Human sirtuin-2 protein (SIRT2) – a host-target providing broad-spectrum effectiveness through multiple mechanisms of viral restriction and immunity

Lillian Chiang, PhD[1], Stacy Remiszewski, PhD[1], Rekha Panchal, PhD[2], Daniel Streblow, PhD[3], Sean Liu, MD PhD[4], Alison Welsh, BS[1], Jay Wells, BS[2], Sean Van Tongeren, BS[2], Xiaoli Chi, BS[2], Kathleen Cashman, PhD[2]

[1]Evrys Bio LLC, 3805 Old Easton Rd., Doylestown PA 18902, [2]U.S. Army Medical Research Institute of Infectious Disease, [3]Oregon Health Science University, [4]Icahn School of Medicine, Mount Sinai

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Abstract

Abstract: Current antivirals and vaccines target the virus. Virus are diverse in their biology and disease pathogenesis. Infection by a given virus-type requires a distinct, direct-acting therapeutic option. In the case of biodefense, this minimally requires development and stockpiling of one therapy/vaccine for each threat. Given biological diversity, the use cases are potentially infinite. Per session topic, "Host-directed therapies can improve host cellular responses to pathogens/biologicals, target disease-causing virulence factors, and activate innate and adaptive immune responses and immunological memory." Small-molecule host-directed therapies are additionally rapidly manufactured, can be taken in shelf-stable pill-form, and do not require cold-chain logistics for distribution. Evrys Bio has developed small-molecules targeting the human sirtuin-2 protein (SIRT2) that are simultaneously effective against diverse virus families: alpha-, arena-, and filoviruses.

An accumulating body of literature shows that small molecule modulation of SIRT2 can provide effective anti-infective activity against diverse viral and non-viral pathogens: herpes viruses, HSV-1, cytomegalovirus and Epstein Barr virus[1-3]; hepatitis A and B virus[4,5]; Zika, other arboviruses (West Nile, Chikungunya, Rift Valley fever, and La Crosse viruses)[6], and intracellular bacteria *Listeria*[7], *Salmonella*[8], and *Mycobacterium tuberculosis*[9]. SIRT2 is a ubiquitously expressed (NAD)$^+$-dependent lysine-deacylase that regulates cellular metabolism, stress response, and epigenetics through post-translational modification of key proteins that regulate cellular processes. Depending on the specific context, the SIRT2-targeted effect results from modulation of infected host-cell metabolism and biosynthetic pathways needed for productive viral infection, epigenetic disruption of pathogen regulation of host-restriction and/or virulence factors, and activation of innate and adaptive immunity.

Given the urgency of biodefense against innumerable pathogens, timely validation and prioritization of host targets as medical countermeasures is crucial. This presentation will discuss the mechanism of SIRT2-targeted antivirals for distinct virus-types. We also will present preclinical in vitro and in vivo pharmacology predicting beneficial clinical effects including reduced morbidity, mortality, and viral dissemination to end organs as exemplified by alphavirus and filovirus challenge studies in mice. Finally, we will present our data supporting combination dosing that may boost effectiveness of existing direct-acting antivirals.

[1] Roche (2023) "An allosteric inhibitor of sirtuin 2 deacetylase activity exhibits broad-spectrum antiviral activity," *J Clin Invest* doi:10.1172/JCI158978.

[2] Cheung (2023) "Inhibition of SIRT2 promotes death of human cytomegalovirus-infected peripheral blood monocytes via apoptosis and necroptosis," *Antiviral Res* doi:10.1016/j.antiviral.2023.105698.

[3] Li (2023) "SIRT2 negatively regulates the cGAS-STING pathway by deacetylating G3BP1," *EMBO Rep* doi:10.15252/embr.202357500.

[4] Kanda (2015) "The sirtuin inhibitor sirtinol inhibits hepatitis A virus (HAV) replication by inhibiting HAV internal ribosomal entry site activity," *Biochem Biophys Res Commun* doi:10.1016/j.bbrc.2015.09.083.

[5] Piracha (2018) "Sirtuin 2 isoform 1 enhances hepatitis B virus RNA transcription and DNA synthesis through the AKT-GSK-38/ß-catenin signaling pathway," *J Virol* doi:10.1128/JVI.00955-18.

[6] Hackett (2019) "Sirtuin inhibitors are broadly antiviral against arboviruses," *mBio* doi:10.1128/mBio.01446-19.

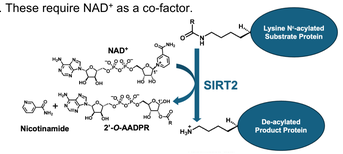
[7] Haig (2013) "A role for SIRT2-dependent histone H3K18 deacetylation in bacterial infection," *Science* doi:10.1126/science.1238858.

[8] Gogoi (2018) "Salmonella escapes adaptive immune response via SIRT2 mediated modulation of innate immune response in dendritic cells," *PLoS Pathog* doi:10.1371/journal.ppat.1007437.

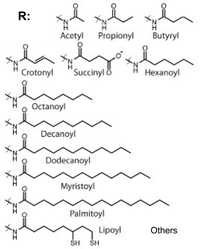
[9] Bhaskar (2023) "SIRT2 inhibition by AGK2 enhances mycobacteria-specific stem cell memory responses by modulating beta-catenin and glycolysis," *iScience* doi:10.1016/j.isci.2023.106644.

Background: Cellular SIRT2 Protein Function

1. SIRT2 belongs to the sirtuin family (SIRT1-7) of cellular Class III lysine de-acylase (KDAC) enzymes.
2. These require NAD$^+$ as a co-factor.



3. The acyl-chain is transferred to the ADP-ribose of NAD$^+$.
4. Nicotinamide and the 2'-O-acyl-ADP-ribose are released…
5. …as is the post-translationally modified de-acylated target protein.
6. Numerous cellular targets of SIRT2 de-acylation have been reported. As a result, numerous cellular functions are impacted by SIRT2 including epigenetics, signal transduction, stress response, metabolism, and cellular organization.
7. The requirement for NAD$^+$ couples SIRT2-mediated regulatory events to the metabolic status of the cell.



Allosteric Modulators of SIRT2 Are Broadly Anti-Infective

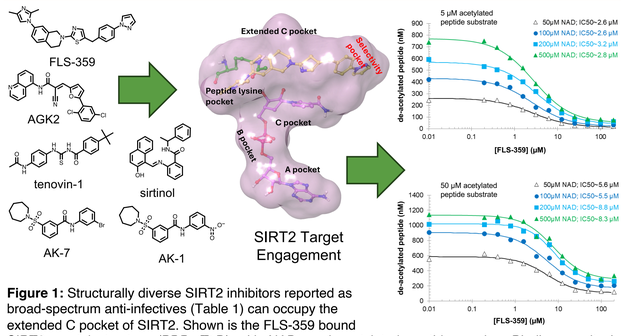


Figure 1: Structurally diverse SIRT2 inhibitors reported as broad-spectrum anti-infectives (Table 1) can occupy the extended C pocket of SIRT2. Shown is the FLS-359 bound SIRT2 crystal structure (PDB:7T1D) with NAD$^+$ and acetylated peptide overlay. Binding results in inhibition of SIRT2 de-acetylase activity that is independent of NAD$^+$ and acetyl-peptide concentration.

Virus/Pathogen	Pathogen Family	FLS-359 µM EC_{50}	EV-100 µM EC_{50}	Comparator µM EC_{50}	Comparator or Standard of Care (SOC)	Other SIRT2 modulator (literature)
Respiratory viruses						
Influenza A	**orthomyxovirus**	1.2	0.86	0.3	**oseltamivir (SOC)**	
Influenza B	**orthomyxovirus**	1.2	0.86	6.4	**oseltamivir (SOC)**	
Influenza A oseltamivir[R]	**orthomyxovirus**	2.5		9	**oseltamivir (SOC)**	
Respiratory Syncytial Virus	**orthopneumovirus**	6.7	3.7	16.1, 9.5	**ribavirin, remdesivir**	
Ad5	**adenovirus**	1.6		3.1	**cidofovir**	
HCoV-229E	**coronavirus (alpha)**	1.6		0.04	**remdesivir**	
HCoV-OC43	**coronavirus (beta)**	0.5	0.4	1.6	**hydroxychloroquine**	
SARS-CoV2	**coronavirus (beta)**	0.6	0.4	0.07	**remdesivir (SOC)**	
MERS	**coronavirus (beta)**		1.3	0.07	**remdesivir (SOC)**	
Biodefense pathogens						
Venezuelan Eq. Encephalitis	**alphavirus**	1.9	1.6	0.78	**β-d-N4-hydroxycytidine**	
Chikungunya	**alphavirus**					**sirtinol, tenovin-1[6]**
West Nile Virus	**alphavirus**					**sirtinol, tenovin-1[6]**
Lassa Fever Virus	**arenavirus**	1.7	0.83	0.015	**ST-193**	
Rift Valley Virus	**bunyavirus**					**sirtinol, tenovin-1[6]**
La Crosse Virus	**bunyavirus**					**sirtinol, tenovin-1[6]**
Ebola (Sudan)	**filovirus**	2.4	1.9	0.04	**remdesivir**	
Zika	**flavivirus**	0.4		3.9	**amodiaquine**	**sirtinol, tenovin-1[6]**
Dengue (DENV2)	**flavivirus**	2.0				**tenovin-1[10]**
Herpes viruses						
Cytomegalovirus	**herpesvirus (beta)**	0.6	1.2	1.4	**ganciclovir (SOC)**	**sirtinol[11]**
Epstein-Barr Virus	**herpesvirus (gamma)**	3.8		43	**ganciclovir**	
Hepatic viruses						
Hepatitis A	**picomavirus**	1.8				**sirtinol[4]**
Hepatitis B	**hepadnavirus**	5.2	3.2	0.03	**tenofovir (SOC)**	**AGK2, SIRT2 shRNA[5]**
Other opportunistic pathogens						
***Salmonella* Typhimurium**	**gram(-) bacteria**					**SIRT2 KO, AK7[8]**
Listeria monocytogenes	**gram(+) bacteria**					**AGK2, SIRT2 siRNA[7]**
Mycobacterium tuberculosis	**mycobacteria**					**AGK2[9]**
AVERAGE ANTIVIRAL EC_{50}		< 2.1	< 1.5	< 5.6		
MEDIAN ANTIVIRAL EC_{50}		1.9	1.3	1.1		

[10] Wan (2021) "Tenovin-1 inhibited dengue virus replication through SIRT2" *Eur J Pharmacol*. doi: 10.1016/j.ejphar.2021.174264.

[11] Mao (2016) "Suppressive effects of sirtinol on human cytomegalovirus (hCMV) infection and hCMV-induced activation of molecular mechanisms of senescence and production of reactive oxygen species" *Mech Age Dev*. doi: 10.1016/j.mad.2015.12.005.

Table 1: Pan-viral profile of FLS-359 and EV-100. EV-100 is a clinical-stage drug that is structurally similar to FLS-359. In cell-based assays, Column 1 lists diverse viruses tested from DNA and RNA virus families shown in Column 2. The next three columns report the concentration of drug in μM providing 50% antiviral effectiveness (EC_{50}) for FLS-359, EV-100, and the comparator. The Evrys drugs are more potent against many viruses compared to standard-of-care. The advantage of host-targeting is evident in Column 6 wherein the comparator was by necessity, different depending on the virus. Note: Underlined indicates EC_{90} reported. The CC_{50} across assays was ≥ 10-100 μM, the highest concentration tested. Other lists SIRT2 modulators reported in the literature.

Anti-infective Mechanisms of SIRT2 Modulators

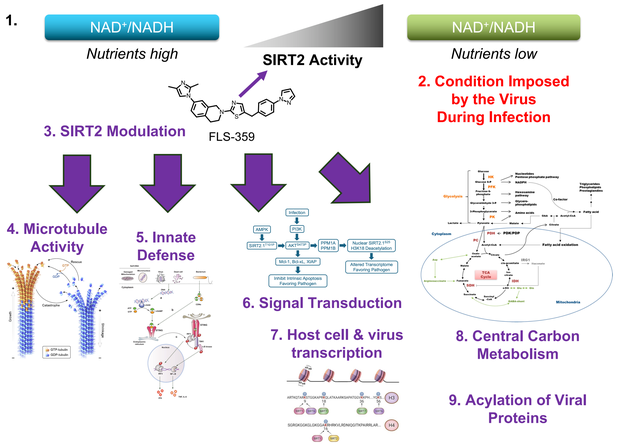


Figure 2: 1. Due to its absolute requirement for co-factor NAD$^+$, the enzyme activity of SIRT2 is tied to the metabolic capacity of the cell. **2.** Virus infection often imposes a low nutrient condition, activating SIRT2 and pushing the cell into glycolysis to fuel viral replication, and, also, cellular immunity. **3.** SIRT2 allosteric modulators like FLS-359 partially inhibit SIRT2 de-acetylase activity in infected cells and can change the relative preference of SIRT2 for the various acyl-chain target protein modifications. Many de-acylation targets downstream of SIRT2 can influence the course of infection. **4.** α-tubulin is a direct target of SIRT2 de-acetylation. AGK2, a SIRT2 modulator, primarily causes hyperacetylation of perinuclear microtubules. This activity can have major consequences for intracellular pathogens[12], controlling the dynamics of intracellular movements of the invader's components. **5.** SIRT2 has been reported to interact with the cGAS-STING pathway by deacetylating G3BP1 and blocking the G3BP1-cGAS interaction[3]. Consistent with a role for SIRT2, AGK2 reduced HSV-1 production in cultured cells several fold, and, also, reduced virus load and extended survival of mice in a lethal model of HSV-1 infection. **6.** The AKT signal transduction pathway has been the best studied in the context of SIRT2 modulation during infection. SIRT2 binds to AKT through its PH and catalytic domains and has been shown to drive its maximal activation. On the other hand, AGK2 prevented AKT hyperphosphorylation at S473 but the basal level of activated AKT S473P was not reduced[13], arguing that elevated but not basal AKT activity is blocked by AGK2. Multiple viruses have been shown to activate AKT[14]. The PI3K/AKT pathway impacts numerous cell processes, including RNA processing and translation, metabolism, cell proliferation and cell survival, and in many cases activated AKT supports viral replication. **7.** SIRT2 resides predominantly in the cytoplasm but can be relocated to the nucleus during infection via an AKT-dependent process. Nuclear SIRT2 imposes a host-cell transcriptional program that favors growth of *Listeria monocytogenes*, and a block to this program by AGK2 inhibits growth of the pathogen in infected cells in culture and in mice[7]. A similar SIRT2 nuclear localization and altered host-cell transcriptome has been reported for *M. tuberculosis*-infected macrophages where AKT is activated[9]. **8.** SIRT2 regulates many aspects of metabolism, including enzymes that drive glycolysis, the TCA cycle, oxidative phosphorylation (OxPhos) and lipid synthesis. Multiple viruses also induce aerobic glycolysis and OxPhos[15]. The combination of SIRT2 modulation plus viral infection can lead to high consumption of glucose, and the resulting buildup of lactic acid in the microenvironment of infected cells could be detrimental to viral replication. **9.** Acetylated lysines have been identified in a variety of viruses[16], so SIRT2 modulators could act directly on the acetylation status of viral proteins.

[12] da Silva (2023) "Microtubules and Viral Infection" In *Advances in Virus Research*; ISBN 9780443193569.

[13] Ramakrishnan (2014) "Sirt2 Deacetylase Is a Novel AKT Binding Partner Critical for AKT Activation by Insulin" *J. Biol. Chem*. 289, 6054–6066.

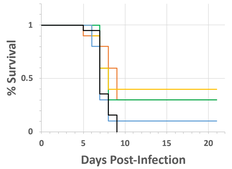
[14] Blanco (2020) "Phosphatidylinositol-3-Kinase-Akt Pathway in Negative-Stranded RNA Virus Infection: A Minireview" *Arch. Virol*. 2020.

[15] Girdhar (2021) "Viruses and Metabolism: The Effects of Viral Infections and Viral Insulins on Host Metabolism" *Annu. Rev. Virol*. 2021, 8, 373–391.

[16] Xue, M (2022) "Protein Acetylation Going Viral: Implications in Antiviral Immunity and Viral Infection" *Int. J. Mol. Sci*. 23, 11308.

SIRT2 Modulation as a Biodefense Medical Countermeasure

Figure 3: SIRT2 modulation protects mice against Ebola Sudan challenge. IFN-α/βR–/– mice were injected i.p. with Ebola Sudan (SUDV; 1,000 pfu). Four SIRT2 modulators, structurally related to FLS-359, were administered at 100 mg/kg p.o. q.d. (n=10 per drug arm) starting 1 h post-SUDV challenge. The data is combined in one Kaplan–Meier graph and significance determined by one-tailed log-rank test. 0/19 infected mice, vehicle treated survived. 3/10, 3/10, 4/10, and 1/10 survived when treated with MCM2 (p=0.03), MCM3, MCM4 (p=0.01), and MCM5, respectively.



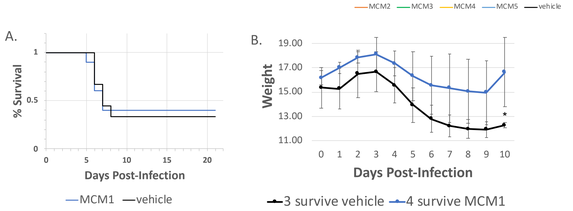


Figure 4: Mice receiving a SIRT2 modulator recover faster than untreated mice that survive Ebola Sudan challenge. Mice were challenged with SUDV as described in Figure 3. 4/10 mice treated with SIRT2 modulator MCM1 (structurally related to FLS-359) survived compared to 3/9 vehicle. Among the survivors, MCM1-treated mice showed statistically significant improved weight gained (*p=0.02).

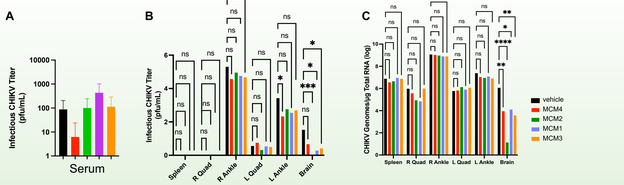


Figure 5: SIRT2 modulation prevents Chikungunya virus dissemination to the brain. Eight-week-old C57BL6 mice were infected with 1000 pfu Chikungunya virus (CHIKV) by foot pad injection. SIRT2 modulating drugs (MCM1-4) or vehicle (0.5% methylcellulose/0.5% Tween 80/water) (n=8 per group with legend at far right) were administered daily 100 mg/kg po starting 1 h after CHIKV challenge. **A.** 2 dpi-viremia was measured for serum by limiting dilution plaque assays on Vero cells. Drug treatment did not differ significantly from vehicle. **B.** 7 dpi tissues were collected at necropsy, homogenized in 500 μl PBS, and clarified by centrifugation. 20 μl of homogenate was titered by limiting dilution plaque assays on Vero cells. **C.** RNA was extracted from 300 μl of homogenate and viral genomes were detected by qRT-PCR. Two to five log less CHIKV was detected in the brain by both plaque assay and RNA genome detection. (*p=0.05, **p=0.01, ***p=0.001, ****p=0.0001, ns=not significant).

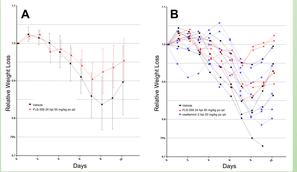


Figure 6: SIRT2 modulation protects mice against influenza A challenge. A. BALB/c mice were challenged intranasally (at non-lethal 0.3 LD50) with influenza A/Singapore/gp1908/2015 IVR-180 (H1N1). Mice received oseltamivir (20 mg/kg) and vehicle daily, 2 h after challenge. Mice received FLS-359 (50 mg/kg) daily, 24 h after challenge. A trend to improved recovery (n = 5) was observed for FLS-359 but not oseltamivir (not shown). **B.** The individual plots show that all animals receiving FLS-359 started gaining weight by d 7 and did not drop below 85% of starting.