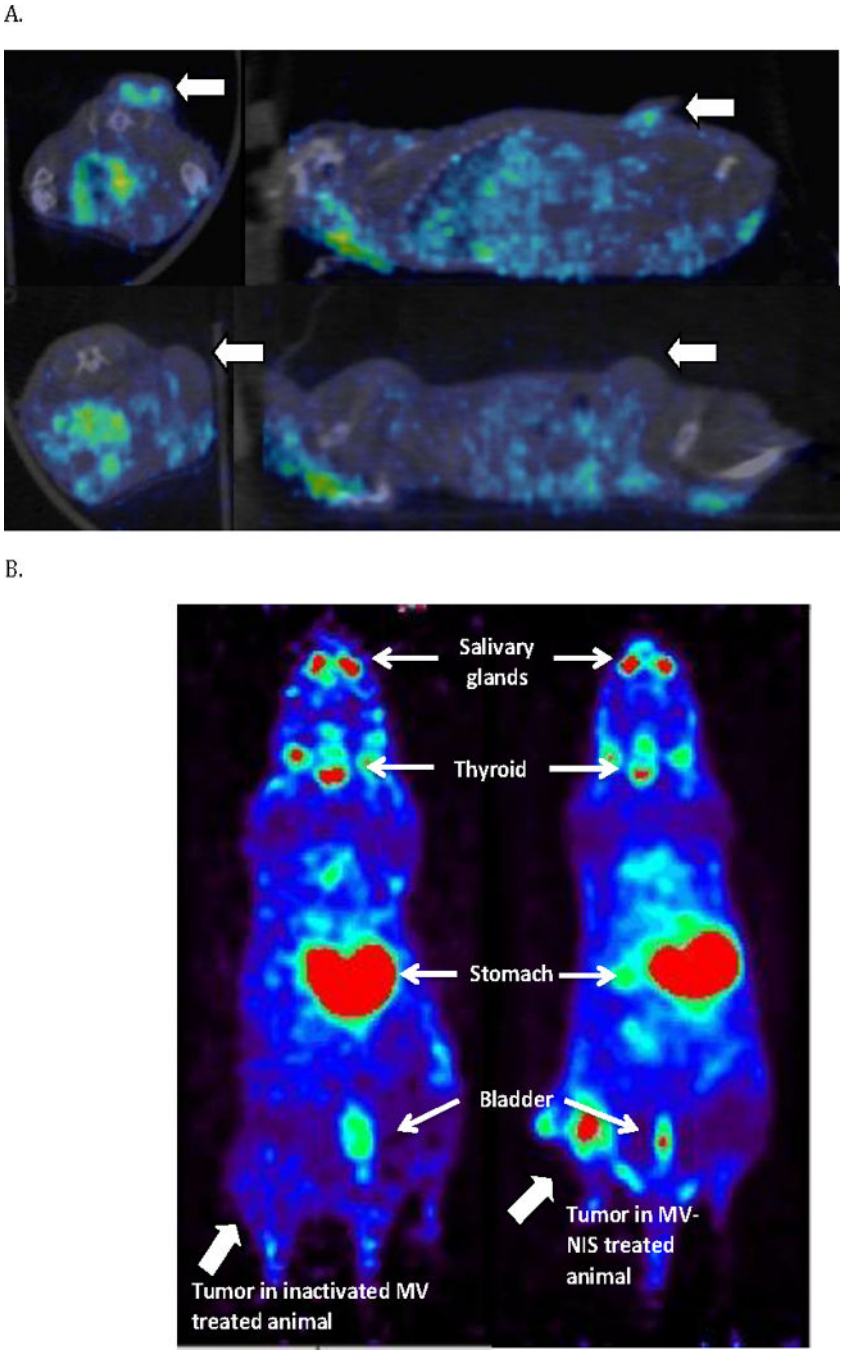


Figure 9.

MV-NIS treatment allows real-time *in vivo* imaging of viral replication. After two weeks of MV-NIS therapy OS xenografts were imaged by CT-SPECT or PET-CT. (A) Flank xenografts treated every four days for two weeks with intratumoral injections of 1×10^6 $TCID_{50}$ were imaged by CT-SPECT. Representative image shows uptake of Tc-99m in flank tumors (large arrows) of MV-NIS treated (9A, upper panel), but not inactivated virus treated animals (9A, lower panel). (B) Orthotopic tibial xenografts were treated intravenously every four days for two weeks with 1×10^6 $TCID_{50}$ MV-NIS. They were

subsequently imaged by PET-CT after F-18 TFB administration. In contrast to MV-NIS treated animals (9B, right panel), no significant uptake was seen in control virus treated mice (9B, left panel).