SUPPLEMENTARY DATA

MATERIALS AND METHODS

Investigational agent

Recombinant oncolytic vesicular stomatitis virus (VSV) expressing human interferon-beta (IFNβ) and human sodium iodide symporter (NIS) was constructed and rescued as previously described.[16] Clinical grade VSV-hIFNβ-NIS was manufactured by the Mayo Clinic Viral Vector Production Labs in Rochester, MN. Briefly, low passage viral stock (rescued VSV-hIFNβ-NIS virus) was subjected to three rounds of limiting dilution subcloning and amplified in a suspension human HEK293 cell line under GMP conditions. Final product was tested for sterility, endotoxin contamination, and functional human IFNβ and NIS gene expression. The wild-type VSV Indiana serotype (VSIV) used in *in vitro* experiments was the San Juan laboratory strain.[4] VSV titers were determined by titration on baby hamster kidney (BHK-21) cells.

Procedures

Cells. BHK-21 [C-13] cells (ATCC® CCL-10™) were used to compare growth kinetics of VSV strains and quantify VSV titer. Fetal porcine kidney cells (FPKC) were derived and cultured locally as previously described.[25] BHK-21 and FPKC cell lines were used to compare replication, IFNβ expression, and cytotoxicity by plaque assay of VSV strains.

Pig inoculation, monitoring, and sample collection. Swine—specifically eight male, approximately 10 week old Yorkshire pigs ($\sim$30kg in weight)—were included in the study. All pigs were seronegative against VSV as indicated by results of serum neutralization tests and were housed in biosafety level 3 isolation units at the Plum Island Animal Disease Center. Pigs were divided into two groups, an inoculated group and contact group, of four pigs each. Inoculation was performed on pigs in the inoculated group by pricking each pig's snout 20 times using a dual-tip skin test applicator (Lincoln Diagnostics, Decatur, IL), and 1×10^7 $TCID_{50}$ (50% tissue culture infective dose) of VSV-hIFNβ-NIS was applied to the scarified area in 100 μL of Dulbecco's modified Eagle's medium. Pigs were monitored for clinical symptoms of infection, including fever by measurement of rectal temperature and appearance of lesions on snout, mouth, lips and hooves. Personal protective equipment was worn during all animal procedures including inoculation and sample collection. Oral, nasal, and rectal swabs were collected in 2 mL minimal essential medium for infectious virus isolation, RNA extraction, and analysis by quantitative real-time reverse transcription polymerase chain reaction (qRT-PCR) RT-PCR (qRT-PCR) at indicated time points following inoculation (Table 2). Whole blood for RNA extraction and qRT-PCR analysis was collected in EDTA-containing Vacutainer tubes or in serum tubes without anticoagulant (Vacutainer).

Assays

Detection of infectious virus and virus RNA. Oral, nasal, and rectal swabs and serum samples were tested to detect presence of infectious virus following clarification by centrifugation, serial dilution of supernatant, and overlay on susceptible BHK-21 cells in 96-well plates and calculation of 50% tissue culture infective dose ($TCID^{50}$) using the Spearman-Karber equation. RNA was isolated from oral, nasal, and rectal swabs; whole blood; and postmortem tissues using an Ambion's MagMax-96 Viral RNA Isolation Kit (Ambion, Austin TX) on King Fisher-96 Magnetic Particle Processor (Thermo Scientific Waltham, MA) as previously described[21]. Samples were analyzed using qRT-PCR to detect VSV-N gene copy number as previously described[22] using the primers N1082F 5-CGGAGGATTGACGACTAATGC-3 and N1148R 5-TCAAACCATCCGAGCCATTC-3 and the probe 5-CCGCCACAAGGCAGAGATGT
GGT-3.

Detection of neutralizing antibodies. Presence of neutralizing antibodies in VSV-hIFNβ-NIS inoculated or contact pigs were monitored in serum samples collected at indicated time points (Table 2). Serum samples were collected using Vacutainer tubes. Serum was subject to complement inactivation by incubation at 56°C for 30 minutes. Serum was diluted and pre-incubated with 500 $TCID_{50}$ VSV-hIFNβ-NIS and the virus/serum mix

was added to BHK-21 cells. Cells were incubated at 37°C and assessed 48 h later. Anti-VSV antibody titer indicates the maximum serum that protected BHK-21 cells from *in vitro* VSV infection.

In vitro studies. *In vitro* assessment of VSV-hIFNβ-NIS or VSIV replication was assessed following infection of BHK-21 or porcine kidney FPKC cells following infection at a multiplicity of infection of 0.1. Cell supernatant was collected at indicated time points to quantify virus titer in $TCID_{50}$ by overlay on BHK-21 cells. Supernatant was also tested to measure human IFNβ expression quantified using the Verikine human interferon-beta ELISA kit (PBL Assay Science, Piscataway NJ). Cytotoxicity of VSV strains was assessed by standard plaque assay on BHK-21 and FPKC cells. Cells were incubated for 48 h at 37°C and stained with crystal violet.

Gene expression studies. FPKC cells were infected with oncolytic VSV-hIFNβ-NIS or VSIV at an MOI of 10. At 5 h postinfection, RNA was isolated from infected cells and gene expression quantification was assessed by qRT-PCR. Values are represented as relative quantities (RQ) of mRNA accumulation (estimated by 2-$\Delta\Delta$ct) with their corresponding standard deviation.