SAND2022-7563C

Sandia National Laboratories

Immunocompromised *Cas9* transgenic mice for the rapid *in vivo* assessment of host factors involved in highly pathogenic virus infection

Nicole Collette[1], Pragyesh Dhungel[2], Sean J. Lund[2], Jennifer L. Schwedler[2], Alexander N. Freiberg[3], Joseph S. Schoeniger[2], Oscar A. Negrete[2]

[1]Physical and Life Science Directorate, Lawrence Livermore National Laboratory; [2]Department of Systems Biology, Sandia National Laboratories; [2]Department of Pathology, University of Texas Medical Branch, Galveston, TX 77555

Background and Model

- Animal models are an important tool in studying infections and development of counter measures
- Host factors involved in viral infection and replication are intriguing targets for antiviral therapies
- Interferon-alpha receptor knockout (Ifnar1$^{-/-}$) mice are permissive hosts for several human highly pathogenic viruses
- The CRISPR/Cas9 system is a powerful tool that has been extensively utilized *in vitro* and *in vivo* to quickly generate knockouts
- **Objective**: Develop new mouse model that is both permissive to highly pathogenic viral infections or their surrogates and can be used to screen involved host factors and their impact on disease progression
- **Model**: Cas9$^{tg/tg}$; Ifnar1$^{-/-}$ mice injected intraperitoneally with ultra-modified NPC1 gRNA, and subsequently infected with VSV-EBOV and WT-EBOV

1. Optimization of sgRNA *in vivo* delivery to the liver of *Cas9* transgenic mice using Invivofectamine 3.0

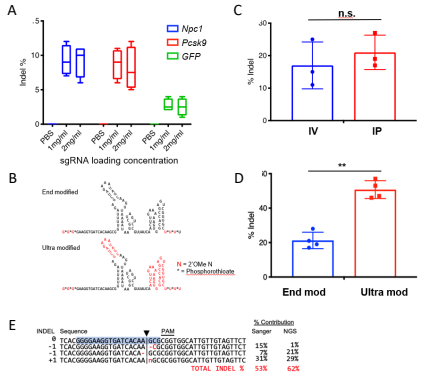

Figure 1. (A) INVFT3.0-based LNPs were loaded with standard end-modified sgRNAs targeting mouse Npc1, Pcsk9 or GFP. **(B)** End- versus ultra-modified sgRNA chemical compositions. **(C)** Performance of ultra-modified Npc1 sgRNA loaded INVFT3.0-LNPs was evaluated by comparing IV versus IP injections at a dose of 0.75mg/kg **(D)** Direct editing efficiency comparison of end- or ultra-modified sgRNAs targeting Npc1 was performed via IP administration at a dose of 1.5mg/kg **(E)** Percent indels are all reported based on decomposition of chromatographs from sanger sequencing using Synthego ICE and a comparison to indel sequences and percentages based on amplicon sequencing using NGS is shown of a single sample

2. Immunocompromised IFNAR1 knockout mice and those crossed with *Cas9* transgenic mice serve as lethal models for VSV-EBOV

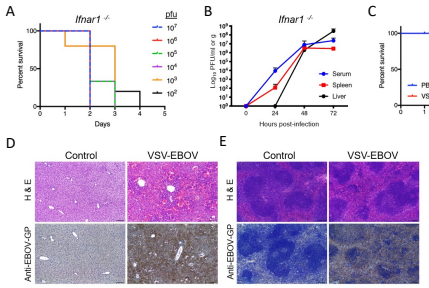

Figure 2. (A) Interferon alpha receptor knockout mice (Ifnar1-/-) were challenged with VSV-EBOV through IP administration. Survival curves are shown with 5 mice per challenge dose. **(B)** A time course experiment of VSV-EBOV tissue and blood dissemination was performed using a challenge dose of 100 PFU. Viral titers from tissues (PFU/g) or serum (PFU/ml) of three mice **(C)** Cas9 transgenic mice crossed with IFNAR1 knockout mice (Cas9tg/tg; Ifnar1-/-) were challenged with VSV-EBOV or mock infected (PBS) and survival was measured. **(D)** Liver and **(E)** Spleen tissues were harvested at day 3 post infection with VSV-EBOV in Cas9tg/tg; Ifnar1-/- mice and subjected to histological analysis via hematoxylin and eosin (H and E) staining or immunohistochemistry using antibodies against the Ebola glycoprotein (Anti-EBOV-GP). Scale bars = 100mm.

3. Multi-dosing of sgRNA-LNPs in *Cas9$^{tg/tg}$; Ifnar1$^{-/-}$* mice results in liver-specific cumulative editing

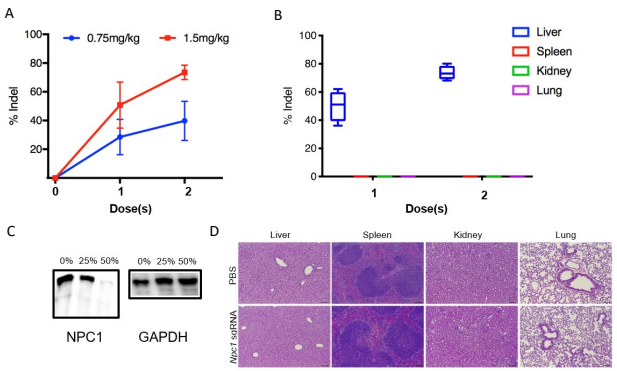

Figure 3. (A) INVFT3.0 LNPs were loaded with ultra-modified sgRNA targeting Npc1 and administered to immunocompromised Cas9 transgenic mice (Cas9tg/tg; Ifnar1-/-) via IP injection at either 0.75mg/kg or 1.5mg/kg doses. Another equivalent dose was given to a subset mice 7 days after the first dose. All mice were subjected to indel analysis of liver tissue on day 14 after the first dose. Data points are average of 4 mice per group +/- standard deviation. **(B)** Percent indels from other tissues in addition to liver are shown for the 1.5mg/kg dosing condition. (n=4). **(C)** NPC1 protein expression from liver homogenates of mice edited at the Npc1 locus at either 0% (PBS), 25% or 50% were analyzed using western blots. GAPDH was used as a loading control. **(D)** Histological analysis via hematoxylin and eosin staining was performed on tissues harvested from Npc1 sgRNA-LNP (double dose-1.5mg/kg) treated mice along with controls (PBS treated). Representative images are shown. Scale bars = 1mm.

4. Liver-specific editing of *Npc1* in *Cas9$^{gtg/tg}$; Ifnar1$^{-/-}$* mice provides complete protection against lethal VSV-EBOV

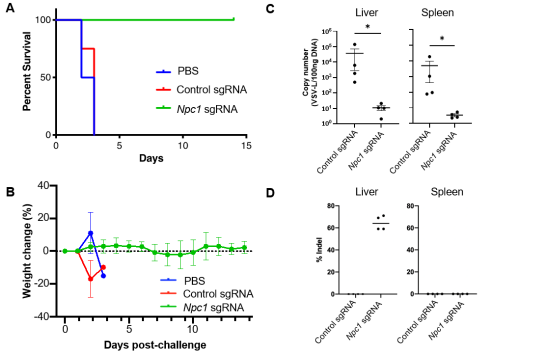

Figure 4. (A) Cas9tg/tg; Ifnar1-/- mice were dosed twice at a 7 day interval with Npc1 targeting sgRNA-LNPs or controls that included PBS or non-targeting sgRNA-LNPs, and subsequently challenged with VSV-EBOV seven days after the second dose (n=4 per group). Survival was measured for 14 days past challenge and **(B)** weights of the PBS or sgRNA-LNP treated mice were recorded daily and averaged as percent change from day 0. **(C)** Copies of the VSV polymerase gene (L) measured by RT-qPCR and **(D)** editing at the Npc1 locus measured using indel analysis from tissues collected at the end point of the study for the Npc1 sgRNA treated mice, or mice that succumbed to disease in the control sgRNA-LNP condition. Data is depicted as mean +/- standard deviation and *=p<0.05.

5. *Npc1* editing in the liver of immunocompromised Cas9 transgenic mice protects against lethal WT-EBOV challenge

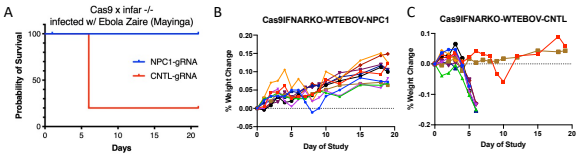

Figure 5. (A) Two doses of the sgRNA NPC1 treatments were administered to Cas9tg/tg; Ifnar1-/- mice (n=10) and spaced seven days apart. A control sgRNA treatment was also administered to these mice in using identical procedures to the experimental group. Seven days after the second sgRNA dose, all mice were challenged with wild type Ebola virus (Mayinga) at UTMB in ABSL-4 containment. Survival curves are shown as well as weight measurements collected through the endpoint of study (Day 21 post challenge).

Conclusions

- Ultra-modified sgRNA delivered in Cas9 tg mice using commercial lipid nanoparticles efficiently edit genes in the liver
- Cas9tg-Ifnar1KO mice are susceptible to VSV-EBOV and WT-EBOV
- Liver-specific editing of NPC1 protects against VSV-EBOV and WT-EBOV

Acknowledgments

Sandia National Laboratories is a multimission laboratory managed and operated by National Technology and Engineering Solutions of Sandia, LLC, a wholly owned subsidiary of Honeywell International, Inc., for the U.S. Department of Energy's National Nuclear Security Administration under contract DE-NA-0003525.