Supplementary Data

Supplementary Materials and Methods

A rapid dose-escalation study in purpose-bred Beagle dogs was performed to identify the adverse event profile, no adverse event level, and the MTD after systemic delivery of a novel oncolytic virus, VSV-hIFNβ-NIS. Briefly, selected dogs were treated with a single IV dose of VSV-hIFNβ-NIS at 10-fold dose escalations. Dogs were monitored for 30–45 days for adverse events, along with serial biologic sampling to monitor physiological changes, routes and duration of viral shedding, and antiviral immunity in response to viral administration.

Shedding studies

A summary of methods employed to evaluate virus shedding in biologic samples are described below.

Urine. Urine was collected sterilely by cystocentesis or urinary tract catheterization in a sterile falcon tube. Urine was briefly centrifuged, and urine cell pellet was collected in and resuspended in 1 ml Qiazol (Qiagen). Samples were stored at −80°C, batched, and processed for analysis. Samples were homogenized using the QiaShredder columns, and RNA was isolated using the Qiagen RNeasy Plus Universal kit.

Buccal swab. Buccal swabs were collected using a C.E.P swab (Fitzco), suspended in 1 ml Qiazol, and stored at −80°C. Thawed samples were centrifuged for 1 min and homogenized on a Qiashredder column, and RNA was isolated using the RNeasy Plus Universal kit.

Feces. Feces were collected during evacuation, batched, and stored at −80°C. About 5 g of feces was resuspended in sterile PBS and vortexed for 2 min. Samples were centrifuged, and the supernatant was filtered through a 70 μm cell strainer (BD Falcon) and subsequently through a 0.45 μm disposable filter disc (Whatman). RNA was isolated using the Qiagen Viral RNA Mini kit.

Blood. Whole blood was collected in RNAprotect animal blood tubes (Qiagen), and RNA was isolated using the Qiagen RNeasy animal blood system. Additional blood was collected in a BD Vacutainer PST tube for isolation of plasma. Viral RNA was isolated from plasma using the Viral RNA mini kit.

Quantitative reverse transcription–polymerase chain reaction analysis. About 25 μl quantitative reverse transcription–polymerase chain reaction (qRT-PCR) was prepared containing 300 n*M* VSV-N forward primer (5′-TGATAGT ACCGGAGGATTGACGAC-3′), 200 n*M* dual-labeled probe (5′-FAM-TCGACCACATCTCTGCCTTGTGGCGGTGCA-BHQ-3′), and 300 n*M* VSV-N reverse primer (5′-CCTTGCAGTGAC ATGACTGCTCTT-3′); nuclease free water; and RNA template. In addition, a synthetic internal positive control was spiked into each reaction to prevent false-negative results. Where possible, tissue RNA samples were diluted to 0.2 μg per reaction. qRT-PCR was carried out using the Lightcycler 480 RNA Master Hydrolysis Probes Kit (Roche). Fluorescence was measured at the annealing–extension step on a Roche Lightcycler 480 multiplex quantitative RT-PCR system (Roche) and quantified on a standard curve. All samples and standards were run in triplicate.

Infectious virus recovery assay. Samples were collected and frozen immediately for detection of infectious virus. Briefly, urine cell pellets, buccal swabs, plasma, and isolated PBMCs were all thawed at room temperature, subject to a single round of freeze–thaw, and centrifuged, and the supernatant was overlayed on susceptible BHK cells to detect infectious virus.

Viral gene expression and immune response

Viral gene expression and antiviral immune response were evaluated in serum and plasma samples isolated at various time points after IV virus administration in dogs.

Viral gene expression. Human IFNβ was measured in dog plasma samples using the Verikine HS enzyme-linked immunosorbent assay (ELISA) against human IFNβ (PBL InterferonSource).

Antibody neutralization assay. Neutralizing antibodies were detected in isolated serum samples. Briefly, serum samples were incubated at 56°C to inactivate complement. Twofold serial dilutions of serum were prepared starting at 1/5 dilution. Serum dilutions were preincubated with 500 $TCID_{50}$ VSV-GFP for 1 hr at 37°C. Vero cells were added to the serum + virus mix, 1.5×10^4 cells/well, with 3 wells plated for each sample. CPE in wells were recorded at 48 hr later. The minimum virus dilution that did not protect Vero cells from virus-induced CPE in 2/3 or 3/3 wells was recorded as the neutralizing antibody titer.

Serum immunoglobulin. Specific antiviral canine immunoglobulins IgG and IgM in serum were measured by ELISA. Briefly, 96-well plates were coated with VSV virus diluted in coating buffer and were incubated overnight at 4°C. Plates were washed and blocked with 1× casein diluted in distilled water. Plates were washed, and dog serum samples diluted in PBS were applied, and incubated for 1 hr at room temperature, followed by an additional wash step. Secondary antibody (either goat anticanine IgM diluted 1:2,000, or sheep anticanine IgG diluted 1:5,000) was plated and incubated for 1 hr at room temperature. Plates were washed and the substrate was added to each well. Color development was stopped after 5–15 min and the plates were read at 450 nm. Immunoglobulin values were quantified as arbitrary units expressed relative to baseline (pretreatment) values.

Investigational agent

The agent under investigation is an engineered recombinant VSV that was previously generated and characterized (Naik *et al.*, 2012b).