GALILEI BioSciences

Innovation in Neuroprotection

CORPORATE PRESENTATION

December 2024

Galilei Biosciences

Executive Summary

- **World-class scientific team** and network
- **Strong rationale for Sirt6** as a therapeutic target in **glaucoma**
- **PoC of Sirt6 activation** confirmed in several **glaucoma disease models**
- **Clear rationale and development plan** towards **neurodegeneration in glaucoma** developed
- Considerable **upside potential** in additional promising **indications with confirmed PoC**
- Unique approach to **age-related diseases** (and **longevity**) due to critical role of Sirt6 in homeostasis
- Several **tool compounds identified** with demonstrated **Sirt6 target engagement**
- Medicinal chemistry **optimizations** are ongoing to identify **suitable candidates for *in vivo* PoC studies**
- **Patent application** has been filed for first-generation Sirt6 activators

Seeking $4.9 M to reach preclinical candidate for glaucoma

Galilei

World-Class Leaders in Sirtuin Research

John M. Denu
Professor at UW-Madison, leading Sirtuin biochemist

Leonard P. Guarente
Professor at MIT, leading Sirtuin biologist and entrepreneur

Raul Mostoslavsky
Professor at HMS, leading Sirtuin cancer biologist

David Sinclair
Professor at HMS, leading Sirtuin biologist and entrepreneur

Our founders are renowned experts and pioneers in sirtuin research, bringing unparalleled expertise in genetics, biology, and biochemistry to advance cutting-edge scientific innovation.

Scientific Background

Sirt6 - Functions

Sirt6 is an NAD^+-dependent histone deacetylase and mono-ADP-ribosyltransferase which has multiple functions and plays a key role in a variety of biological processes for maintaining cellular and organismal homeostasis.

PoC in mice, using Sirt6 activator tool compound MDL-800, confirmed[1]:

Retinal diseases (glaucoma)

Liver diseases: Acute liver failure (ALF)

Heart failure

Cutaneous wound healing

Osteoarthritis

Non small cell lung cancer (NSCLC)

Hepatocellular carcinoma (HCC)

Strong rationale for Sirt6 as a therapeutic target in multiple diseases

[1)]see backup

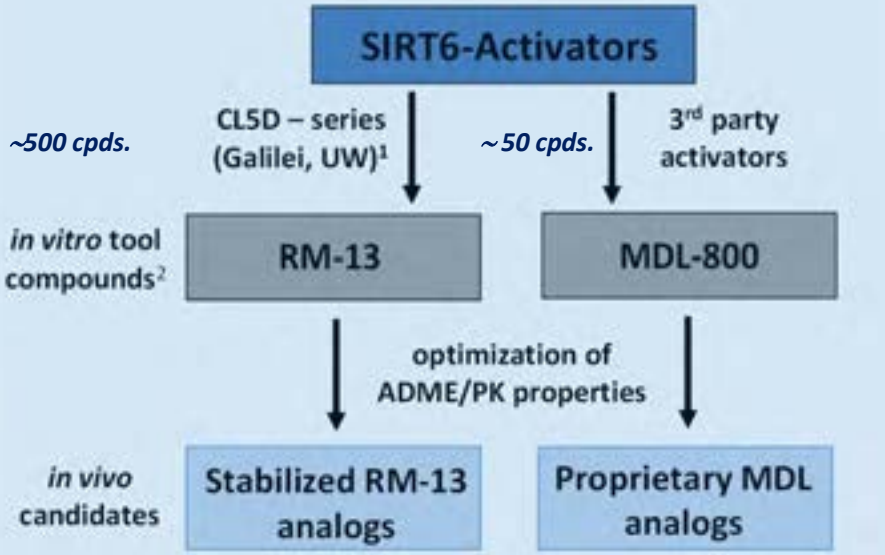
SIRT6 – Activator

Strategy - Status

- Galilei successfully developed structurally diverse small molecule Sirt6 activators
- Most advanced tool compounds showed good *in vitro* properties and confirmed Sirt6 target engagement
- Medicinal chemistry efforts are focused on improving biochemical activity and PK properties
- Important biological functions have been investigated and small molecule Sirt6 activation has demonstrated beneficial effects in:
  - Neuroprotection
  - Mitochondrial function
  - Anti-Inflammation
  - Metabolic regulation
  - DNA Repair

Sirt6 is an NAD$^+$-dependent histone deacylase:

Medicinal chemistry strategy:



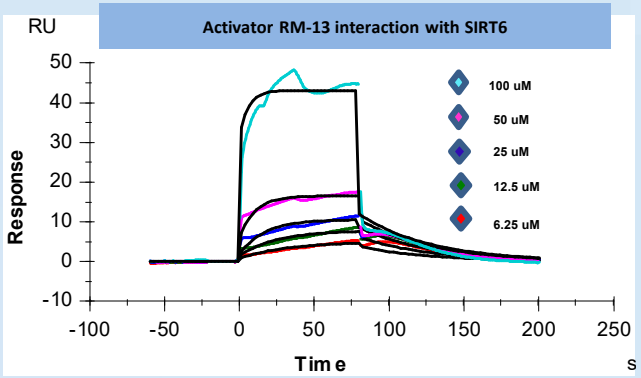
[1)]Klein 2020
[2)]EC_{50} ~10 µM; $EC_{1.5}$ ~5 µM, metabolically labile
$EC_{1.5}$: 1.5-fold activation

SIRT6 – Activator

Target Engagement

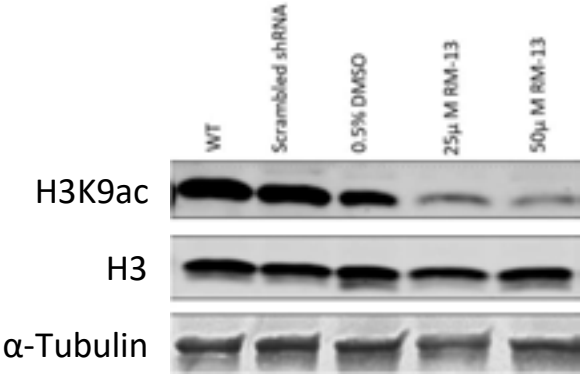
X-ray soaking experiments confirm binding of activators to Sirt6 allosteric site

Concentration dependent binding of activator RM-13 to Sirt6 (SPR)



Sample	ka (1/Ms)	kd (1/s)	KD (M)	Rmax (RU)
RM-13	1488	0.02125	1.428E-05	13.62

RM-13 activated histone H3 deacetylation (H3K9ac) of Sirt6 in HepG2 cells



Target engagement of multiple Sirt6 activators has been validated through crystallography, SPR and Western blot.

SPR surface plasmon resonance

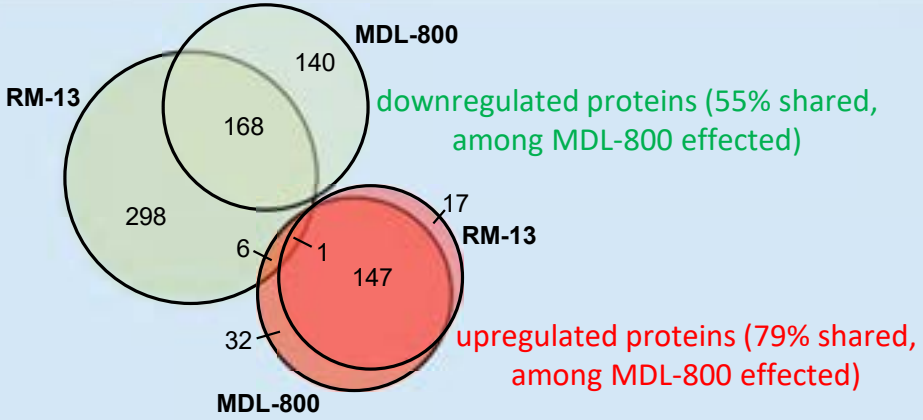
SIRT6 – Quantitative Proteomics

Sirt6 Activators RM-13 and MDL-800 Restore the Proteome of Sirt6-deficient Cells

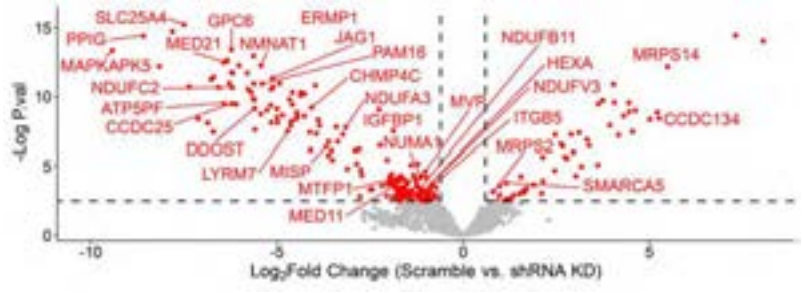
In HepG2 cells with 70% reduction in Sirt6 levels:

- Sirt6 activators RM-13 and MDL-800 (10-50 µM) were applied for 24 hours
- Quantitative proteomics assessed changes in protein expression

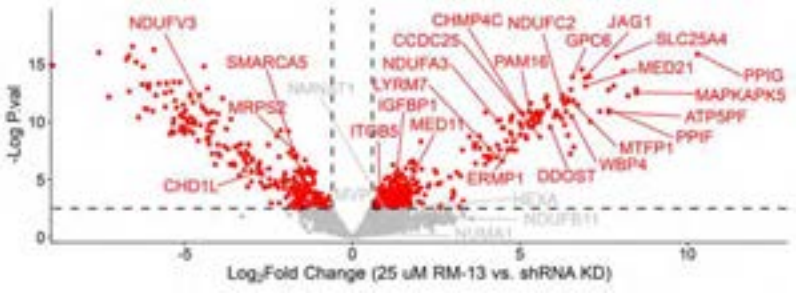
→ **53% of the overall proteome is restored,** including 65% of proteins that are positively regulated by Sirt6



SIRT6 genetic knockdown to 30%:



SIRT6 activator treatment (RM-13):



Mitochondrial functions are the most consistently affected pathways
(complex I & II assembly, mitochondrial protein import, ATP/ADP and pyruvate transporters, oxidative and fatty acid metabolism)

Scientific Background

Rationale for Sirt6 in Glaucoma[1]

- **Sirt6 deletion** results in progressive **degeneration of RGCs and optic nerve** during aging and in mouse models of high-tension glaucoma (HTG)
- **Sirt6 deletion** contributes to **microglial recruitment/activation and inflammatory cell infiltration** and loss of myelin sheaths
- **Overexpression of Sirt6** in mouse models **protects against high-IOP-induced RGC degeneration**
- **Pharmacological activation of Sirt6** with small molecule activators is effective in **reducing RGC loss and inflammation in high-IOP-induced glaucoma models**
- **Sirt6 expression is high in the ganglion cells** in the retina and **low in the inner and outer nuclear layer**
- **Loss of Sirt6 activity** accelerates **RGC senescence** and induces **mitochondrial dysfunction**

Strong rationale for Sirt6 as a therapeutic target in glaucoma and as a potential marker

[1)]Xia 2024
IOP intraocular pressure
RGC retinal ganglion cell

SIRT6 – Activator

Glaucoma: A Condition with Unmet Medical Needs (Neuroprotection/Neuroregeneration)

- **Glaucoma affects millions of people globally**, with a significant percentage remaining undiagnosed or inadequately treated (prevalent population in 7MM in 2020/2030: 46/50M)[1]. US Glaucoma market $2.8 Billion 2022 rising to $3.76 Billion by 2030.
- Glaucoma is characterized by **progressive optic nerve damage** and the **loss of retinal ganglion cells** (RGCs).
- Current treatments, primarily focused on **lowering IOP**, do not address the underlying causes of **optic nerve damage** or progression of the disease. Some patients may not achieve adequate IOP control with existing medications, leading to **disease progression**.
- Current glaucoma development is focused on traditional **drug combinations** and **reformulations** (sustained-release implants (PGA), FDC)), not addressing optic nerve degeneration.

Ideally, a glaucoma drug would not only lower IOP but also provide **neuroprotective** effects to safeguard **retinal ganglion cells** and the **optic nerve from degeneration**.

A complementary **novel mechanism** can enhance current treatments by targeting pathways beyond IOP reduction, such as **inflammation** or **neuroprotection**.

SIRT6 activators show great potential to meet essential requirements for a groundbreaking and effective glaucoma treatment by offering neuroprotection, reducing inflammation and promoting tissue repair.

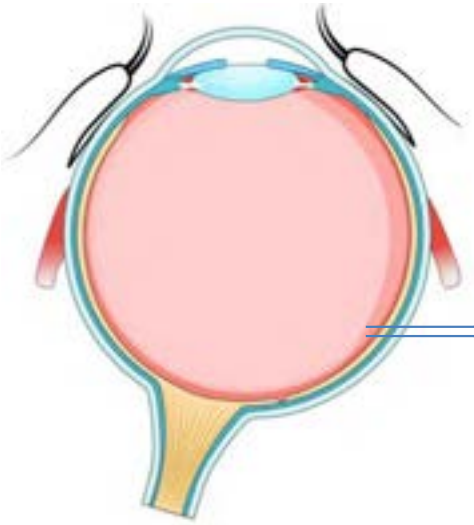
[1]Global Data, report GDHC238PIDR, January 2022, Grand Review Research - Glaucoma marker report 2022
GlobalData expects that if a neuroprotective drug were developed for glaucoma, it would achieve a large market share and possibly blockbuster status.

PGA prostaglandin analog
FDC fixed-dose combination

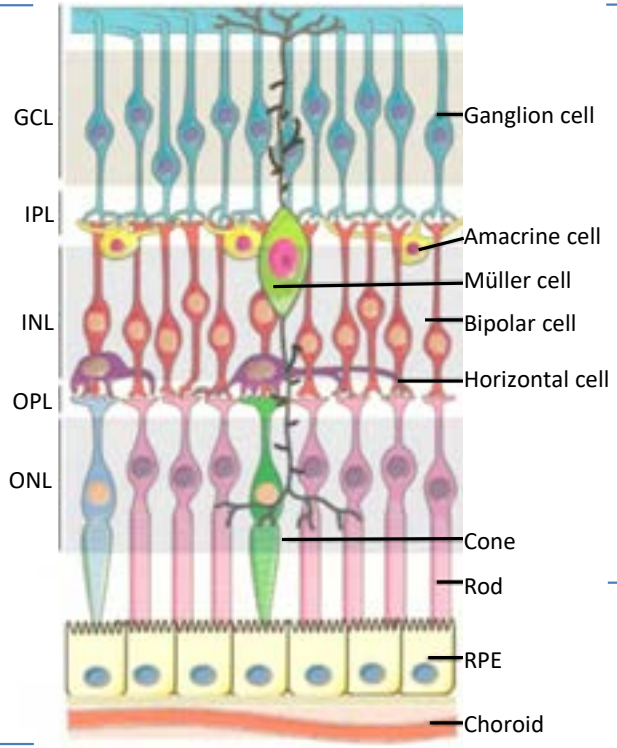
SIRT6 – Activator

Glaucoma and Sirt6 – Key Results[1]

light

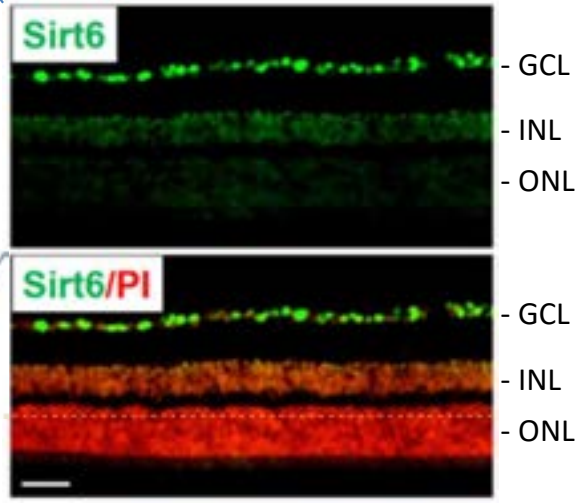


cross section of eye



cross section of retina

Expression of Sirt6 in the retina:
Retinal frozen sections were stained with Sirt6 (green) antibody, and counterstained with PI (red) for the nuclei.



stained retinal frozen sections

Sirt6 is highly expressed in retinal ganglion cells (RGCs) and helps protect them from degeneration.

GCL	ganglion cell layer.
IPL	inner plexiform layer
INL	inner nuclear layer
OPL	outer plexiform layer
ONL	outer nuclear layer
RPE	retinal pigment epithelium

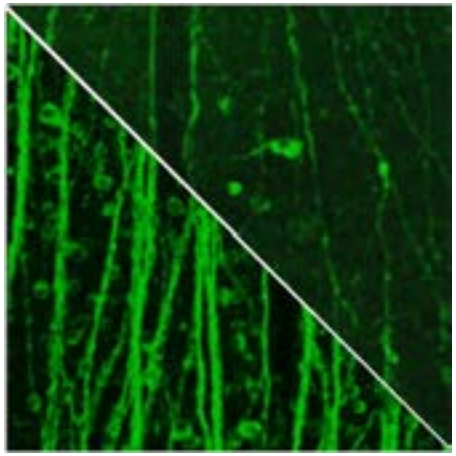
[1)]Xia 2024

SIRT6 – Neuroprotection

Sirt6 is an Attractive Target for the Treatment of Glaucoma[1]

Sirt6 overexpression is **neuroprotective** in the glaucoma IR model

RGC axon bundles are highly degenerated in the mouse IR model (6-month-old mice)



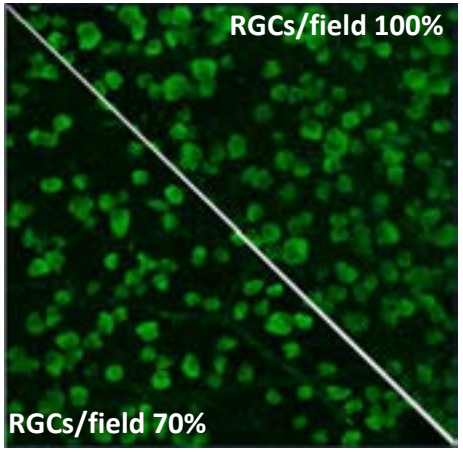
Sirt6 overexpression protects axons from degeneration (6-month-old mice)

Deactivation of Sirt6 leads to progressive **degeneration of optic nerve**

Sirt6 deletion induces optic nerve degeneration (7-month-old mice)

Pharmacological activation[2] of Sirt6 leads to **reduced RGC injury** in the glaucoma IR model

Dramatic reduction of RGC loss by Sirt6 activation (w/ tool compound)



Decreased RGC density in the retina in the mouse IR glaucoma model

Sirt6 protects retinal ganglion cells and optic nerve from degeneration.

[1)] Xia 2024
[2)] tool compound: MDL-800
IR ischemia reperfusion

Disease-associated Genes Can Be Used to Predict Clinical Efficacy

Targets Genetically Linked to a Disease Found in GWAS Have a Higher Success Rate in Clinical Trials

BIOBUSINESS BRIEFS

TRIAL WATCH

Impact of genetically supported target selection on R&D productivity

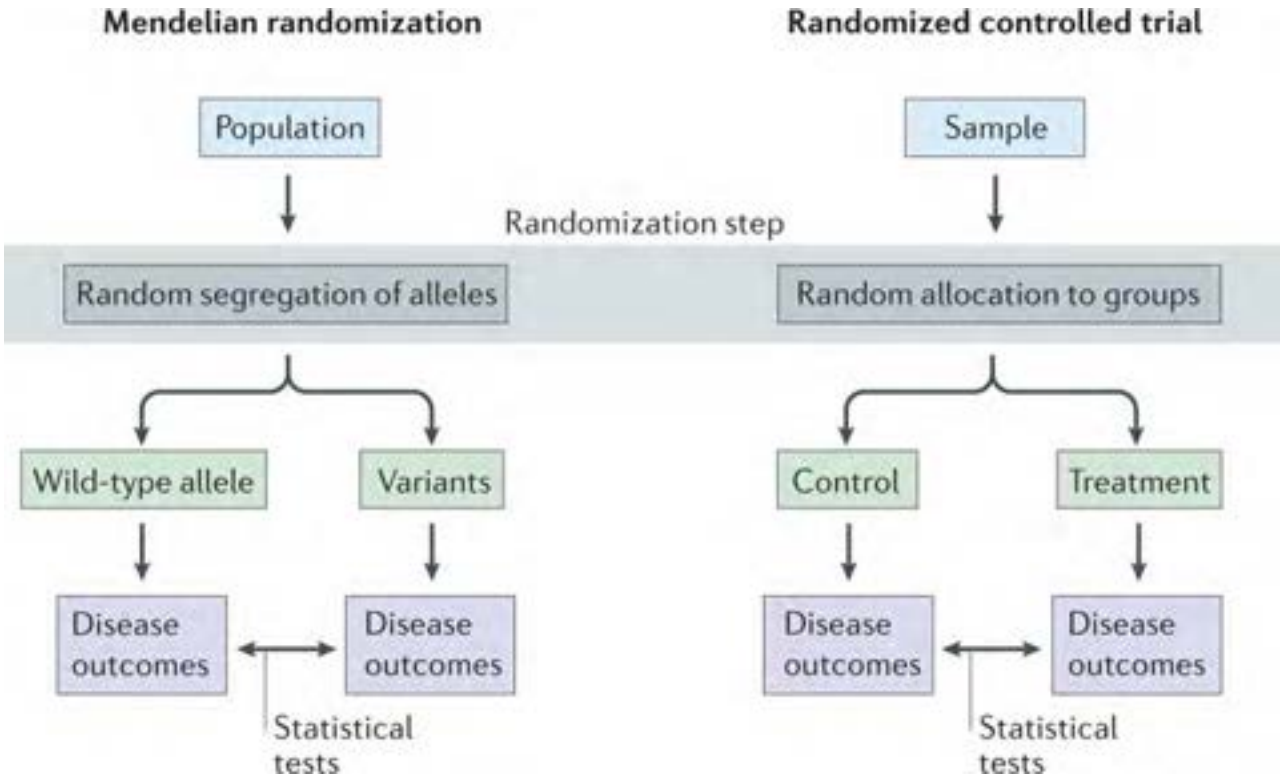
gsk

PLOS | GENETICS

RESEARCH ARTICLE

Are drug targets with genetic support twice as likely to be approved? Revised estimates of the impact of genetic support for drug mechanisms on the probability of drug approval

Emily A. King*, J. Wade Davis, Jacob F. Degner



Strong evidence of Sirt6 expression associated with decreased risk of glaucoma, pancreatic cancer and autoimmune thyroid disease (impact of Sirt6 expression on thyroid)

GWAS genome-wide association studies

SIRT6 – Activator

Towards PoC in Glaucoma

1. Lead structure optimization to meet target research profile for *in vivo* candidate
2. *In vivo* PK study including tissues distribution (mice, rats)
   - orally (IP or IVT), single dose, sampling: plasma, retina, vitreous
3. *In vivo* efficacy in glaucoma model
   - final model will be selected based on scientific rational and partner interest

Most used *in vivo* ocular hypertension and normal-tension glaucoma models[1]:

- ischemia-reperfusion
- microbead
- optic nerve crush

Example: microbead model (mice, rats) - intracameral injection of magnetic microbeads (orally or IP or IVT), daily (2 weeks)

Model:
1. IOP measurement
2. intracameral injection (microbeads)

Grouping and dosing:
1. IOP measurement
2. drug dosing

Testing (*in vivo*):
1. IOP measurement
2. ffVEP or pattern ERG
3. optomotor response

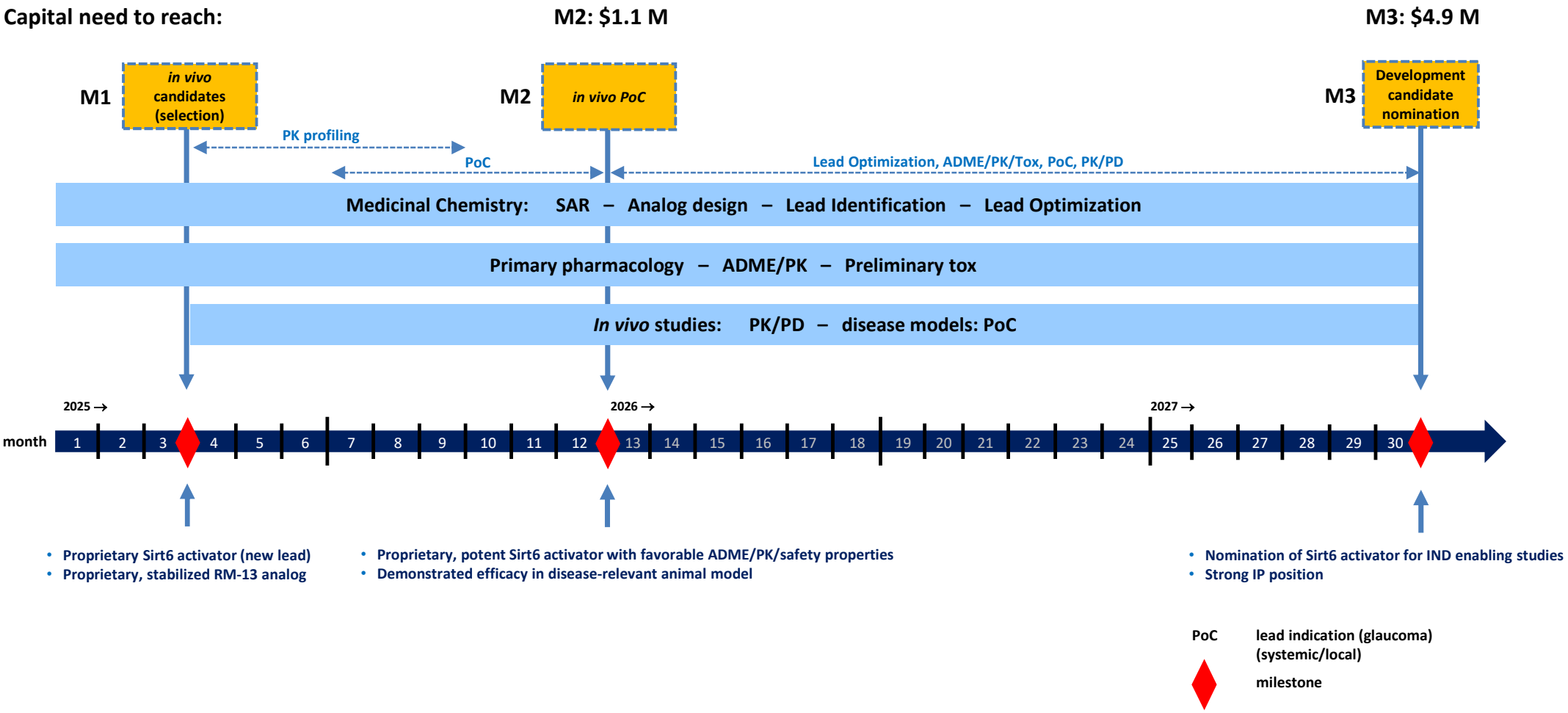
Testing (*ex vivo*):
1. Retinal wholemount (RBPMS staining/counting)
2. Axon slice (PPD staining/counting)

[1] "neuroprotective" control compounds used: brimonidine or BDNF

IOP	intra ocular pressure	ERG	electroretinography
IP	intraperitoneal	RBPMS	RNA-binding protein with multiple splicing
IVT	intravitreal	PPD	paraphenylenediamine
ffVEP	full field visual evoked potential		

SIRT6 – Activator

Development Timelines/Cost Towards Development Candidate Nomination for Glaucoma



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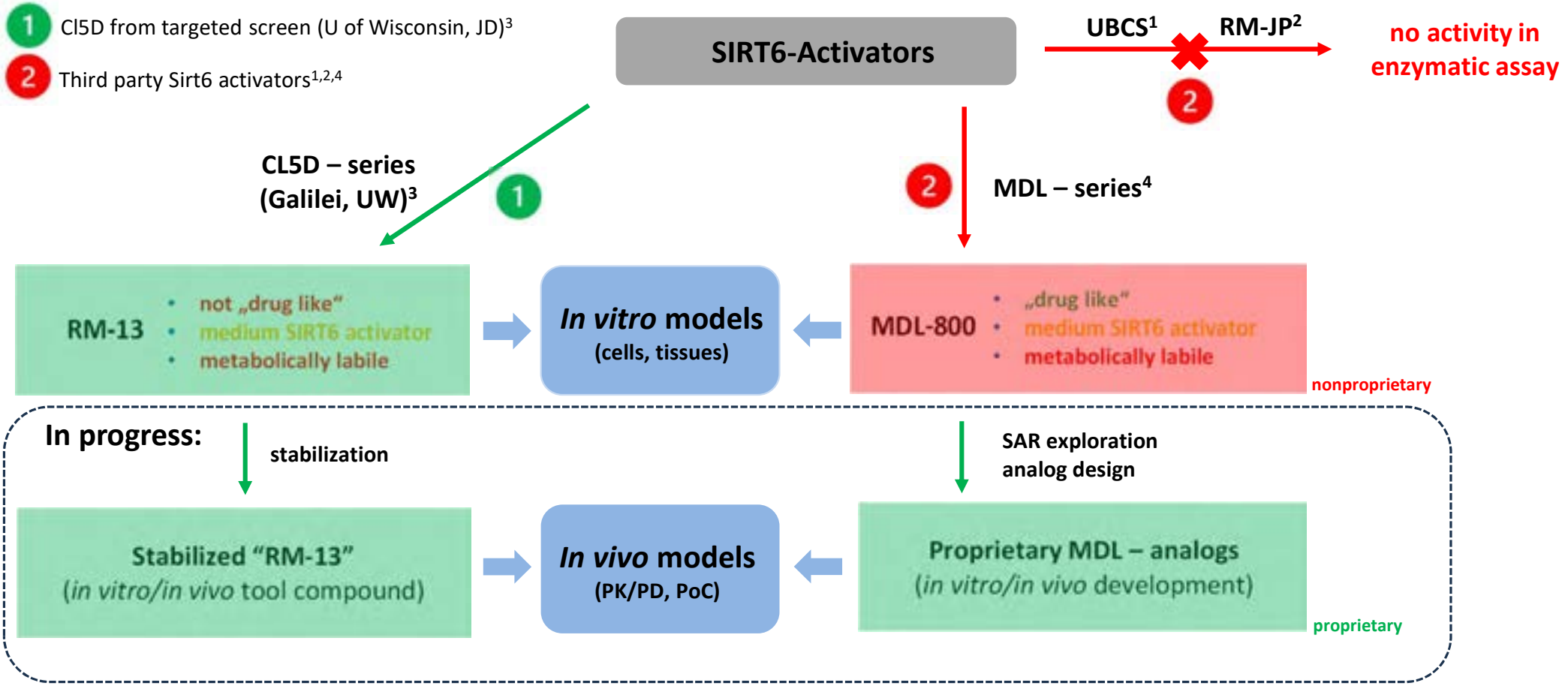
Backup

SIRT6 – Activator

Strategy - Progress

Starting points:

1. Cl5D from targeted screen (U of Wisconsin, JD)[3]
2. Third party Sirt6 activators[1,2,4]



[1]You 2017, Xu 2023
[2]WO2020153434A1
[3]Klein 2020
[4]Huang 2018

SIRT6 – Activator

Status and Goals

Aim: proprietary, small molecule, selective SIRT6 activator (EC_{50}<1µM), orally available, high permeability/tissue distribution, PoC in lead indication

- **Primary and secondary assays established** (biochemical HPLC, biophysical SPR, cellular WB)
- **SAR** of several "drug-like" lead series, including third-party SIRT6 activators, has been **explored**:
  - **well-established SAR** and identified key positions are driving promising optimizations
  - **proprietary activator qualified** as *in vitro* tool compound
- **Strong therapeutic indications** with confirmed positive impact of SIRT6 activation identified
- **Glaucoma identified as primary indication**, supported by robust Mendelian randomization analysis[1], strong publication[2], and a well-defined development plan for rapid progress.
- Specialized CROs in ophthalmology with **established disease models and expert network team** identified

SIRT6 activator small molecule optimization is in progress with first promising therapeutic focus on glaucoma treatment.

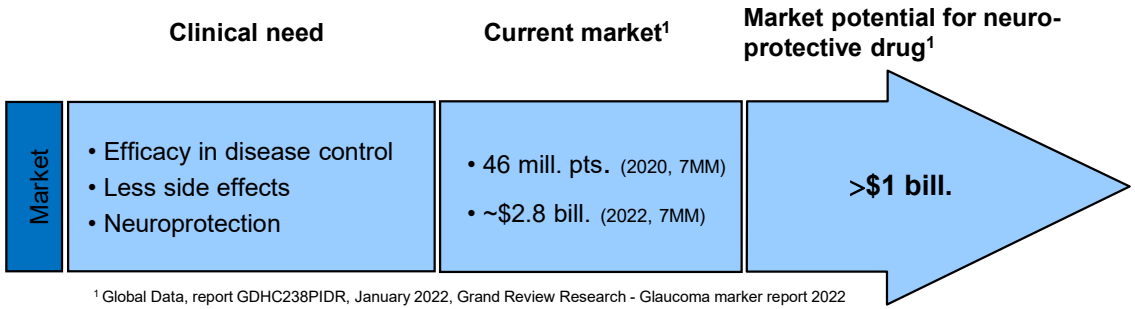
SPR surface plasmon resonance
WB Western blot

[1)] BioTx
[2)] Xia 2024

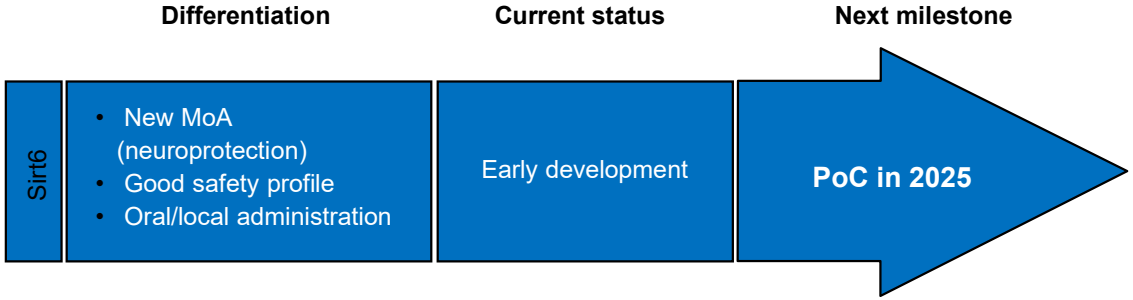
SIRT6 – Activator

Target Indication Glaucoma: A Condition with Unmet Medical Needs

- Gaucoma is a common ophthalmic disorder characterized by optic nerve damage and vision loss[2].
- Glaucoma is the leading cause of irreversible blindness worldwide.
- Prevalence in the U.S.: ~1900 cases per 100,000 persons aged >40 years
- Responsible for over 9 million clinic visits annually in the U.S.
- Annual U.S. spending on optic nerve disorders: Estimated at $5.8 billion



[1] Global Data, report GDHC238PIDR, January 2022, Grand Review Research - Glaucoma marker report 2022



[2] Downs 2022

SIRT6 – Activator

Sirt6 Gene KO Studies - Key Results[1]

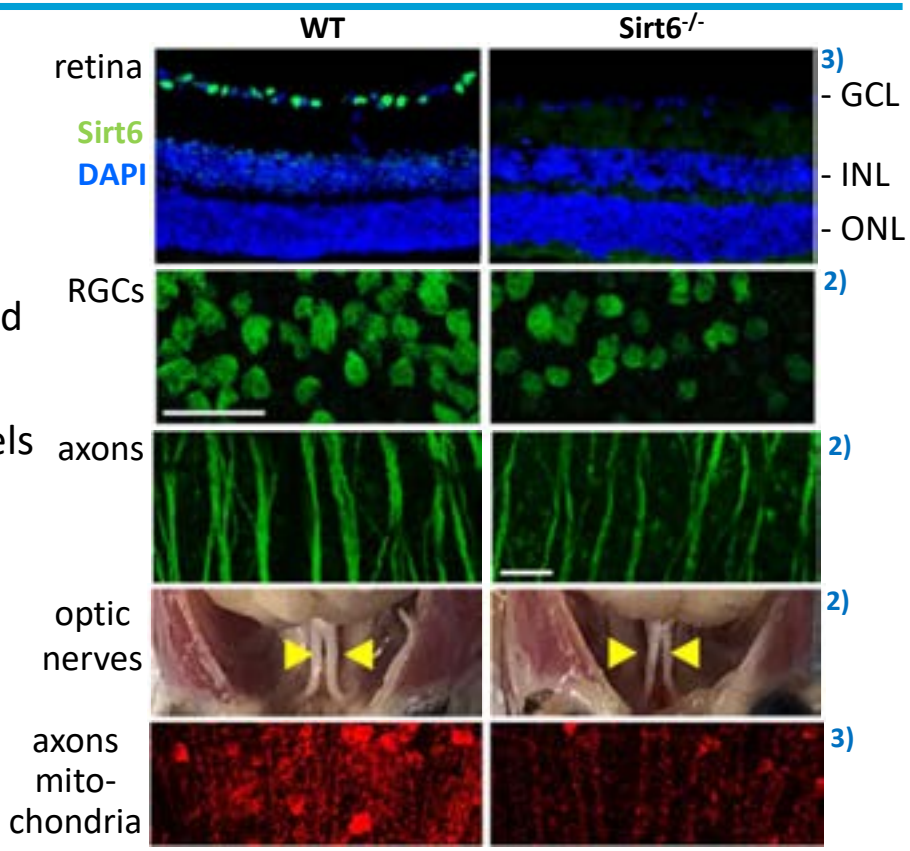
1. **Global Sirt6 knock out (Sirt6$^{-/-}$) mice on mixed genetic background (C57BL/6 and 129/SvJ; 2 months)[2]**
2. **Local Sirt6 deletion in the retina using AAV2-Cre in Sirt6$^{flox/flox}$ mice (C57BL/6; 7 months)[3]**

Validation

- Retinal Sirt6 deletion confirmed by Western blot and immunostaining
- functional loss shown by elevated H3K9/H3K56 levels

Results

- Reduced RGC number and density
- Thinner and shorter RGC axon bundles
- Increased microglial activation and immune cell infiltration
- Thinner optic nerve with fewer intact axons and reduced thickness of myelin sheath



Loss of Sirt6 leads to significant retinal ganglion cell loss, axonal damage, and increased neuroinflammation, indicating its crucial role in retinal and optic nerve health.

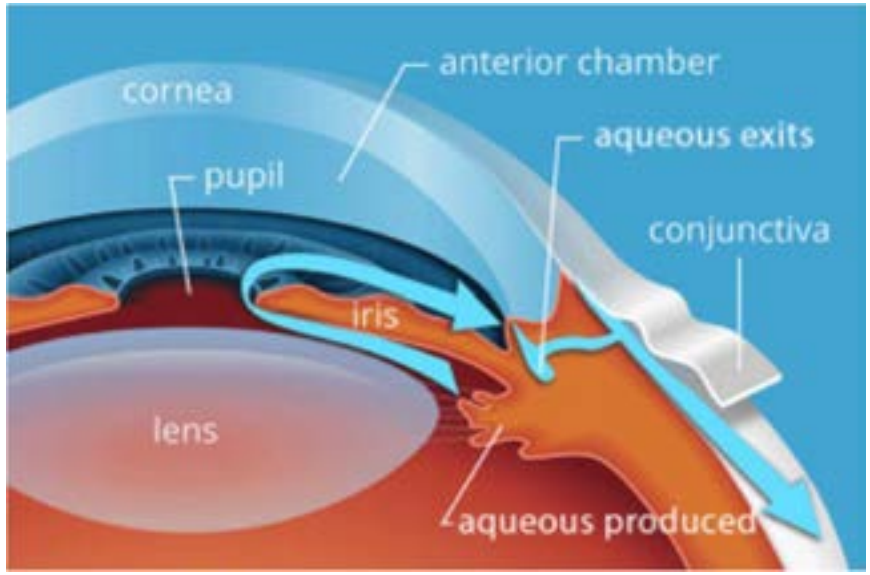
[1)] Xia 2024

[2)] Mixed-background Sirt6$^{-/-}$ mice exhibit extended life span of up to 1 year compared to 4 weeks for pure C57BL/6 background

SIRT6 – Activator

Glaucoma – Different Forms – Risk Factor

- **Forms of glaucoma:**
  - **Primary open-angle glaucoma (POAG):**
    Most common form, typically associated with elevated IOP .
  - **Normal-tension glaucoma (NTG):**
    Optic nerve damage occurs despite normal IOP[1].
  - **Angle-closure glaucoma (ACG):**
  - Less common, involves sudden increase in IOP due to blocked drainage canals.
  - **Secondary glaucoma:**
    Results from other conditions such as eye injuries, inflammation, or tumors.
  - **Congenital glaucoma:**
    Present at birth, due to abnormal development of the eye's drainage system.



All forms of glaucoma are characterized by the loss of the retinal ganglion cells (RGCs) and their axons that make up the optic nerve, which is currently not directly addressed by any treatment.

[1] ~30% - 60% of people with POAG have normal IOP

SIRT6 – Activator

Considerable Upside Potential in Additional Promising Indications

- Association with SIRT6 activation is increasingly recognized in a growing number of peripheral diseases, like:

  - Liver diseases: Acute liver failure (ALF)[1,2]
  - Heart failure[3]
  - Cutaneous wound healing[4]
  - Osteoarthritis[5]
  - Non small cell lung cancer (NSCLC)[6]
  - Hepatocellular carcinoma (HCC)[7]

  } PoC in mice, using Sirt6 activator MDL-800 shown

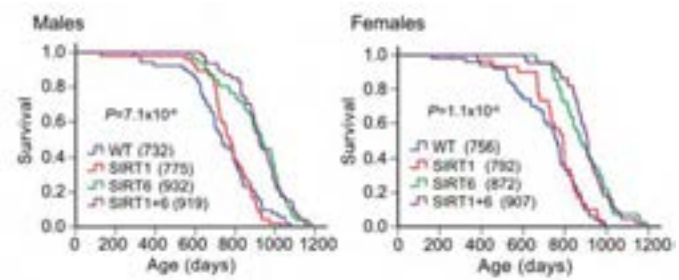
  - Pancreatic cancer
  - Non-alcoholic fatty liver disease (NAFLD), non-alcoholic steatohepatitis (NASDH)
  - Type 2 diabetes (T2D)
  - Cardiovascular disease (CVD)
  - Metabolic syndrome (MeS)
  - Other age-related diseases: Parkinson's disease (PD), Alzheimer's disease (AD)
  - "Longevity"

1) Lancaster 2015; Blieden 2014
2) Luo 2024
3) Wu 2022
4) Jiang 2022
5) Collins 2022; Copp 2023
6) Shang 2021;
7) Huang 2018

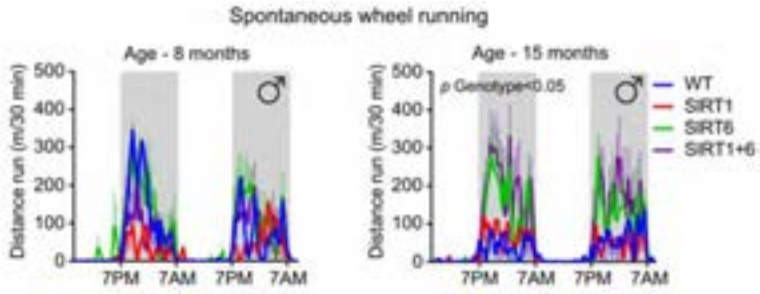
SIRT6 and Aging

SIRT6 Overexpression Extends Lifespan, Improves Frailty and Cognitive Function in Aged Mice[1,2]

Prolonged lifespan:



Improved physical activity:



Sirt6 overexpression in mice results in:

- increased median lifespan by 27% (males) and 15% (females) and enhanced maximal lifespan
- preserved physical activity, reduced frailty indices, and maintained energy homeostasis in old age
- reduced chronic inflammation by downregulating inflammatory pathways
- prevention of age-related lipid accumulation and metabolic dysfunction; maintaining healthy LDL/HDL ratios
- increased mitochondrial biogenesis and antioxidant responses, reduced oxidative damage

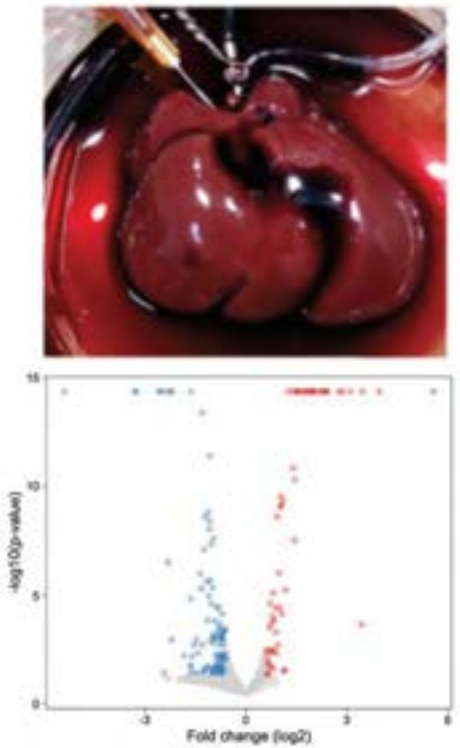
SIRT6 is a master regulator of aging with therapeutic potential to preserve function, combat frailty, and extend health span.

[1] Roichman 2021
[2] Kanfi 2012

Sirt6 – Ex Vivo Studies

Rat Liver Perfusion (UW: JMD and David P. Al-Adra)

- **Rat liver perfusion (UW collaboration: JMD and David P. Al-Adra)[1]**
  - Normothermic *ex vivo* liver perfusion (NEVLP) as an organ preservation method for liver graft functional assessment prior to transplantation.
  - The effects of adding a Sirt6 activator to the perfusion solution are assessed (e.g. immune cells, chemokines, cytokines, liver function, histology, proteomics).
  - Normothermic perfusion solution contains an oxygen carrier to provide oxygen to the liver to sustain metabolic activities.
  - Method allows liver graft functional assessment prior to transplantation, and use of SIRT6 activators to assess endpoints.



Protein level changes (50 µM MDL-800 versus vehicle control, N=3)

SIRT6 activator (MDL-800) induces significant proteomic changes in whole liver *ex vivo* perfusion studies

[1]Jennings 2022

SIRT6 – Activator

Research – TPP (Preliminary)

Property	TPP	RM-13	MDL-800
EC_{50} [μM]	nM range	12.5	12.2
k_{100}/k_0 (fold activation @100 μM)	> 2	5.2	10.8
$EC_{1.5}$ [μM]	nM range	6.5	1.75
MW [g/mol]	< 500	718	626
cLogD	< 3	4.8	4.3
Solubility [μM]	> 100	258	2
Plasma protein binding (m) [%]	< 95	65	99.84
Plasma stability (m) [%]	> 70% remaining @ 1h	100	66
Microsomal stability (m) [%]	> 70% remaining @ 1h	**18**	**1**
Caco-2 Papp [$x10^{-6}$ cm/s] (efflux)	> 10 (< 2)	55 (1.7)	26 (2.8)
MDR1-MDCK Papp [$x10^{-6}$ cm/s] (efflux)	> 5 (< 2)	3.8 (0.86)	4.9 (0.9)

RM-13 and MDL-800 are effective *in vitro* tool compounds with limited microsomal stability, preventing further development.

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References (1/2)

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