

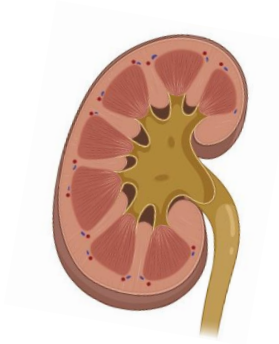
Upregulation of SIRT1 as a therapeutic approach for Chronic Kidney Disease

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Background



?

What is CKD

Chronic kidney disease (CKD) is a condition characterized by a gradual loss of kidney function over time.

10%

adults worldwide
affected by CKD

2M

deaths each year

5th

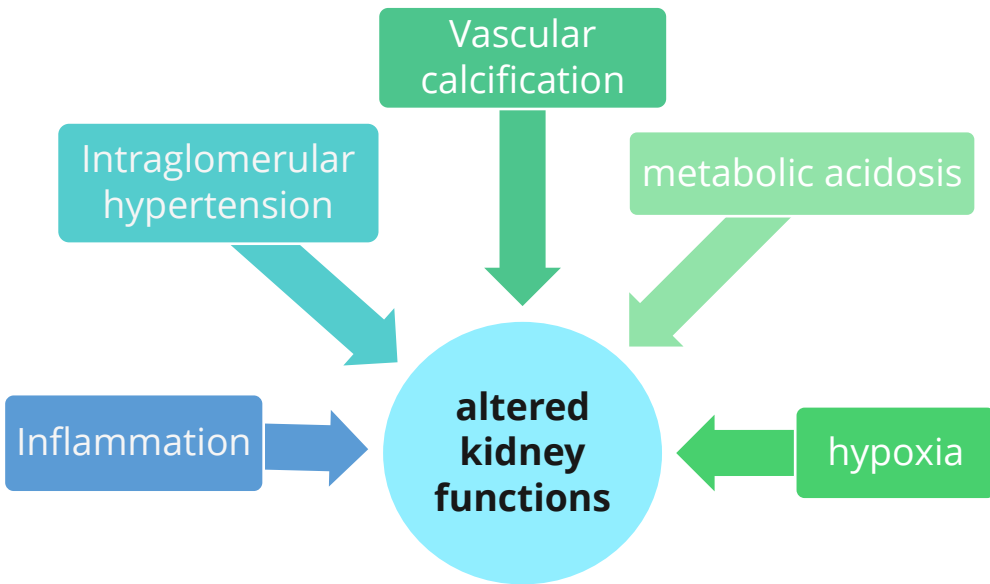
leading cause of death
globally by 2040

Aging disease

Fatal outcomes: kidney failure or
cardiovascular disease

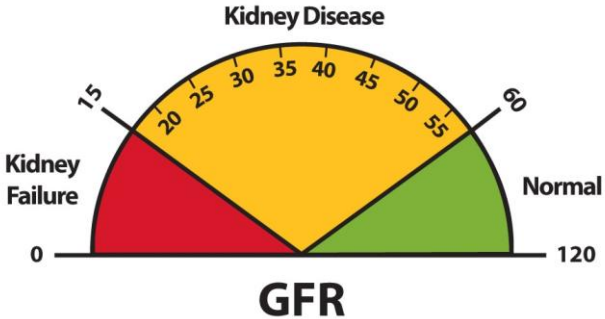


How does CKD affect the kidney



CKD associated markers are:

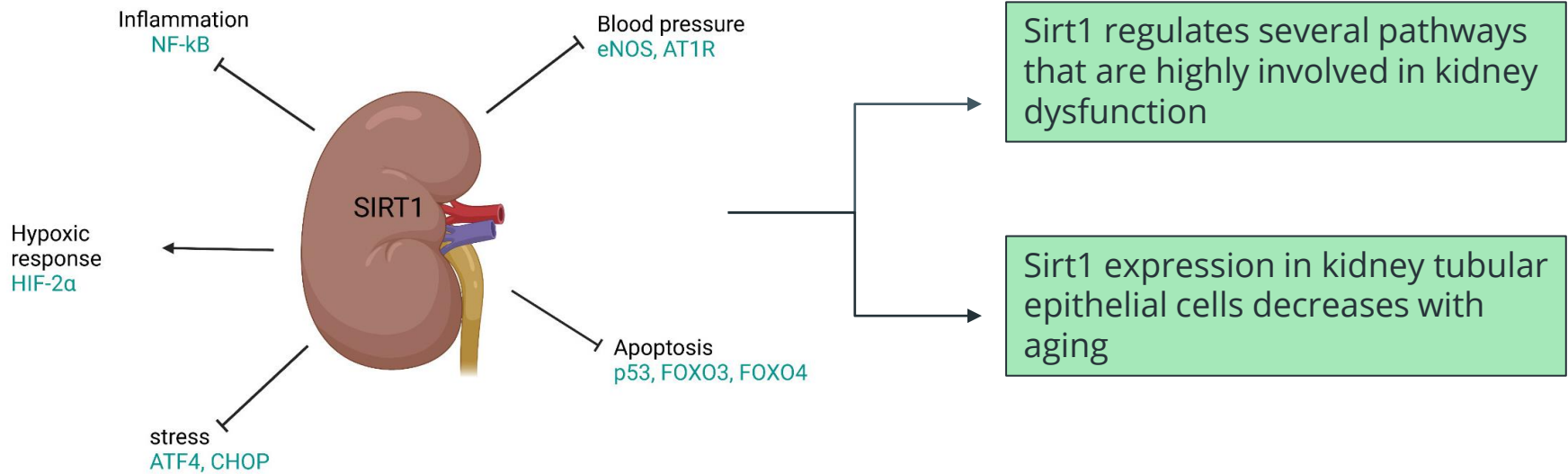
- **low eGFR**
- **albuminuria**
- haematuria
- proteinuria





What is SIRT1

Sirtuin 1 is a nuclear nicotinamide adenine dinucleotide (NAD)⁺-dependent enzyme with deacetylase and mono-ADP-ribosyltransferase activity.



Aim of the project



WHAT?

The overexpression of SIRT1 will reduce the progression of CKD partially restoring the normal functions of the affected kidneys, alleviating symptoms.



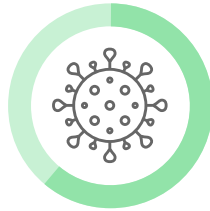
WHERE?

Tubular epithelial cells (TECs).



WHY?

SIRT1 is an optimal target due to its relevance in most of CKD associated molecular pathways.



HOW?

Adeno-associated viral vector 9 (AAV9) expressing SIRT1 transgene.

Tools

Vector

low immunogenicity

safe

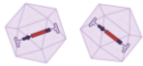
AAV9

selective tissue tropism

stable long-term expression

Transgene

Sirt1



Animal model

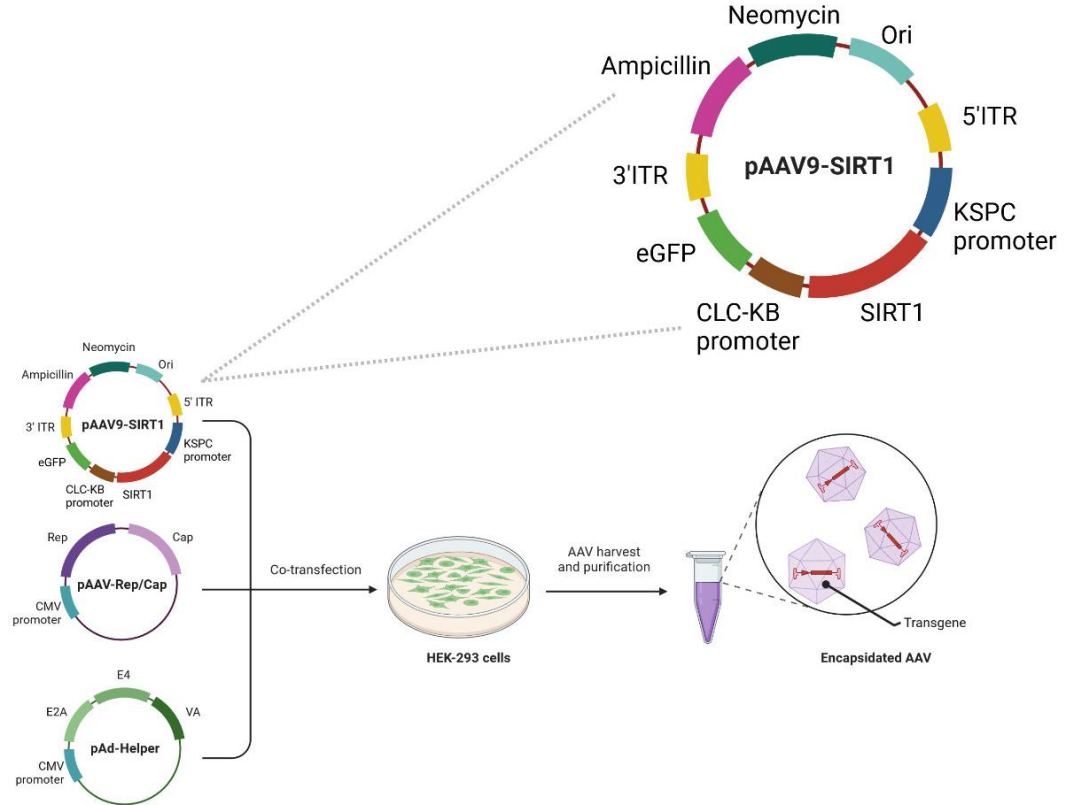


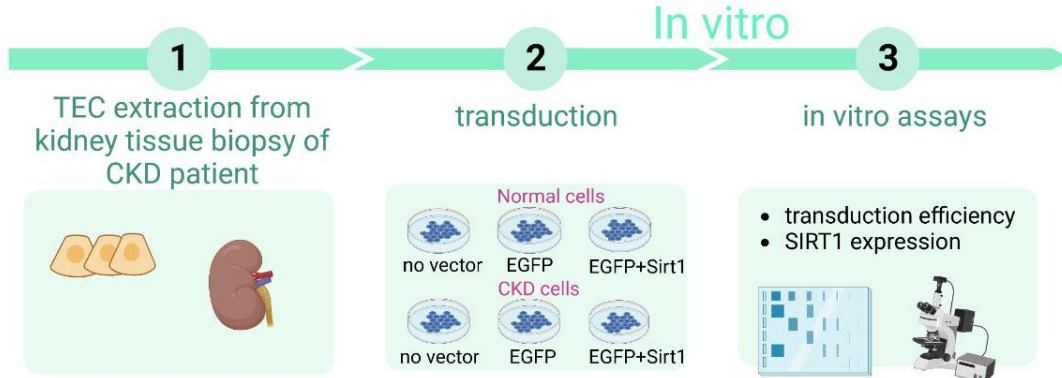
48 ten-weeks old
CKD C57BL/6 mice

Route of administration



retrograde intra-
ureter injection





Experimental plan

Results: In vitro

1. Transduction efficiency

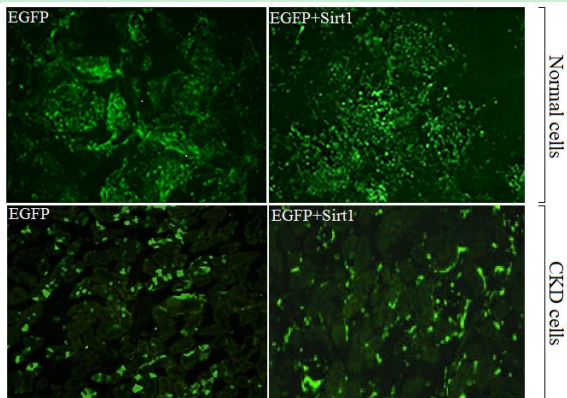
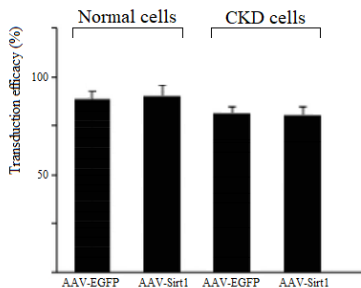


Fig adapted by [10]

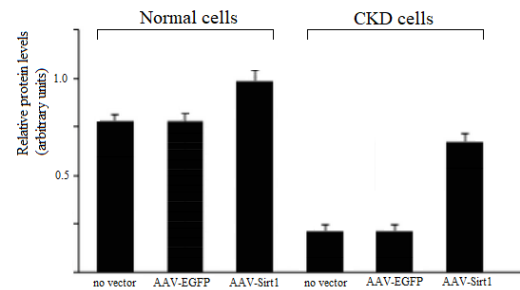
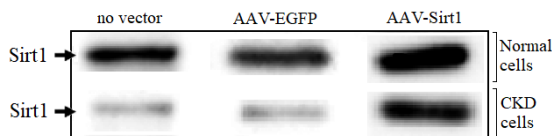
Where?
Tubular
Epithelial cells
(TECs)



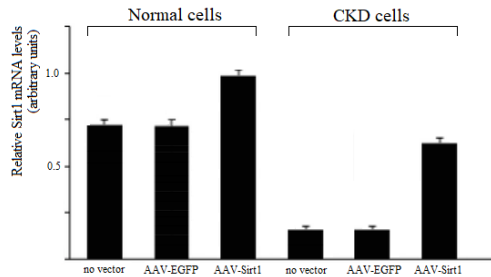
2. Sirt1 expression



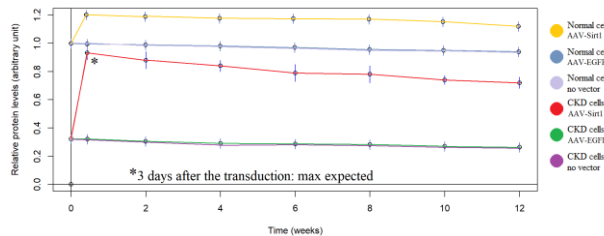
a. Protein levels



b. mRNA levels



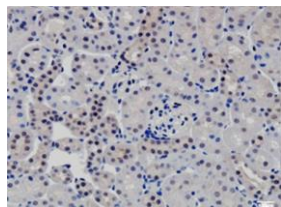
c. Expression over time



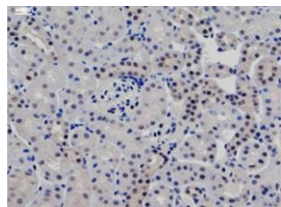
Results: In vivo

1. Immunohistochemistry

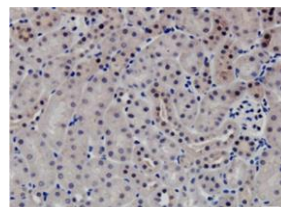
Normal



No vector

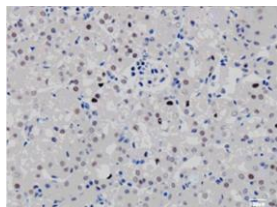


AAV-EGFP

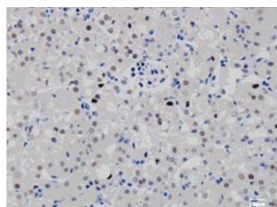


AAV-Sirt1

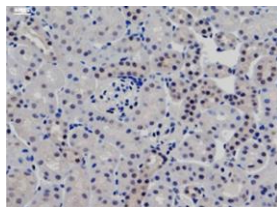
CKD



No vector



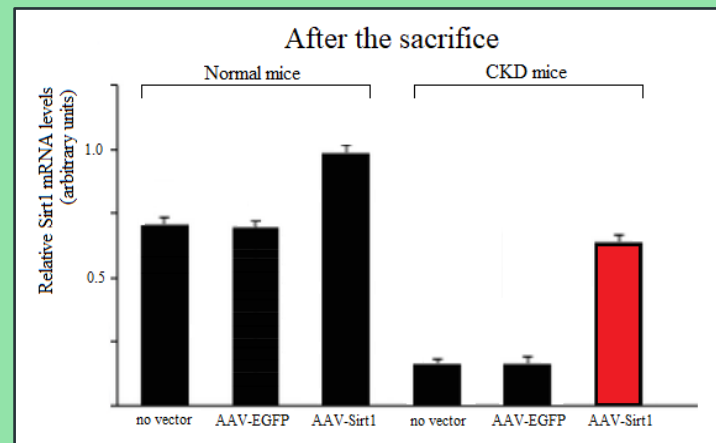
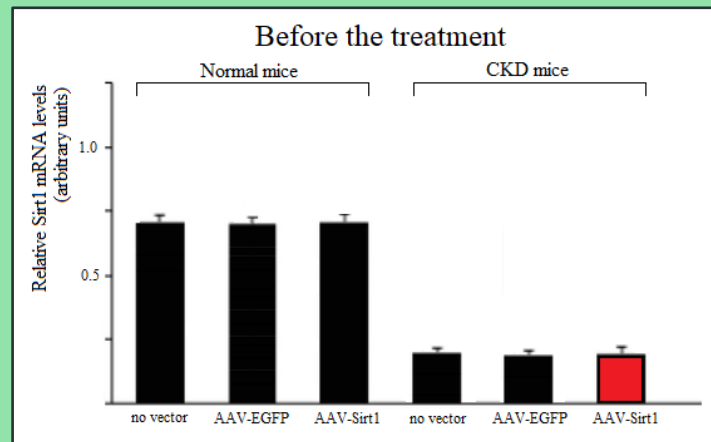
AAV-EGFP



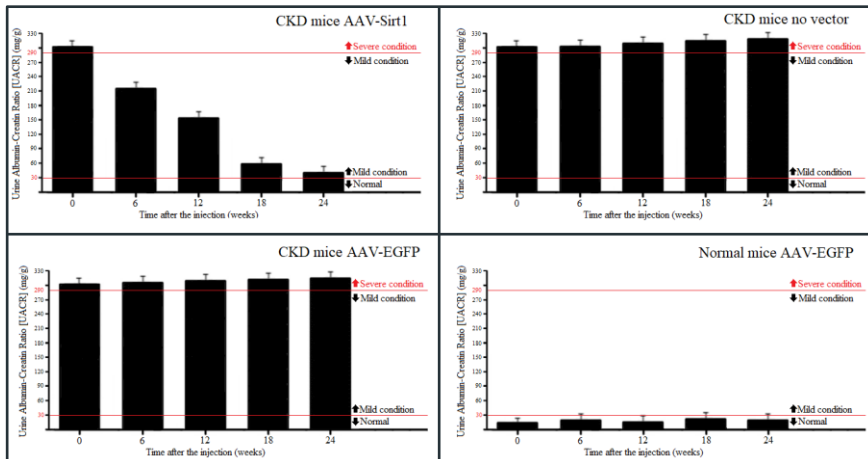
AAV-Sirt1

Fig adapted by [11]

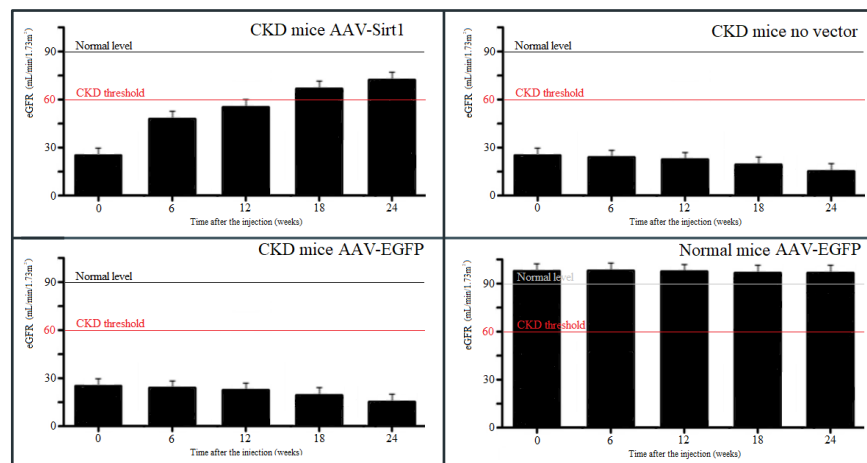
2. qRT-PCR



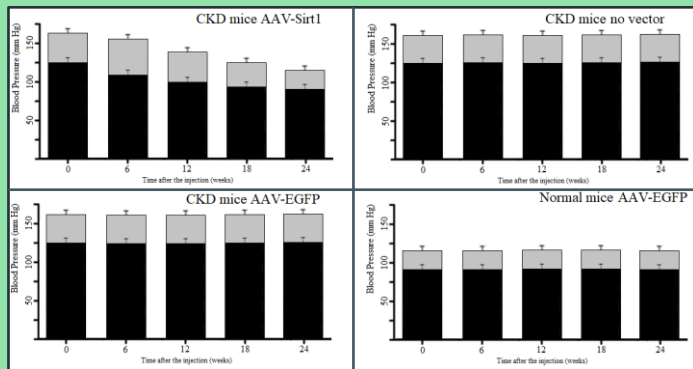
3. Albuminuria control



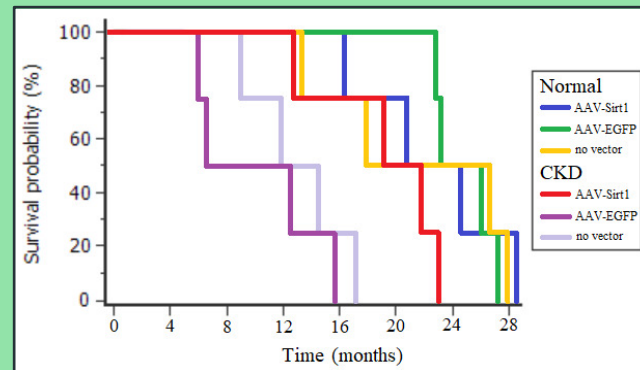
4. eGFR control



5. Blood pressure measurement



6. Survival analysis



Conclusions



What has been found

Our results show that the overexpression of Sirt1 in TECs has positive effects on CKD, stopping its progression and partially restoring kidneys normal functions.



So...

Therefore upregulation of Sirt1 can be considered as a new effective, safe and promising therapy approach for Chronic Kidney Disease.

Pitfalls and solutions

1 Inefficiency of purified AAV due to loss of the transgene during cell division



Many copies of the transgene DNA in each TEC through a higher number of vectors

2 Translatability between pre-clinical models and human patients



The therapy has to be tested on humans in clinical trials

3 Overexpression of Sirt1 could activate new pathways via neomorphic effects



Phenotypic control and experiments to assess new putative molecular pathways

Materials and Costs

| | |
|---|----------|
| • Western Blot kit | 820 € |
| • qRT-PCR kit | 1.424 € |
| • Animal facilities | 24.000 € |
| • AAV packaging plasmids x 2 | 3.600 € |
| • Immunohistochemistry x 24 samples | 8.400 € |
| • Albuminuria analyzer | 380 € |
| • SIRT1 antibodies (300 µL) | 640 € |
| • Helper plasmid x2 | 954 € |
| • C57BL/6 mice (10 weeks old) x 24 | 710 € |
| • CKD C57BL/6 mice (10 weeks old) x 24 | 1.056 € |
| • Vector plasmid AAV9 (EGFP, EGFP+SIRT1) x2 | 5.900 € |
| • CODA blood pressure analyzer | 5.215 € |
| • Salaries 1 PhD (20.500 €) + 1 Post-Doc (26.500 €) | 94.000 € |
| • Blood analysis for eGFR x 130 | 2.600 |

Total costs for **2 years:**

149.700 €



**Thanks for
your
attention!**

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