New Therapy for Pancreatic Cancer?

Biochemical and Structural Studies of novel Sirt2 Inhibitors

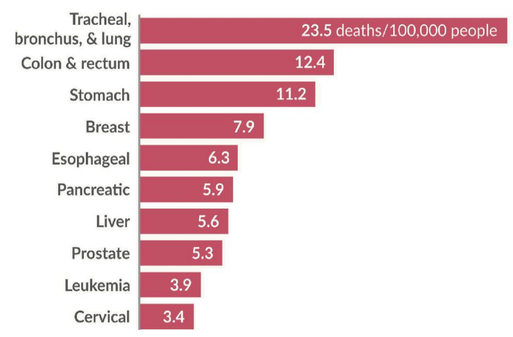
Sonja Schmidt[1,2] , Clotilde Charrier[1], Marcel Gischke[2], Ali Aghdassi[2] and Michael Lammers[1],

[1]University of Greifswald, Institute of Biochemistry, Germany, [2]University Medicine Greifswald, Department of Internal Medicine A, Germany

INTRODUCTION

Pancreatic ductal Adenocarcinoma (PDAC) is one of the most deadly cancers worldwide. Despite decades of intense research, we don‘t have effective treatment and it‘s predicted to become the second leading cause of cancer related death by 2030.

Sirtuin2 (Sirt2) is an intracellular lysin-deacetylase. It regulates many main cellular proteins and therefore might be critical for carcinogenesis.
In order to gain better understanding of how Sirt2 contributes to pancreatic cancer development and to highlight its potential as a therapeutic target we aim to inhibit it in pancreatic cancer cells using specific Nanobodys (Nbs).

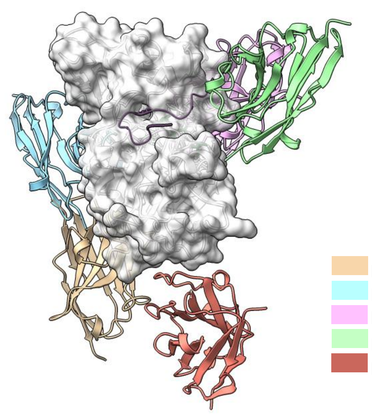


Estimated death Rate from different Cancer types (age-standardized) per 100.000 people, Data for 2021.

Data source: IHME, Global Burden of disease (2024). OurWorldinData.org/cancer

BINDING OF NANOBODY TO SIRT2

Predicted Structural Relations

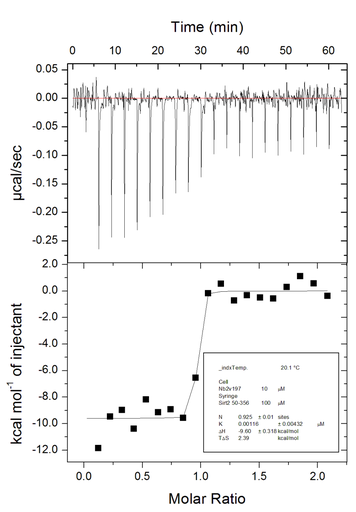


Sirt2 and Nb2v210, Alpha fold.

model 0 ; ranking score 0.71
model 1 ; ranking score 0.70
model 2 ; ranking score 0.70
model 3 ; ranking score 0.70
model 4 ; ranking score 0.70

ITC Measurements:
High affinity binding of Nbs to Sirt2 and Sirt3.
No binding to Sirt1.

Thermodynamic characterization

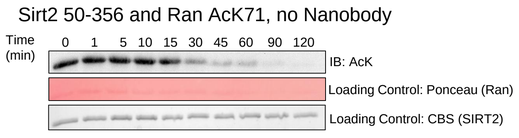


Sirt2 50-356 and Nb2v197, ITC.

INHIBITION IN ASSAYS

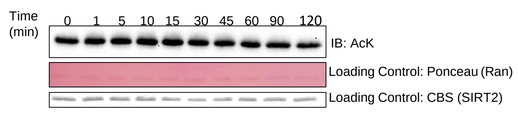
Fluor-de-Lys-Assays

Sirt2 50-356 and Ran AcK71, no Nanobody

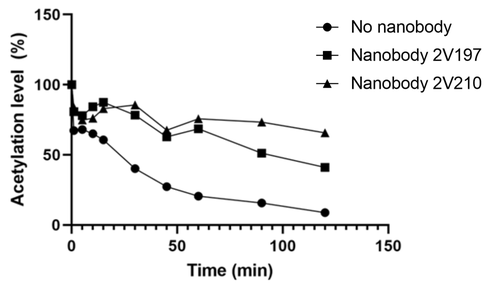


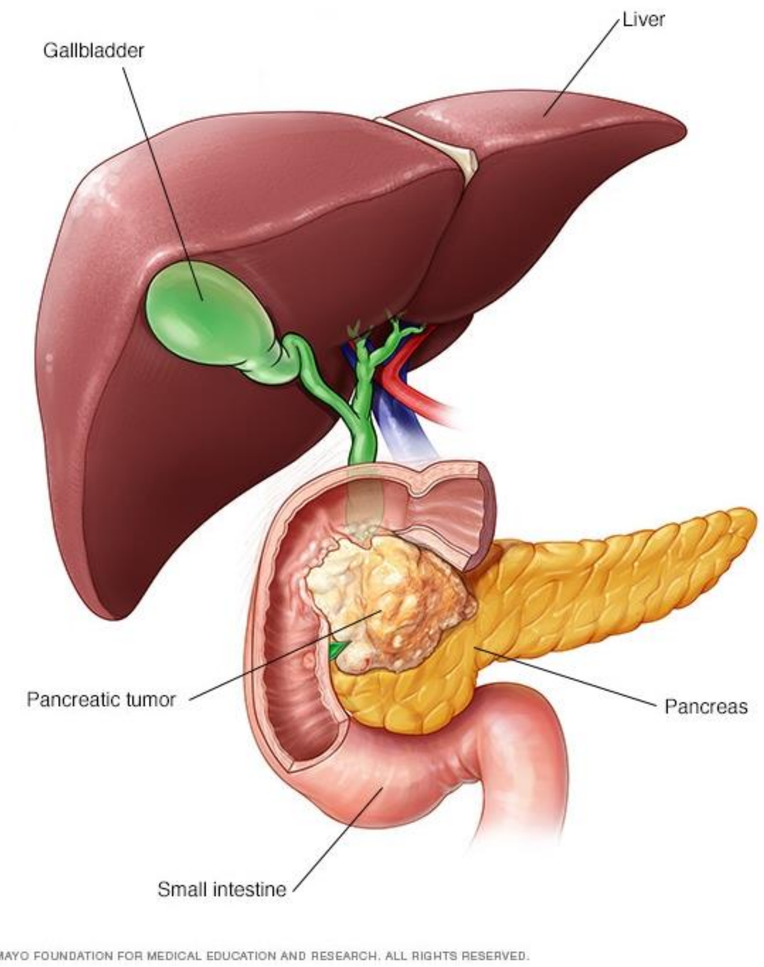
Using RanAcK71 as a specific substrate for Sirt2, time dependent deacetylation activity, when incubated with Nanobody.

Sirt2 50-356 and Ran AcK71,with Nb2v 210



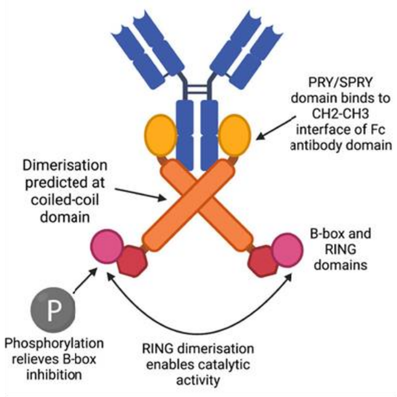
Time-dependent deacetylation of Ran at K71 by SIRT2



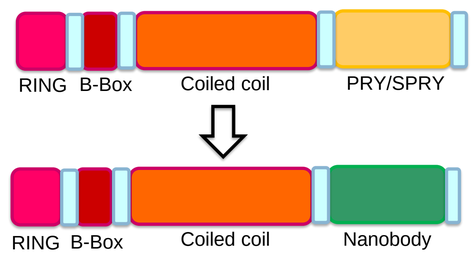


INHIBITION IN CELLS

Modifying TRIM21



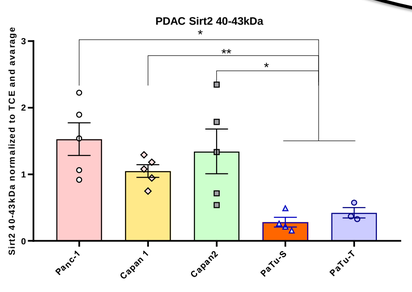
Jones *et al.* TRIM21/Ro52_Roles in Innate Immunity and Autoimmune Disease. 2021

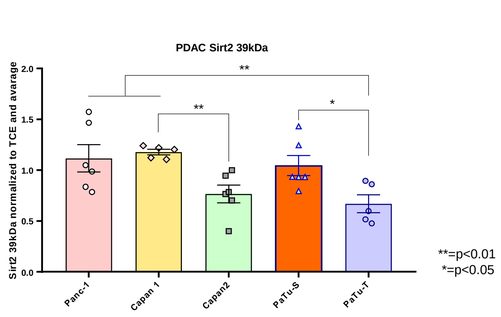


Replacing PRYS/PRY domain by Nanobody leads to digestion of new target molecule Sirt2 in the proteasome.

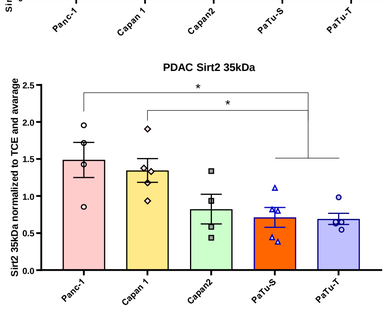
SIRT2 IN PDAC

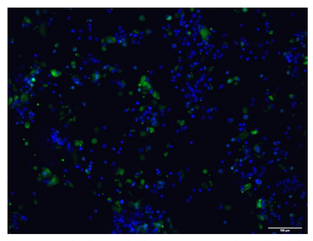
Immunoblots of five different PDAC cell lines show the different endogenous expression of Isoform 1 (41kDa), 2 (39kDa), 3 (43kDa) and 5 (35kDa) of Sirtuin 2.



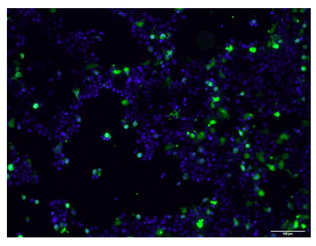


**=p<0.01
*=p<0.05





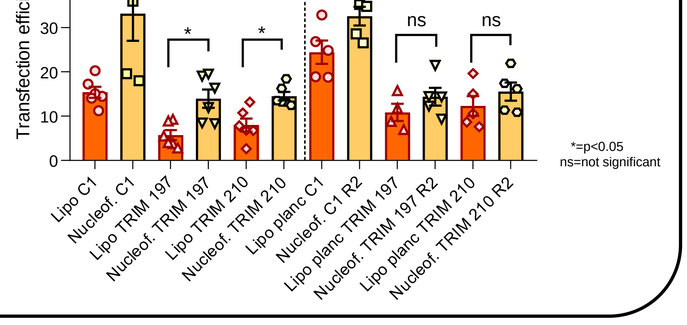
C1 Nuclefect (Electroporation 24h)



C1 Lipofectamine (24h)

TRANSFECTION

Transfection with Electroporation is more effective than using Lipofectamine. However, transfection-efficiency only reaches a maximum of 35%. Cells need to be sorted before further use.



*=p<0.05
ns=not significant

WHAT´S NEXT?

- Sorting transfected cells to perform viability and migration/invasion assays
- Optimazing crystal structure of Sirt2 with attatched Nanobody
- Including Data in future projects to use Sirt2 modulation as therapeutic strategy in vivo

sonja.schmidt@stud.uni-greifswald.de

Funding

Funded by DFG Deutsche Forschungsgemeinschaft German Research Foundation

GERHARD DOMAGK Nachwuchsförderprogramm

Reference

Abb. 1: Estimated Death Rates from different Cancer Types. IHME, GlobalBurden of disease (2024). OurWorldinData.org/cancer.
Abb. 2: Pancreatic Cancer. Mayo Foundation for Medical Education And Research (2024). mayoclinic.org/diseases-conditions/pancreatic-cancer/symptoms-cause
Abb. 3: Jones *et al*. TRIM21/Ro52_Roles in Innate Immunity and Autoimmune Disease. 2021