Evaluation of Seneca Valley Virus Levels Mouse to Man

EMEA/IHC Meeting on Virus Shedding
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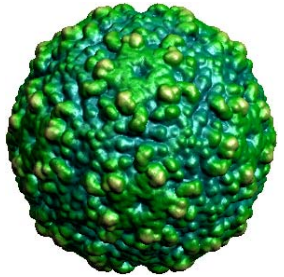
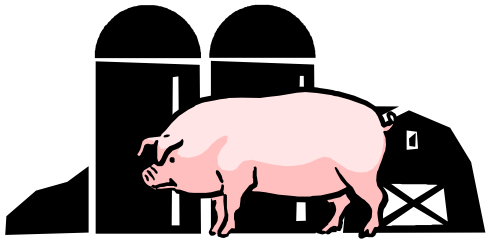
Introduction

- Brief background of Seneca Valley Virus (SVV)
- Assays used to detect SVV in vivo.
- Kinetics/Biodistribution in Mice
- Kinetics/Shedding in Patients treated with SVV in Phase I Clinical trial
- Other assays used to detect nature of viral signal in vivo
- Lessons learned

Discovery of Seneca Valley Virus (SVV-001)

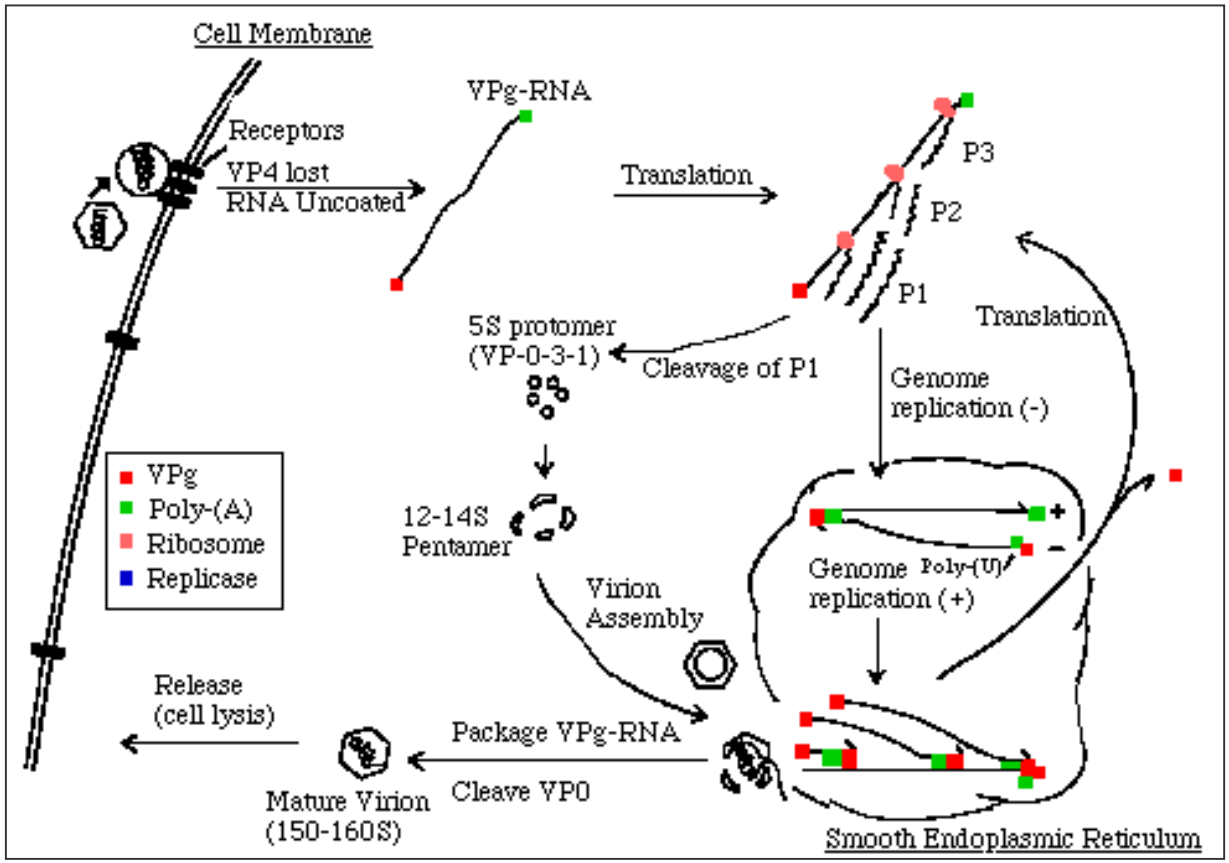
- Discovered serendipitously as a contaminant in an adenoviral vector preparation
  - Fetal Bovine Serum/Swine Trypsin
- Broad species tropism; A Natural host is swine
- In picornaviridae family; possibly new genus
- Selective tropism for tumors with neuroendocrine properties
- Not linked to any specific disease





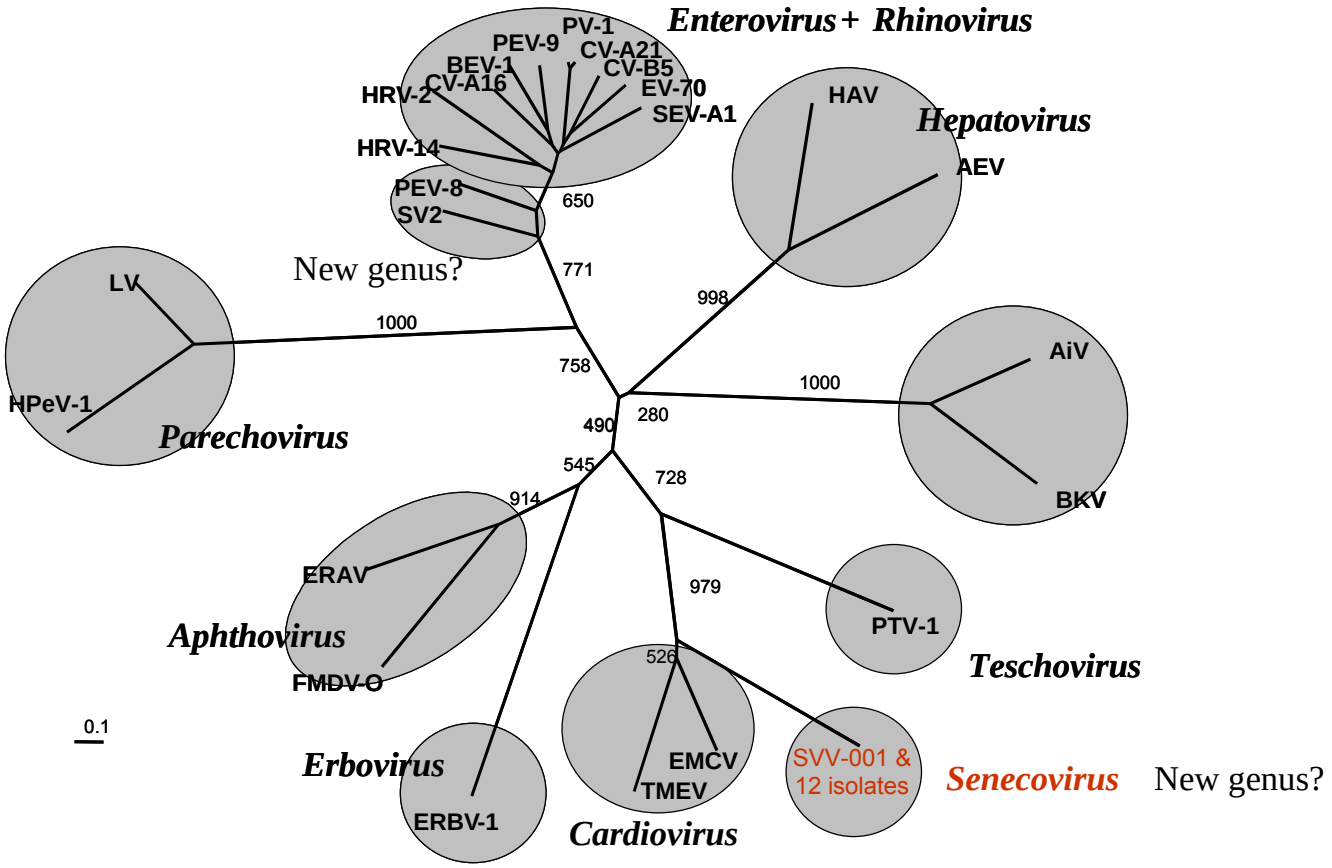
Life Cycle


Cloverleaf
Structural Region
Non-Structural Region
P1
P2
P3
3′ NTR
VP2
VP3
VP1
2A
2B
2C
3A
3C
3D
VPg
VP4
VPg
AAAn

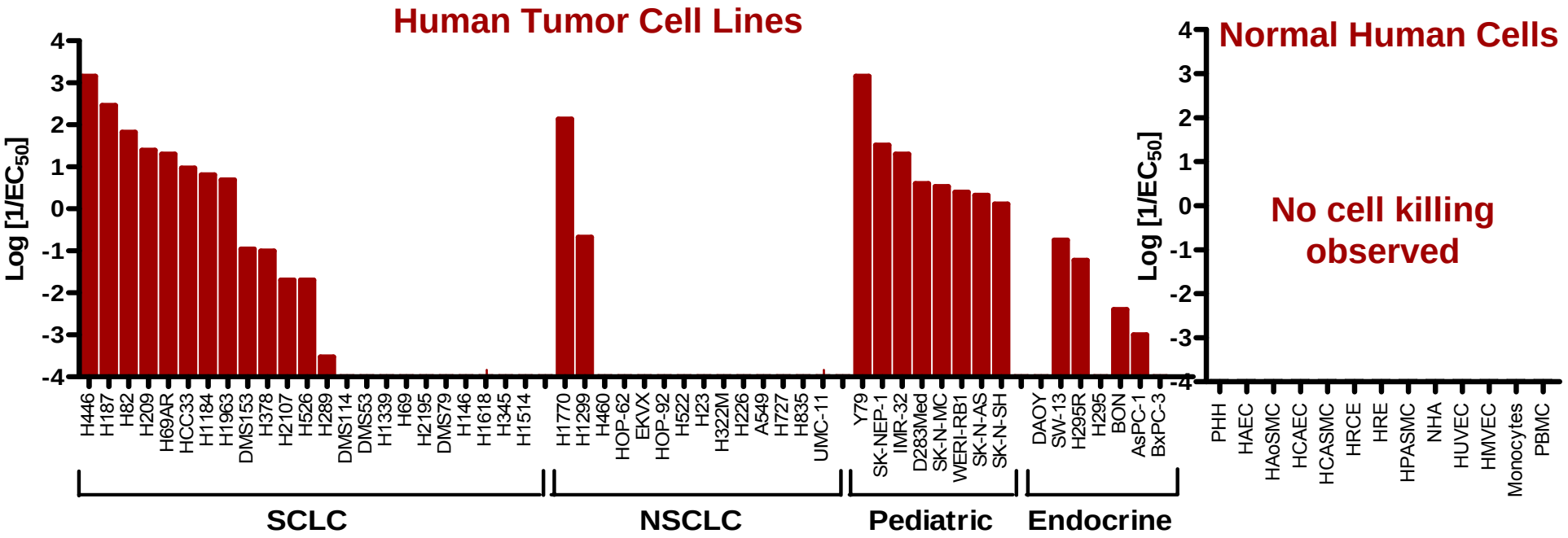

Cell Membrane
VPg-RNA
Receptors
VP4 lost
RNA Uncoated
Translation
P3
P2
P1
5S protomer
(VP-0-3-1)
Cleavage of P1
Translation
Genome
replication (-)
VPg
Poly-(A)
Ribosome
Replicase
12-14S
Pentamer
Virion
Assembly
Genome Poly-(U)
replication (+)
Release
(cell lysis)
Package VPg-RNA
Cleave VP0
Mature Virion
(150-160S)
Smooth Endoplasmic Reticulum

Phylogeny of SVV-001

- Sequence comparisons of P1, 2C, 3C & 3D and unique features indicate a likely new genus called Senecovirus



Selectivity of NTX-010 for Human Cancers

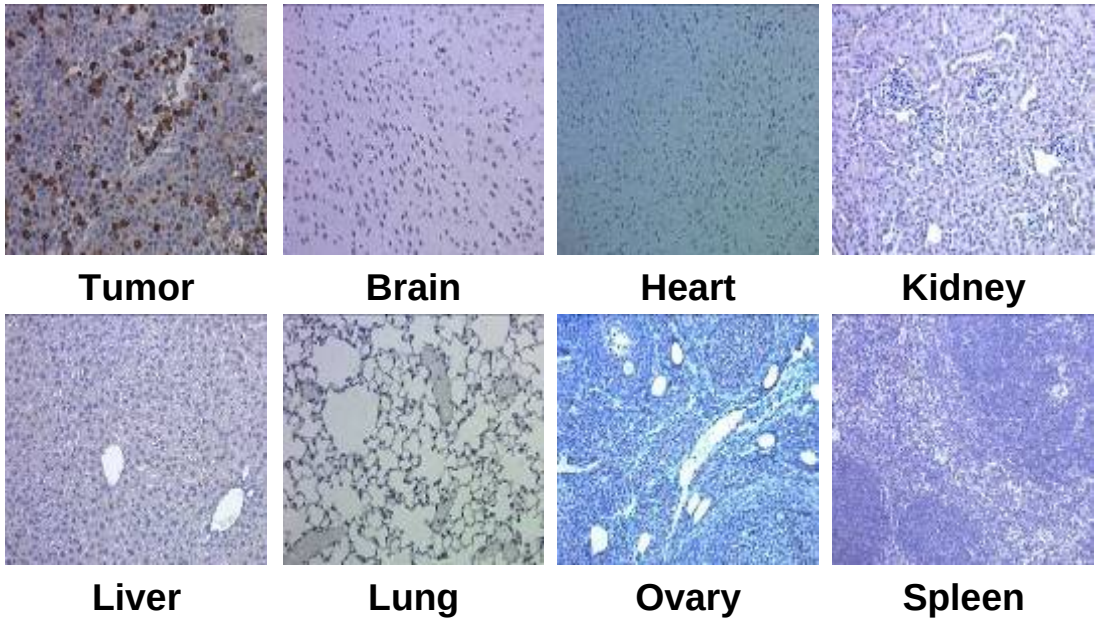


- Potent and highly selective cell killing in human tumor cells
  - 50% of tested SCLC are permissive
  - 100% of tested pediatric cancers permissive
- No cell killing or viral replication observed on normal human cells
- ≥10,000 fold selectivity in cell killing of highly permissive tumor vs. normal cells

Minimal Toxicity in Pre-Clinical Models

Murine Model

- No dose-limiting toxicity up to 10^{14} vp/kg
- No microscopic pathology at 10^{14} vp/kg
- Tumor-specific replication observed



Porcine Model

- Up to 3 x 10^{11} vp/kg
- No dose-limiting toxicity
- No infectious virus detected in tissues 7 days after dosing

Non-human Primate Model

- Up to 5 x 10^{11} vp/kg
- No dose-limiting toxicity

NTX-010 Efficacy in Tumor Models

- **Anti-tumor activity in 13/13 models tested**
  - Xenograft
  - Syngeneic (immunocompetent)
  - Orthotopic
  - Metastatic

- **NTX and 3 collaborators**
  - St. Jude/NCI (Pediatric Preclinical Testing Program)
    - Pediatric models shown to predict clinical activity
    - Durable complete responses in 3/3 models tested
  - Baylor College of Medicine
    - Eradication of metastases in orthotopic retinoblastoma
  - Johns Hopkins
    - Primary SCLC model

Measuring Virus Levels in Vivo

- Broad based
  - Tissues, serum, plasma, whole blood
  - Excreta samples
    - Stool, urine, serum, sputum, nasal swabs
- Sensitivity
- Reproducibility
- Linearity
- Practicality
- Collection
- Storage
- Shipping
- Stability
- What assay tells you

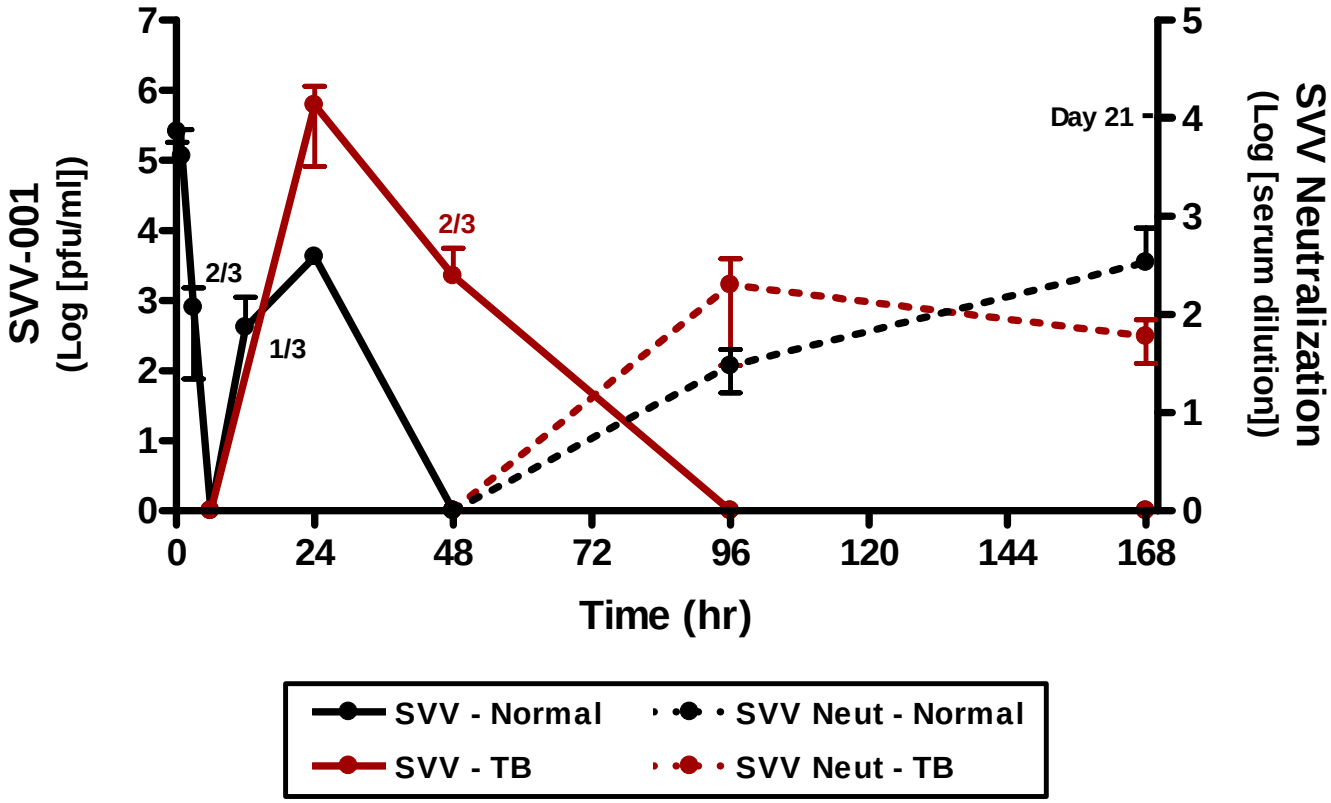
Assays

- Real Time PCR
  - Quantitatively measures small piece of viral genome
    - Sensitive assay-measures maximum signal
    - Detects virus in the presence of some functional inhibitors
      - Example-Antibodies
    - High throughput
    - High reproducibility
    - High specificity
    - Does not measure infectious virus-only measures small piece of genome
    - Subject to contamination and false positives
    - Subject to degradation
  - Specific considerations necessary depending upon sample type
    - Example-Stool
      - PCR inhibitors in stool requiring sample dilution
- Infectious virus
  - TCID50
    - Measures infectious virus
    - Two logs less sensitive then RT-PCR assay
    - May be inhibited or inactivated by numerous cmpds
      - Example-antibodies
    - Labor intensive and expensive
    - Low throughput
    - Lower specificity
  - Specific considerations still necessary depending upon sample type
    - All samples filtered due to sterility issues

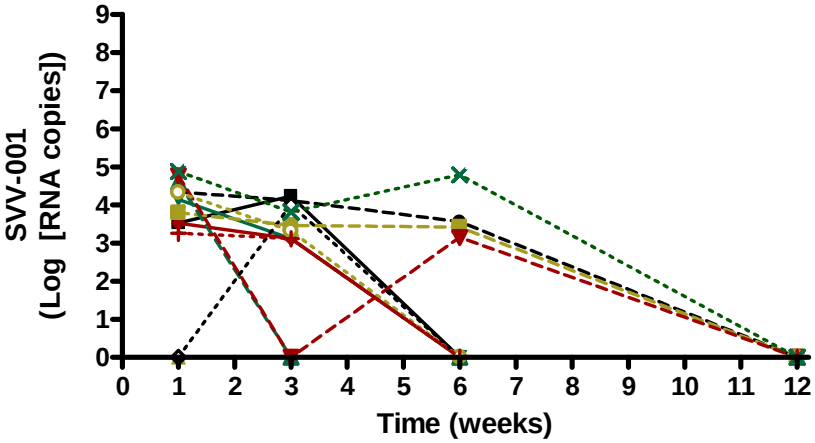
Kinetics/Biodistribution of SVV-001 RNA in A/J Mice

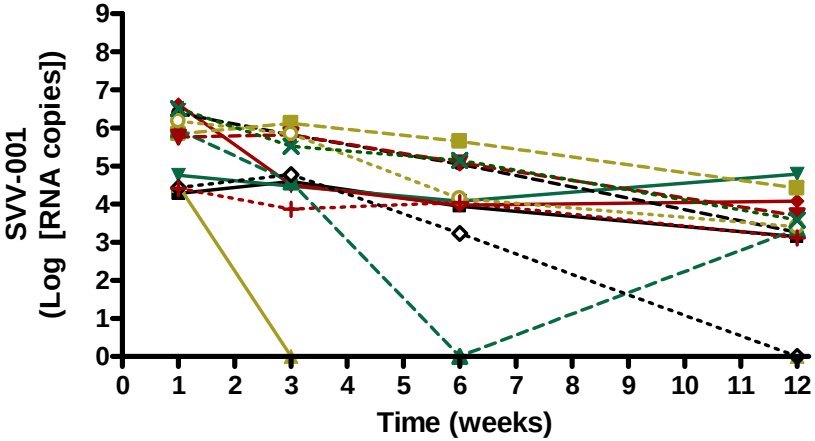
- Kinetics in serum and tissues in mice +/- tumors
- Single IV injection
  - Doses analyzed
    - 10E9, 3 x 10E11, 10E14 vp/kg
  - Multiple tissues collected
    - Over 2 weeks, 3, 6 and 12 weeks
- Assays
  - Genomic RNA by quantitative real-time RT-PCR
  - Infectious virus by TCID50
  - Neutralizing antibodies

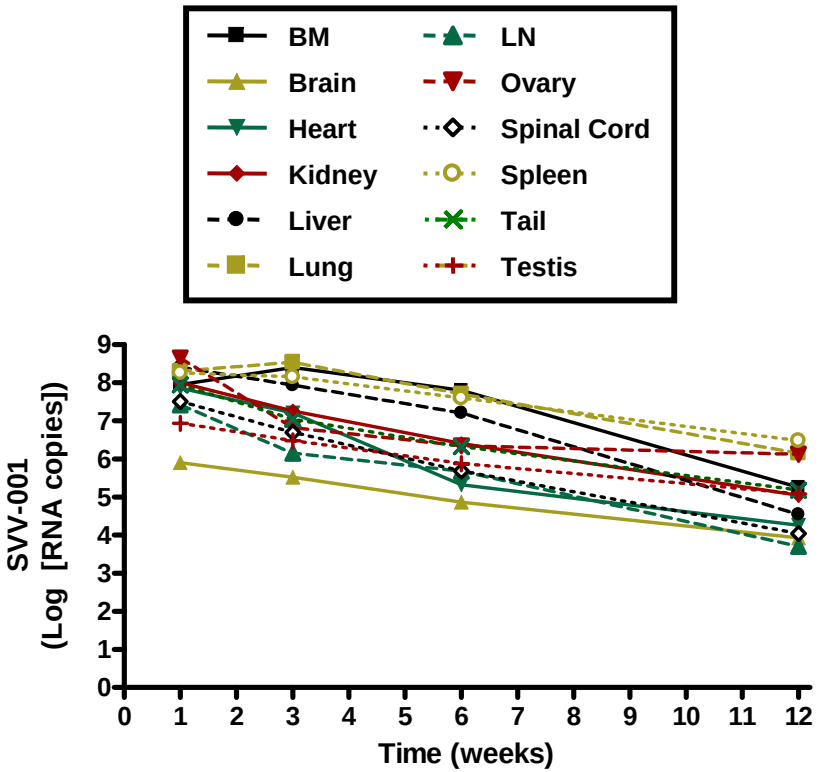
SVV Kinetics in A/J tissues



Biodistribution of SVV-001 RNA in A/J Mice





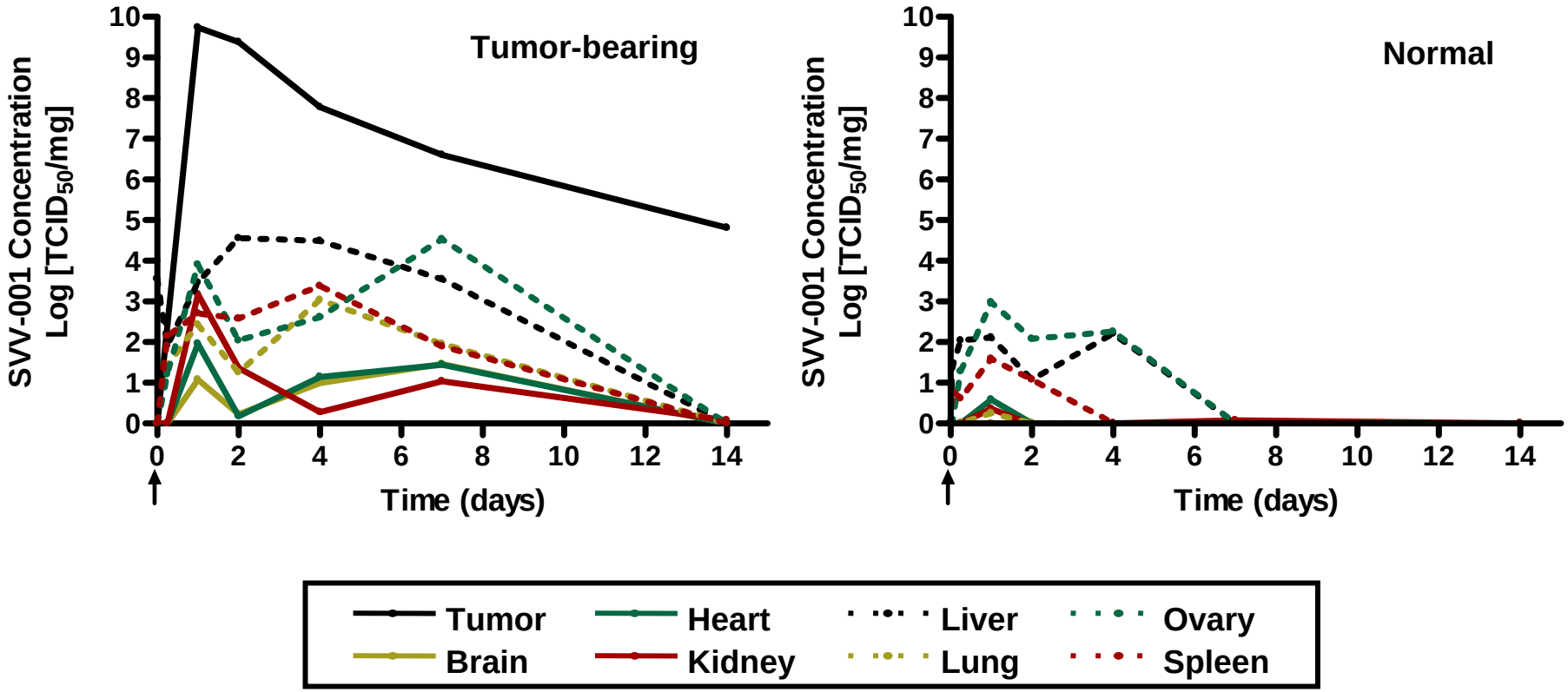


Subsequent analysis demonstrates that no infectious virus or full length genome exists post neutralizing antibody titer presence

Slow degradation apparent

Tumor-selective Replication in Mouse Tissues

10^9 vp/kg IV bolus



Phase I Clinical Trial Design

- Tumors with one or more neuroendocrine markers
  - Small Cell (Lung, Elsewhere), Carcinoid, Others
- Arm 1-Carcinoid Patients
  - Single i.v. infusion in outpatient setting
  - 3 + 3 Dose Escalation in log increments
  - Dose level 1 = 10E7 viral particles/kg to 10E11 vp/kg
  - Low permissive tumors
- Arm 2-Small cell Patients
  - Single i.v. infusion in outpatient setting
  - 3 + 3 Dose Escalation in log increments
  - Dose level 1 = 10E7 viral particles/kg/2 logs behind carcinoid arm
  - High permissive tumors-Elicits efficacy in high permissive tumors in mice
- Intensive Monitoring of Viral kinetics/Clearance
  - Sputum, stool, urine, serum, nasal swab
  - Infectious Virus, qRT-PCR, neutralizing antibody titer
    - Clearance for two consecutive timepoints separated by 48 hrs
    - No less then two weeks of full monitoring
    - Daily (days 1-5) week one; M, W, F (weeks 2-3), M, Th (weeks 4-6), M (weeks 7+)
  - Other assays as appropriate
- Sites
  - Johns Hopkins – Charlie Rudin
  - US Oncology – 9 sites

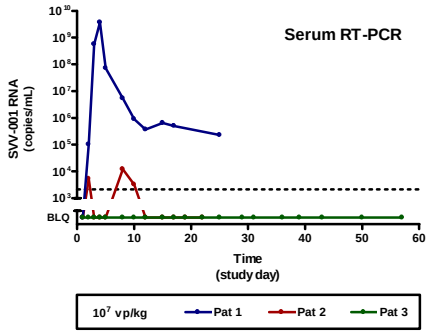
Phase I Clinical Trial-Biosafety Considerations

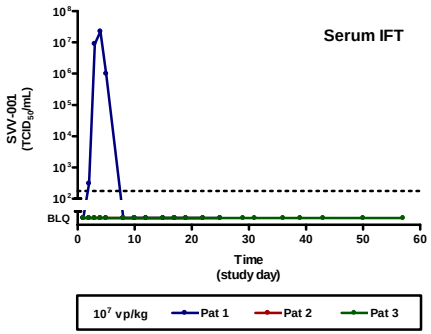
- Isolation of patients
  - Children, pregnant women, limited travel, private bedroom and bathroom
- Disinfect excreta
- Monitor viral shedding
- Epidemiology amongst family members and direct caregivers
- Contraception in men/Sterility in women

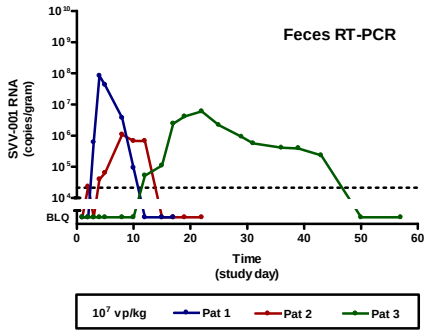
How we use assays

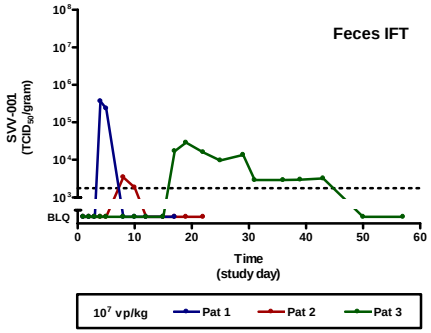
- Monitor dosing
  - Verify input dose
- Monitor replication to distinguish high and low replicators
  - Presence of permissive tissue (tumor)
  - Monitor for safety
- Monitor route and kinetics of shedding
  - Clearance for safety
- Monitor immune response to virus
  - Patient safety
  - Correlative with input virus and viral replication

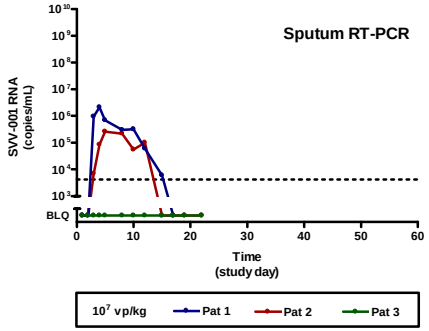
Viral Load – Cohort 1 (10^7 vp/kg)

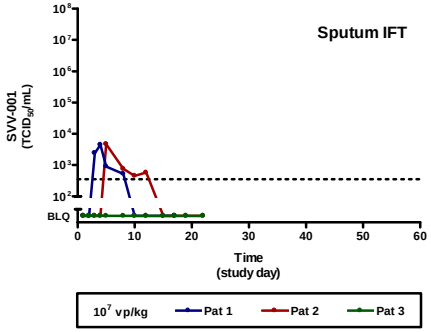




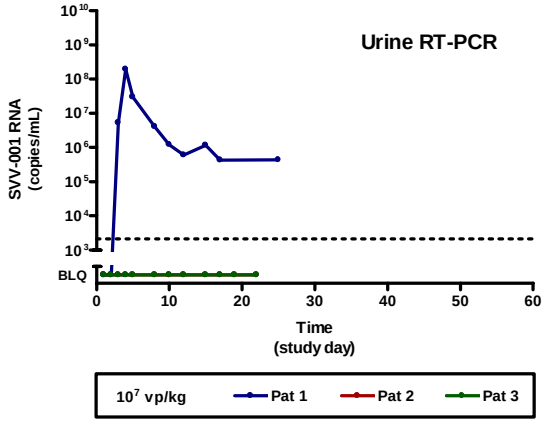


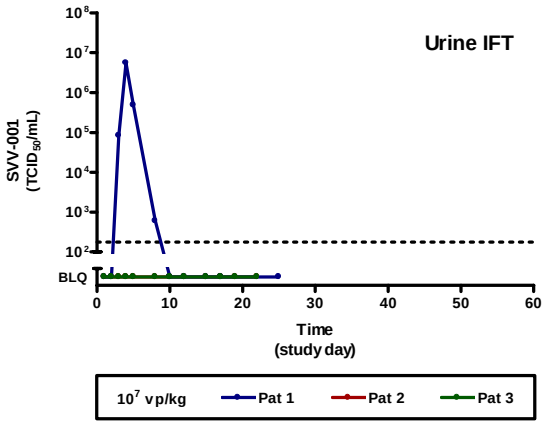


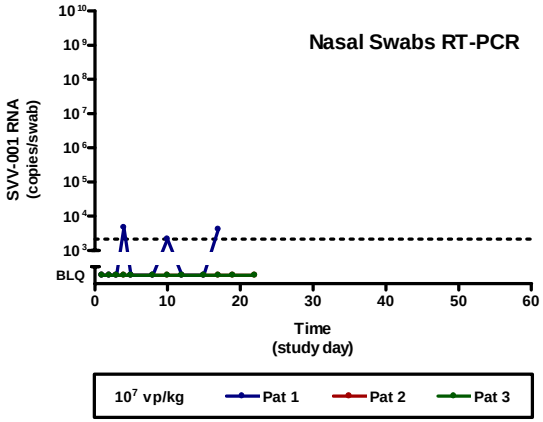


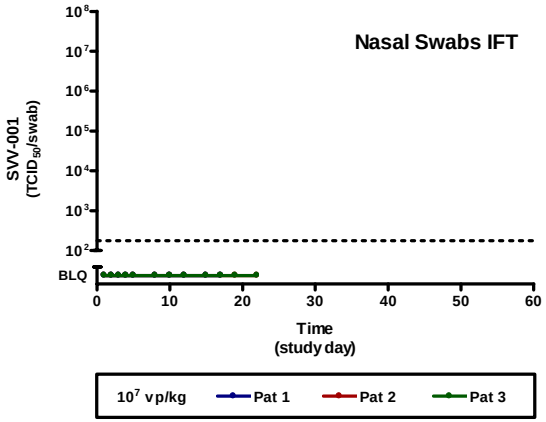


Viral Load – Cohort 1 (10^7 vp/kg)

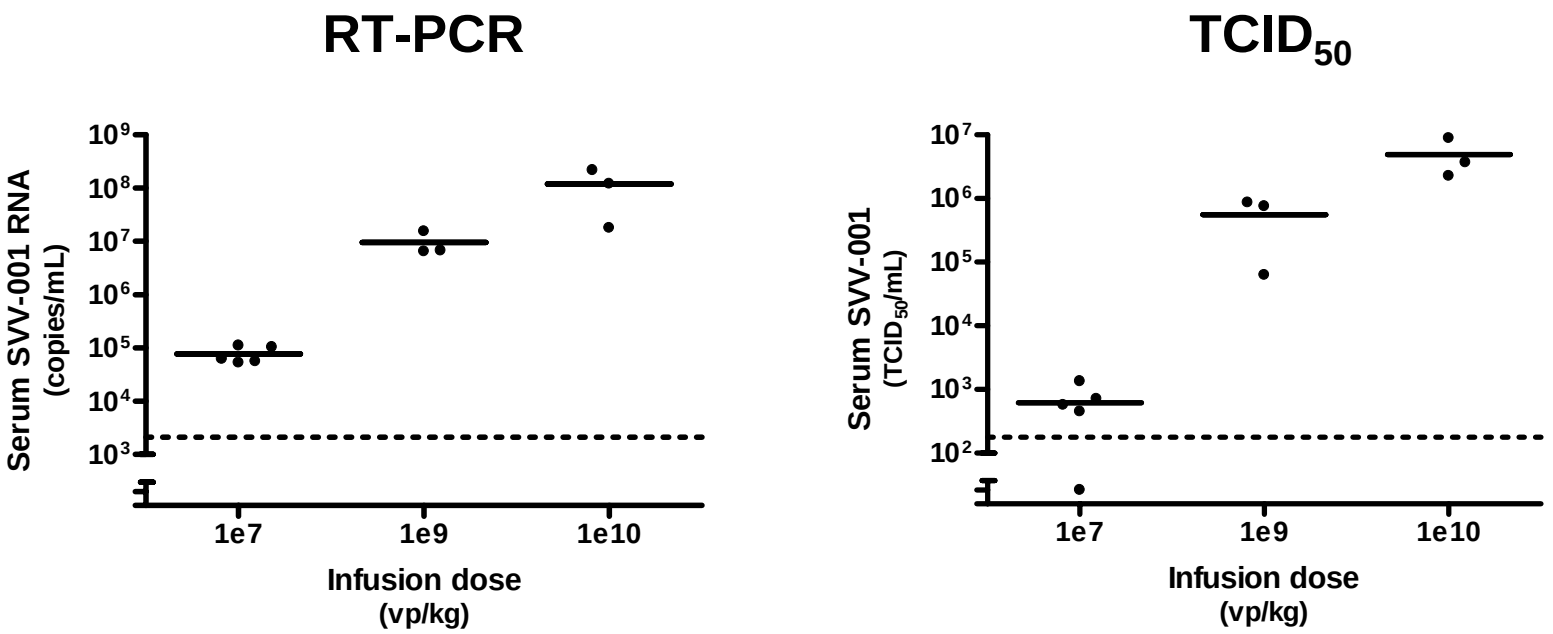




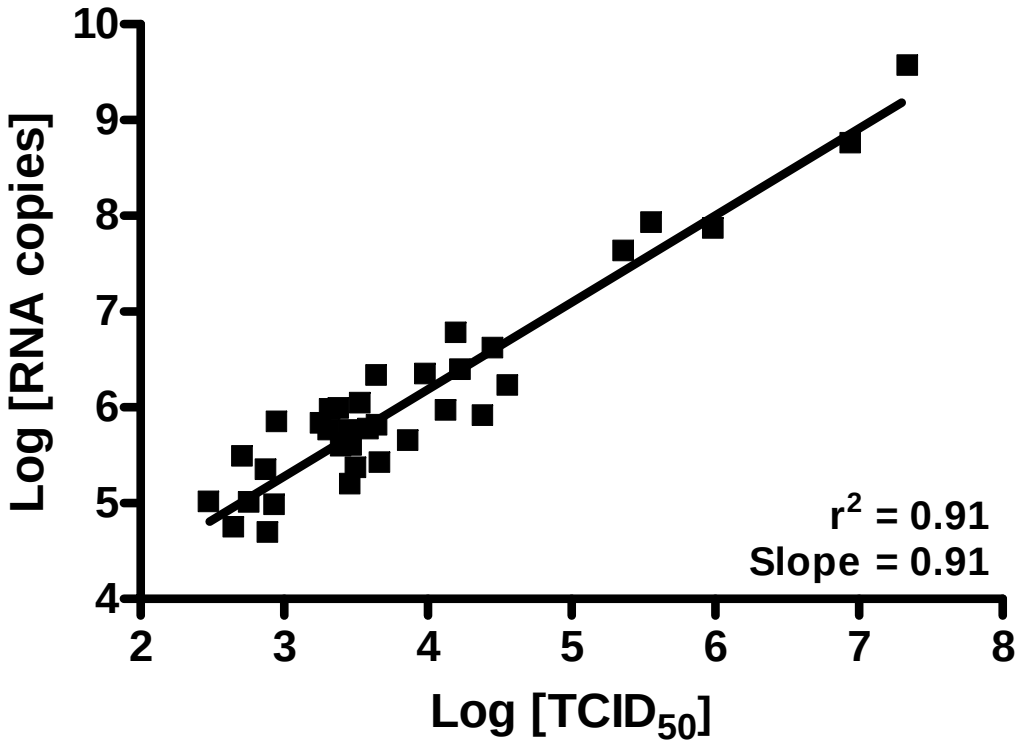




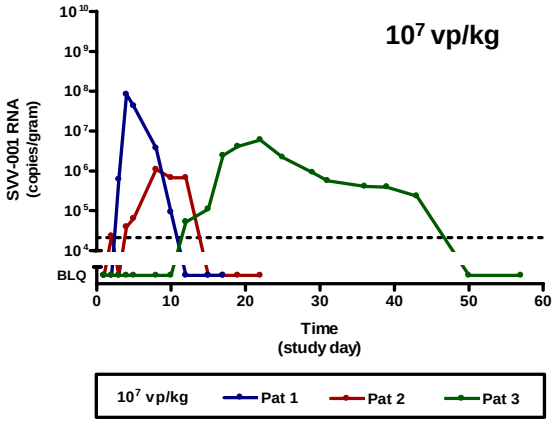
Monitoring Input Dose

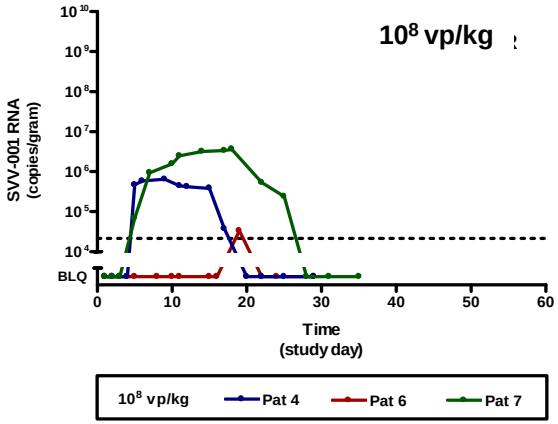


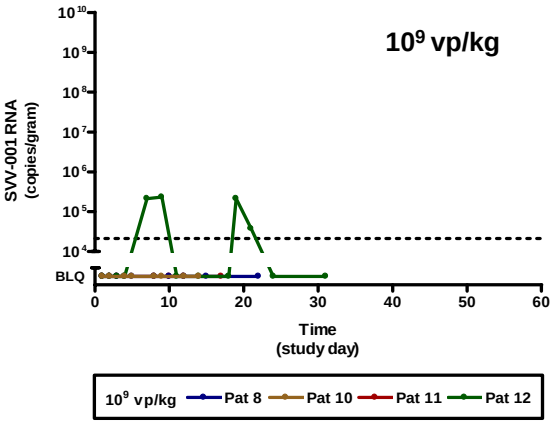
Correlation of $TCID_{50}$ vs. RT-PCR

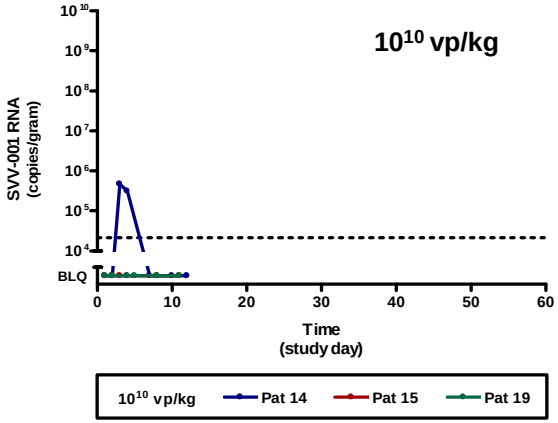


Shedding in Feces Decreases with Dose

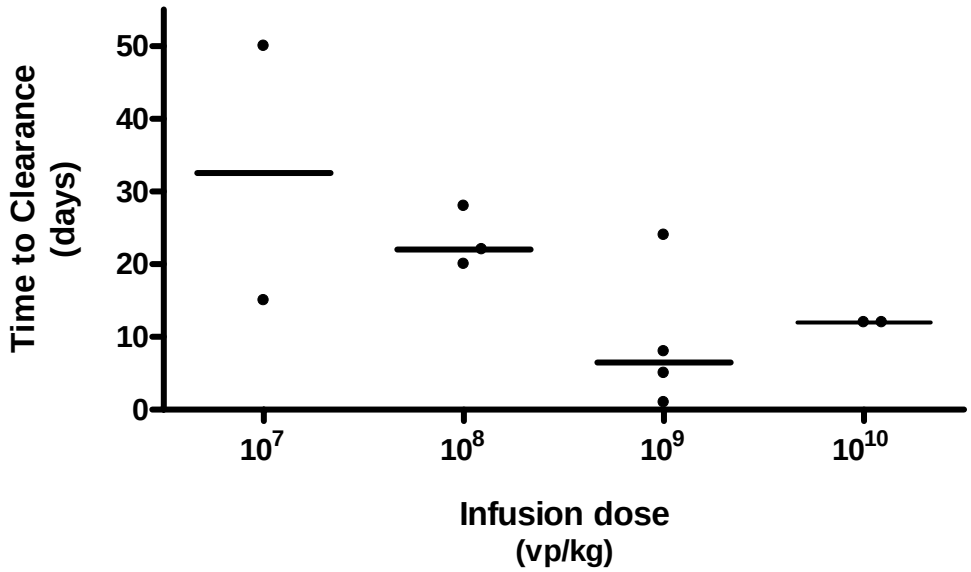




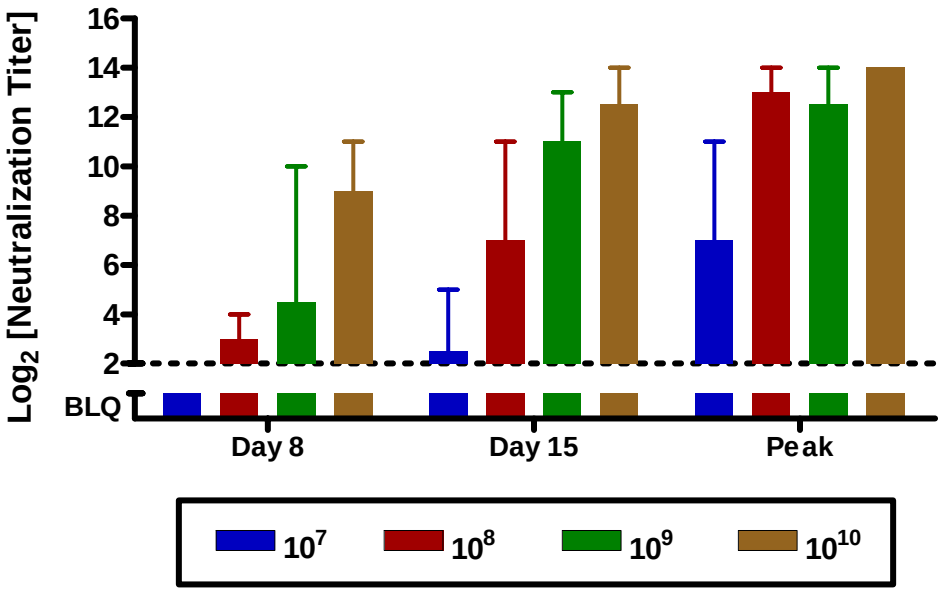




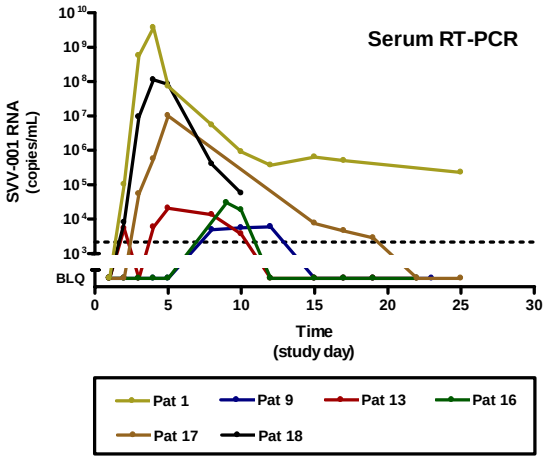
Trend of Shorter Clearance Time with Dose

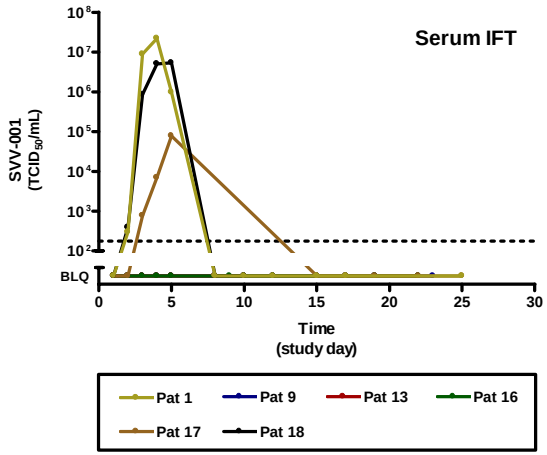


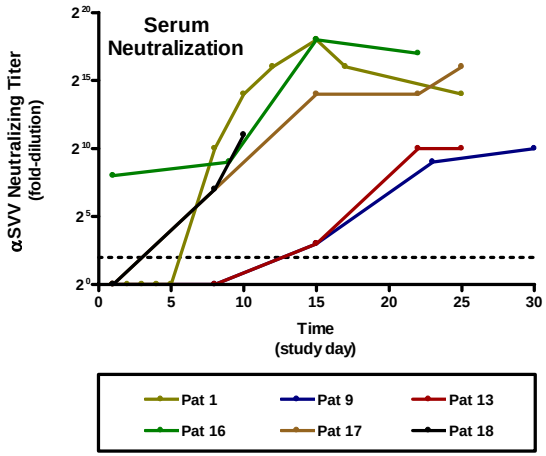
Neutralization Response with Dose



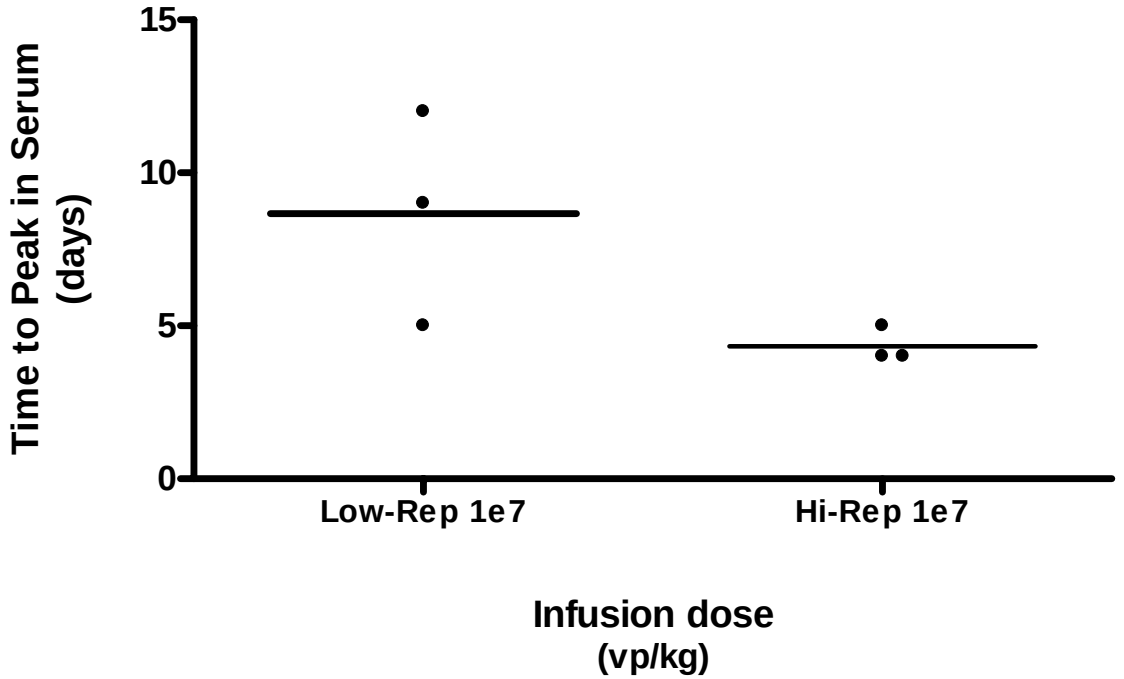
Viral Load – Small Cell Carcinoma (10^7 vp/kg)







Peak in Serum – Small Cell Carcinoma (10^7 vp/kg)



Viral Shedding Conclusions

- Tumor selective replication in small cell as predicted by preclinical
  - Low titers in 14 carcinoid patients despite high doses (max 10e10 vp/kg)
  - High titers in 3/6 small cell patients despite low doses (max 10e 7 vp/kg)
- Shorter times to peak virus, shorter times to clearance, lower levels secreted in excreta samples, and higher neutralization titers in patients **with higher doses**
- RT-PCR is a reliable and sensitive indicator of maximum theoretical signal
- Excellent correlation between RT-PCR and Infectious virus assay in most samples
- No issues of transmission
- Viremia appears before shedding
- Discordance between RT-PCR signal and TCID50 in rare patients.
  - In two patients RT-PCR signal persists but infectious virus signal does not
  - Other assays (Long PCR, Northerns, IHC) indicate that RT-PCR signal in discordant cases in mice and man is from degraded genomic RNA

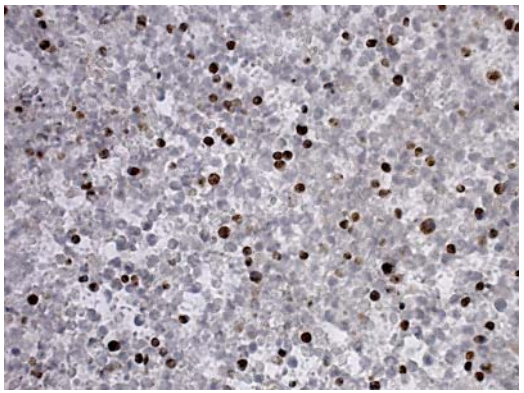
Acknowledgements

- Neotropix
  - John Neefe: Senior VP Clinical and Regulatory
  - Kevin Burroughs: Director of Non-Clinical Studies
  - Seshidhar Reddy: Director of Technology and New Products
- Johns Hopkins
  - Charles Rudin, PI
- US Oncology

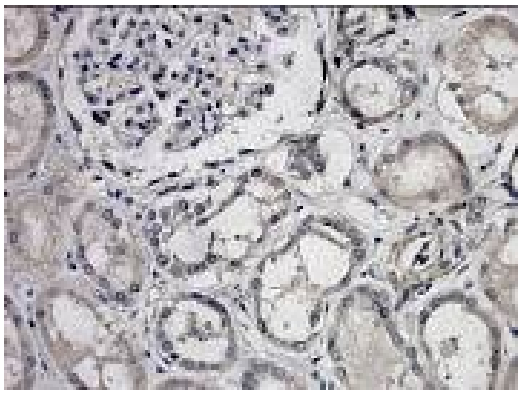
Other Methods

- Is virus infectious assay not detecting infectious virus in tissues
  - Sensitivity
  - Neutralizing ab response
  - Production equals output
  - Slow degradation
  - Does RNA signal indicate infectious virus?
    - Long PCR
    - Northerns
    - In Situ Hybridization
    - IHC

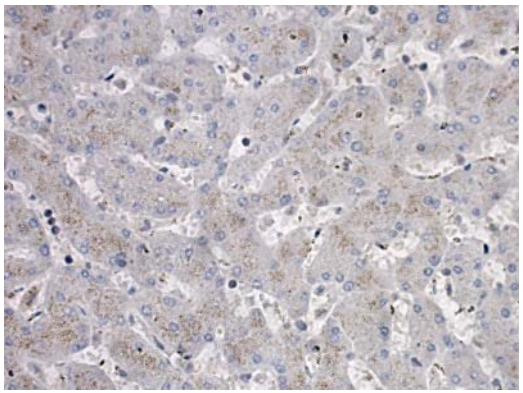
Patient 001 Tumor-selective Replication



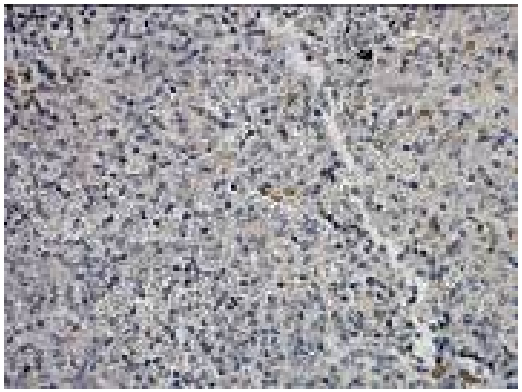
Liver Metastasis



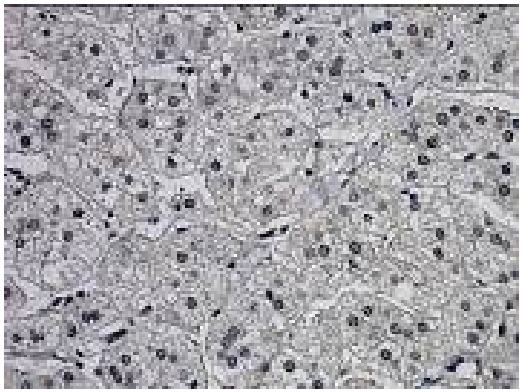
Kidney



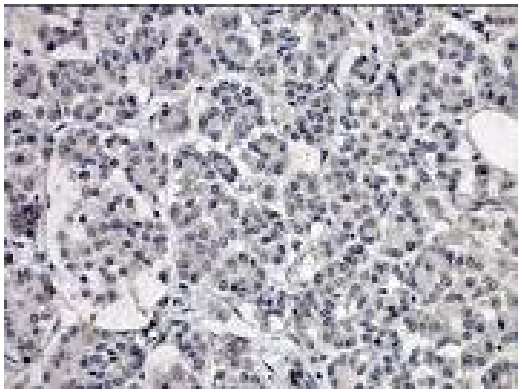
Normal Liver



Spleen



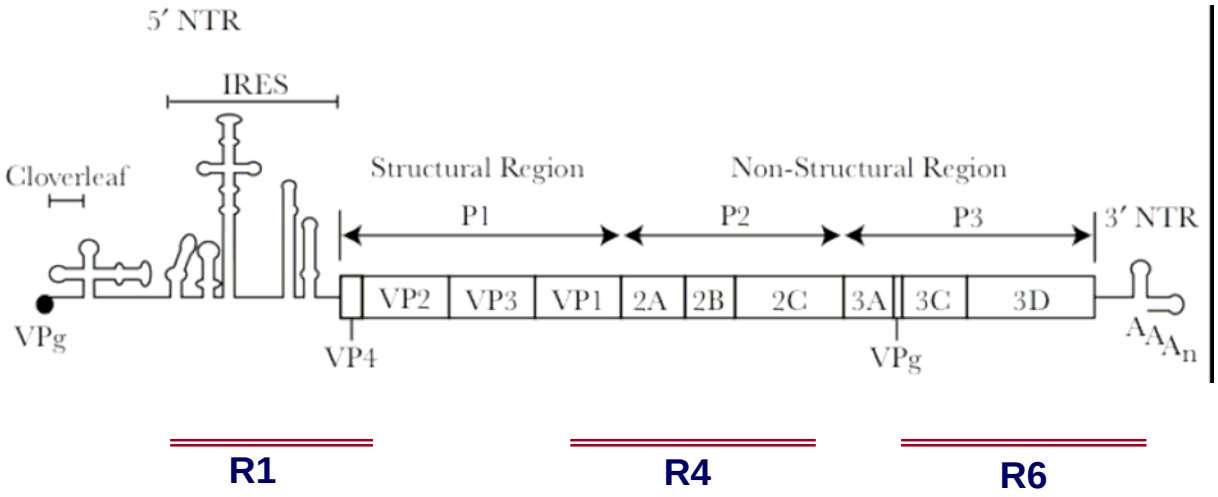
Adrenal Gland



Pancreas

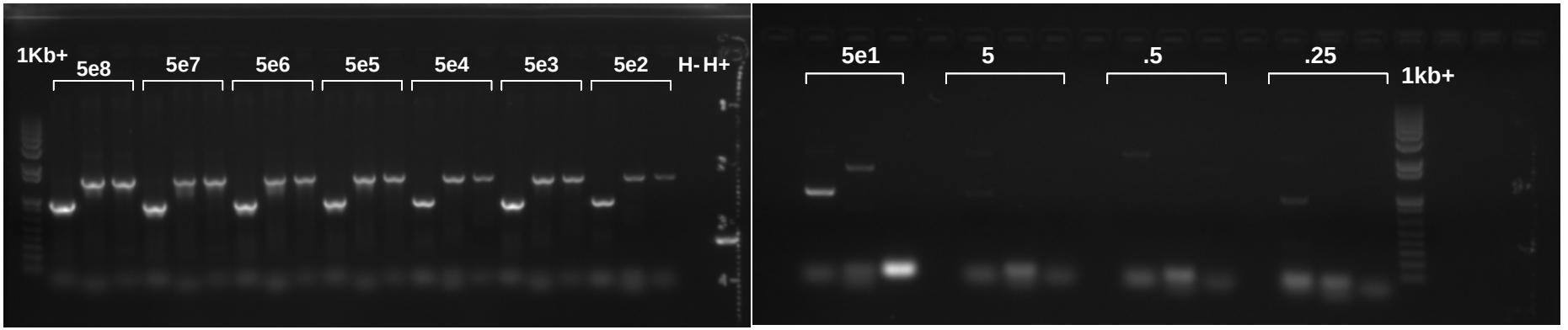
Intactness of Genomic RNA in serum - RT-PCR Assay

Primers for long RT-PCR reactions:



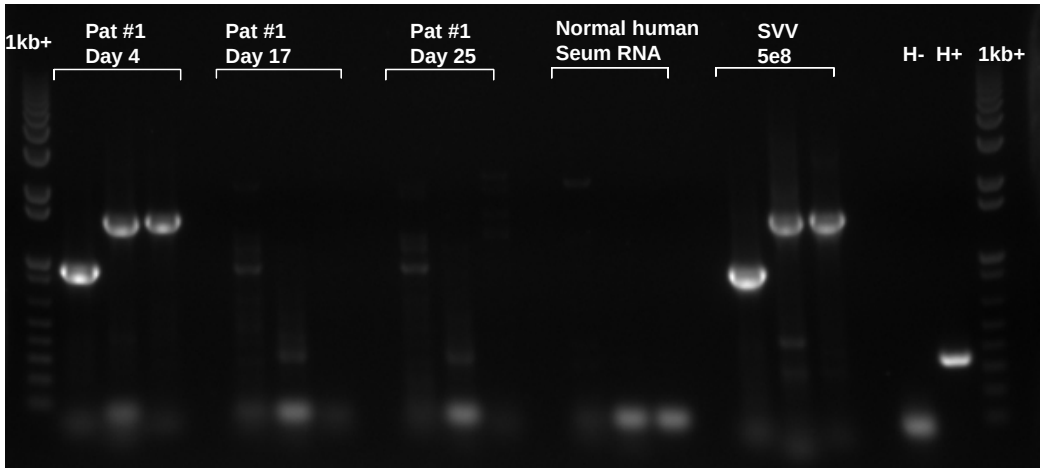
Reaction	Primer set	Position	Product size
R1	F2/ R5	348 / 1309 (5'end of the genome)	961 bp
R4	F11 / R16	3052 / 4609 (Middle of the genome)	1557 bp
R6	F19 / R24	5452 / 7009 (3'end of the genome)	1557 bp

Determination of SVV-001 RT-PCR assay sensitivity



- SVV-001 RNA ranging from 0.25 to 5e8 copies per reaction amplified by three primer pairs
- 353-bp product of human beta-actin gene from HeLa cells served as positive control
- HeLa RNA without primers served as negative control
- Two out of three fragments amplified at 50 copies/reaction, thus sensitivity of assay is 50 copies of SVV-001 genome per reaction.

Analysis of RNA in Patient 0001 Serum



- RT-PCR reactions performed with RNA isolated from equal volumes of Patient 0001 serum at study days 4, 17 & 25)
- Genomic RNA (5e8 copies) from SVV-001 served as primary positive control
- 353-bp product of human b-actin gene from HeLa cellular RNA served as a secondary positive assay control (H+)
- HeLa RNA containing no primer serves as negative control (H -)
- Only study day 4 serum sample gave positive signal