Allosteric sirtuin-2 (SIRT2) modulators are pathogen-agnostic anti-infectives that inhibit both viruses and bacteria, and have application beyond infectious disease

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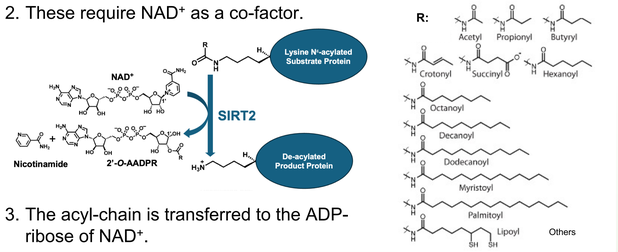
Abstract

Sirtuin-2 (SIRT2) is an NAD⁺-dependent lysine de-acylase that regulates cellular metabolic reprogramming by post-translationally removing marks from histones and many other nuclear and cellular protein targets. A metabolic switch occurs during many physiological processes, including somatic reprogramming, tumorigenesis, and immune cell differentiation, activation, and tolerance. Indeed, intracellular infection by viruses or bacteria, often invokes metabolic reprogramming that the pathogen needs to coordinate nuclear and cellular events with the changing host-cell metabolic status to achieve optimal productive infection. Evrys Bio has developed a portfolio of >1000 SIRT2-selective small molecules that do not compete with NAD⁺ or the (peptide) target of SIRT2 post-translational modification. Instead, these are allosteric modulators that change the relative rate of de-acylation mediated by SIRT2 for targets differing in acyl-chain modification and/or peptide sequence. Additionally, even at saturating concentrations, these allosteric modulators never completely shut down SIRT2 enzyme function. As a result, Evrys is developing multiple anti-infective drugs that provide "fit-to-purpose" SIRT2 modulation and therapeutic window (compared to uninfected cells) for (1) opportunistic viruses that threaten immunocompromised patients including actively immunosuppressed transplant recipients, (2) chronic hepatitis B, (3) pan-biothreat RNA viruses, and (4) diverse gram +/- intracellular bacteria. Preclinical data from cell-based and animal models of tolerability and pathogen challenge will be presented including for Evrys' first IND-ready candidate, EV 100. Interestingly, exploratory research indicates that Evrys' SIRT2 portfolio provides bioavailable and well-tolerated SIRT2 modulators as proof-of-concept for application to non-infectious disease conditions.

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

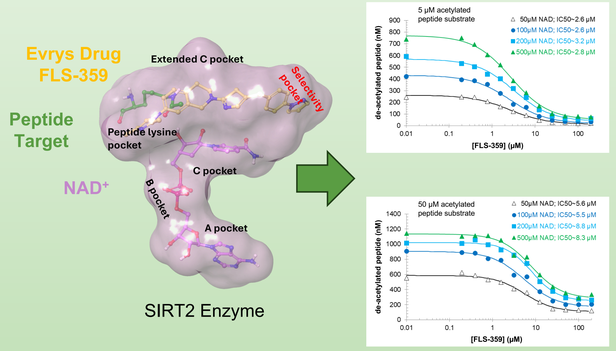


Figure 1: Evrys Bio has validated > 1000 allosteric modulators of SIRT2. The co-crystal structure for early lead FLS-359 occupies the extended C pocket of SIRT2 (PDB:7T1D). NAD⁺ and acetylated peptide overlay show both maintain access to the catalytic domain in the presence of FLS-359. Binding results in inhibition of SIRT2 de-acetylase activity that is independent of NAD⁺ and acetyl-peptide concentration.

Anti-infective Mechanisms of SIRT2 Modulators

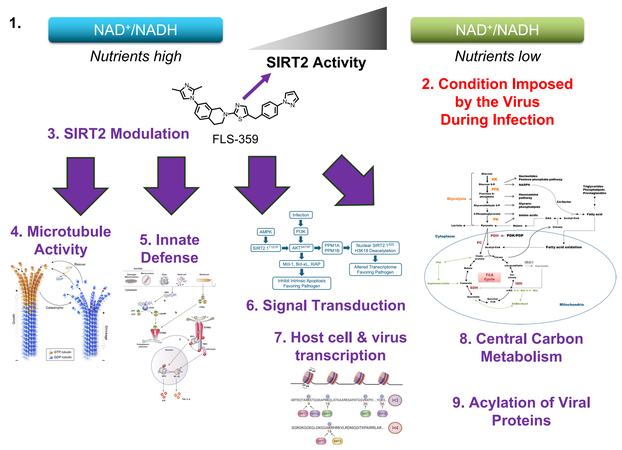


Figure 2: 1. Due to its absolute requirement for co-factor NAD⁺, the enzyme activity of SIRT2 is tied to the metabolic capacity of the cell. **2.** Virus infection often imposes a low nutrient condition, activating SIRT2 and pushing the cell into glycolysis to fuel viral replication and cellular immunity. **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 targets. Many de-acylation targets downstream of SIRT2 can influence the course of infection. **4.** α-tubulin is a direct target of SIRT2 de-acetylation. SIRT2 modulator AGK2 causes hyperacetylation of perinuclear microtubules, having major consequences for dynamics of intracellular pathogen movement[10]. **5.** SIRT2 has been reported to interact with the cGAS-STING pathway by deacetylating G3BP1 and blocking the G3BP1-cGAS interaction[11]. **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 to drive its maximal activation. AGK2 prevented AKT hyperphosphorylation at S473 but the basal level of activated AKT S473P was not reduced[12], thus blocking elevated but not basal AKT activity. Multiple viruses have been shown to activate AKT[13] impacting numerous cell processes, including RNA processing and translation, metabolism, cell proliferation and cell survival. **7.** SIRT2 can translocate to the nucleus during infection. Nuclear SIRT2 imposes a host-cell transcriptional program that favors growth of *Listeria monocytogenes.* AGK2 blocks this program and inhibits growth of the pathogen in cell culture and in mice[7]. SIRT2 nuclear localization and altered host-cell transcriptome has also been reported for *M. tuberculosis*-infected macrophages[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[14]. The combination of SIRT2 modulation plus viral infection leads to high consumption of glucose resulting in buildup of lactic acid in the microenvironment of infected cells that is detrimental to viral replication. **9.** Acetylated lysines have been identified in a variety of viruses[15], so SIRT2 modulators also can act directly on the acetylation status of viral proteins.

Table 1: Pan-viral profile of FLS-359 and EV-100.

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)
Herpes viruses						
Cytomegalovirus[1]	herpesvirus (beta)	0.6	1.2	1.4	ganciclovir (SOC)	sirtinol[2]
Epstein-Barr Virus[1]	herpesvirus (gamma)	3.8		43	ganciclovir	
Hepatic viruses						
Hepatitis A	picornavirus	1.8				sirtinol[3]
Hepatitis B[4]	hepadnavirus	5.2	3.2	0.03	tenofovir (SOC)	AGK2, SIRT2 shRNA[5]
Respiratory viruses						
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)	
HCoV-229E	coronavirus (alpha)	1.6		0.04	remdesivir	
Influenza A	orthomyxovirus	1.2	0.86	0.3	oseltamivir (SOC)	
Influenza B	orthomyxovirus	1.2	0.86	6.4	oseltamivir (SOC)	
Influenza A^R	orthomyxovirus	2.5		9	oseltamivir (SOC)	
Ad5	adenovirus	1.6		3.1	cidofovir	
Respiratory Syncytial Virus	orthopneumovirus	6.7	3.7	16.1, 9.5	ribavirin, remdesivir	
Biodefense viruses						
Zika	flavivirus	0.4		3.9	amodiaquine	sirtinol, tenovin-1[6]
Dengue (DENV2)	flavivirus					tenovin-1[7]
Venezuelan Eq. Encephalitis	alphavirus	1.9	1.6	0.78	β-d-N4-hydroxycytidine	
Chikungunya	alphavirus					sirtinol, tenovin-1[6]
Lassa Fever Virus	arenavirus	1.7	0.83	0.015	ST-193	
West Nile Virus	alphavirus					sirtinol, tenovin-1[6]
Rift Valley Virus	bunyavirus					sirtinol, tenovin-1[7]
La Crosse Virus	bunyavirus					sirtinol, tenovin-1[7]
Ebola (Sudan)	filovirus	2.4	1.9	0.04	remdesivir	
Other intracellular pathogens						
Salmonella Typhimurium	gram(-) bacteria					SIRT2 KO, AK7[7]
Listeria monocytogenes	gram(+) bacteria					AGK2, SIRT2 siRNA[8]
Mycobacterium tuberculosis	mycobacteria					AGK2[8]
AVERAGE ANTIVIRAL EC_{50}		< 2.1	< 1.5	< 5.6		
MEDIAN ANTIVIRAL EC_{50}		2.2	1.8	2.3		

EV-100 is a game changer for cytomegalovirus prophylaxis

Drug: *mechanism*	Pan-Viral Profile	EC_{99} (μM)	MAX INH at EC_{99}	Viral Genes conferring resistance	Length of U.S. exclusivity from Orange Book
EV-100: *human SIRT2 inh*	CMV, EBV, respiratory & hepatic viruses	0.7	>100-fold	None	1st issued patent expiry in 2038
Marketed drugs:					
valganciclovir: *nucleoside analog*	CMV, HSV	2.6	28-fold	UL54 UL97	generic
maribavir: *viral protein kinase inh*	CMV, EBV	0.25	24-fold	UL97	Takeda 2026
letermovir: *viral terminase inh*	CMV	0.003	4-fold	UL56 terminal resistance	Merck 2024 (oral) 2033 (injectable)
cidofovir: *viral DNA pol inh* (tox-limited)	CMV, HSV	0.64	>100-fold	UL54	generic
foscarnet: *pyrophosphate mimic* (tox-limited)	CMV, HSV	200	n.d.	UL54	generic

Table 2. EV-100 is an allosteric inhibitor of human SIRT2 protein. Compared to marketed drugs, EV-100 is broadly active against multiple opportunistic viruses; it is equivalent or more potent compared to most in antiviral EC_{50}; it exhibits tighter virologic control at maximum inhibitory concentrations; and viral gene mutations are not expected to confer drug-resistance. In September 2024, Evrys completed an FDA Type B pre-IND meeting providing alignment on GLP-tox species (rat and mini-pig), CMC (with 3 kg pilot batch ready for GLP-tox studies), virology plan, and first-in-man trial design for clinical phase 1 single ascending/multiple ascending dose trial.

SIRT2 modulation to functionally cure chronic hepatitis B

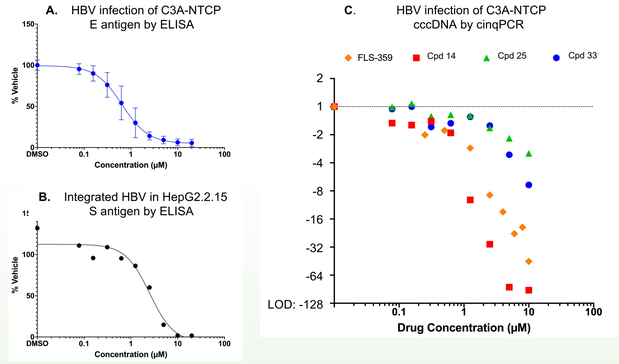
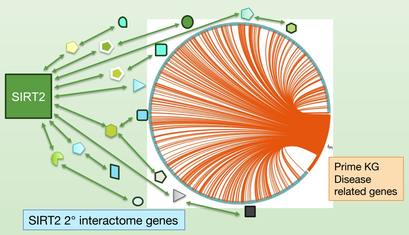


Figure 3. HBV E antigen (**A**), S antigen (**B**), and cccDNA (**C**) were assayed in HBV infected C3A-NTCP or in HepG2.2.15 cells. FLS-359 reduced both E and S antigen, EC_{50} = 0.7 and 1.5 μM, respectively. FLS-359 inhibited cccDNA by 45-fold. Leads improved from FLS-359 also inhibit cccDNA up to 128-fold, the limit of detection, for Cpd 14.

Exploring use of SIRT2 modulators for non-infectious indications



Disease	SIRT2 Interactome Disease Overlap (%)
Anemia	96% (24/25)
Melanoma	94% (45/48)
HIV infection	91% (29/32)
B cell lymphoma	86% (49/57)
Myocardial ischemia	81% (132/163)
AML	79% (137/174)
Breast cancer	**78% (110/142)**
Gastric cancer	74% (224/302)
Lung cancer	70% (389/556)

p < 0.001 FDR adjusted PrimeKG overlap

Marker	CDK4/6^R Breast Cancer Rational	Ref. and Evrys Validation
SIRT2	SIRT2 is upregulated in tumor cells. Cyclin E/CDK2 can phosphorylate SIRT2 at S331, attenuating its activity	Zhao 2014 *J.Clin.Invest.*; Pandithage 2008 *J.Cell.Biol.*
c-Myc	SIRT2 modulation induces c-Myc degradation in ER⁺ MCF7 and triple-negative MB-231	Jing 2016 *Cancer Cell* Liu 2013 *Cell Death Differ.* Evrys SIRT2 modulators confirm in cells and xenografts
pRB	Myc induces CDK4/6i resistance by promoting pRB degradation	Kim 2023 *Cell Death Differ.*; Ma 2024 *Nat.Commun.*
Aurora kinase A	Aurora kinase A interacts with c-Myc; SIRT2 modulation reduces Aurora A levels in MB-231 cells	Dauch 2016 *Nat.Med.*; Liu 2013 *Cell Death Differ.* Evrys SIRT2 modulators confirm in multiple cell lines
Mcl-1	FLS-359 blocks induction of Mcl-1 by virus infection in primary human monocytes	Cheung 2024 *Antiviral Res.* Evrys SIRT2 modulators confirm in cells and xenografts
Akt	PI3K pathway is commonly activated in breast cancer	Vasan 2019 *Annals Oncology* Evrys SIRT2 modulators confirm in cells and xenografts
Slug	SLUG induces tumor cell migration and contributes to malignancy in multiple cancers, including SUM149 breast cancer cell; SIRT2 stabilize SLUG	Zhou 2016 *Cell Rep.* Evrys SIRT2 modulators reduce tumor cell migration
Cell Cycle Status	SIRT2 modulator induces cell cycle arrest, MCF-7 and triple-negative MB-468 cells	Wawruszak 2022 *Cells*
Apoptosis	FLS-359 induces apoptosis in primary human monocytes; SIRT2 modulation activates caspase 3 in multiple breast cancer cell lines	Cheung 2024 *Antiviral Res.* Wawruszak 2022 *Cells*

SIRT2 Modulation as a Biodefense Medical Countermeasure

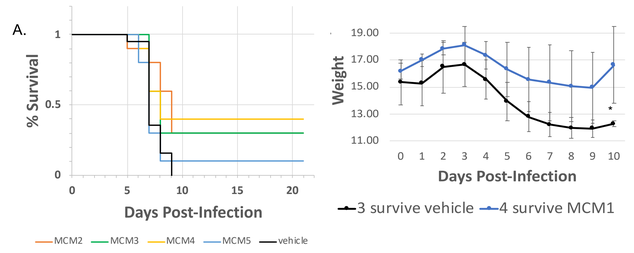


Figure 4: SIRT2 modulation protects mice against Ebola Sudan challenge. IFN-α/βR–/– mice were injected i.p. with Ebola Sudan (SUDV; 1,000 pfu). Four SIRT2 modulators, structurally related to FLS-359, were administered at 100 mg/kg p.o. q.d. (n=10 per drug arm) starting 1 h post-SUDV challenge. The data 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. **B.** Among survivors, MCM1-treated mice showed statistically significant improved weight gained (*p=0.02).

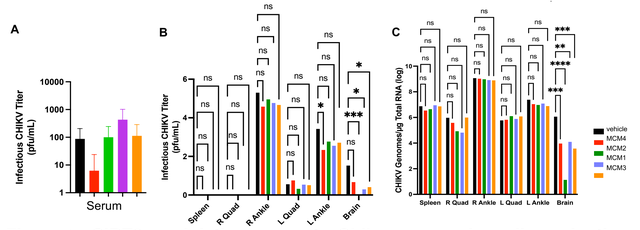
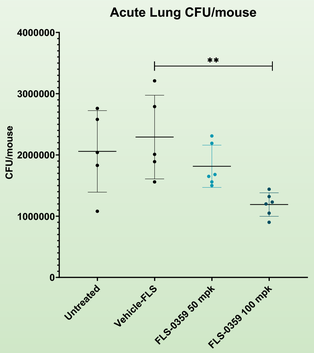


Figure 5: SIRT2 modulation prevents Chikungunya virus dissemination to the brain. Eight-week-old C57BL6 mice were infected with 1000 pfu Chikungunya virus (CHIKV) by foot pad injection. SIRT2 modulating drugs (MCM1-4) or vehicle (0.5% methylcellulose/0.5% Tween 80/water) (n=8 per group with legend at 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=0.02, ***p=0.001, ****p=0.0001).

Figure 6: SIRT2 modulation reduces *Mycobacterium tuberculosis* (*M.tb*) bacterial load in infected mice. Female BALB/c mice were challenged with 100-200 CFU M.tb (Erdman strain) by aerosol challenge. Infection proceeded with CFU check at d 10 post-infection to ascertain bacterial load. Vehicle or FLS-359 (50 mg/kg/d) was then given orally d 11-30 post-infection. Shown is CFU/mouse. **p <).001 by 1-way ANOVA. Note that FLS-359 does not inhibit free growing *M.tb* (MIC_{90} > 100 μM).



All animal research was conducted under an Institutional Animal Care and Use Committee (IACUC) approved protocol in compliance with the Animal Welfare Act, Public Health Service Policy on Humane Care and Use of Laboratory Animals, and other federal statutes and regulations relating to animals and experiments involving animals. The facility where this research was conducted is accredited by the AAALAC International and adheres to the principles stated in The Guide for the Care and Use of Laboratory Animals, National Research Council, 2011.

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