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TITLE: Mesoscale Nanotechnology: A Novel Therapeutic Strategy in Polycystic Kidney Disease

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13. SUPPLEMENTARY NOTES

14. ABSTRACT

The ***central hypothesis*** of this proposal is that the ***targeted tubular delivery of novel therapeutic agents is an effective strategy for the treatment of polycystic kidney disease*.**

Aim 1. Evaluate therapeutic efficacy of nanoparticle-targeted mTOR inhibition in a rat model of PKD

Mesoscale nanoparticles will be synthesized to encapsulate an ASO against mTOR. We will use the PCK-*Pkhd1*^{pck} rat model of PKD that closely mimics ADPKD in humans. Efficacy will be evaluated by longitudinal serial live imaging via MRI, as well as post-mortem phenotypic surrogate markers of renal cystogenesis.

Aim 2. Evaluate therapeutic efficacy of nanoparticle-targeted miR-17 inhibition in a rat model of PKD

Mesoscale nanoparticles will be synthesized to encapsulate an oligonucleotide against miR-17. Just like in Aim 1 we will use the Pkhd1pck/pck rat model of PKD that closely mimics ADPKD in humans. Efficacy will be evaluated by longitudinal serial live imaging via MRI, as well as post-mortem phenotypic surrogate markers of renal cystogenesis.

15. SUBJECT TERMS
None listed.

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			USAMRDC
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1. INTRODUCTION

In this project we are developing novel therapeutic strategies for adult polycystic kidney disease (ADPKD) utilizing newly-developed mesoscale nanoparticles encapsulating an anti sense oligonucleotide against mTOR or an oligonucleotide against miR-17. These studies are being performed in the *Pkhd1*PCK/PCK rat which closely resembles ADPKD in humans and was the pre-clinical model of ADPKD used in the studies that led to the clinical trials with tolvaptan, the only drug currently approved for the treatment of ADPKD. The investigators will employ mesoscale nanoparticles to target an ASO against mTOR or an oligonucleotide against miR-17 specifically to site of injury in order to slow cystogenesis without triggering dose-limiting toxicities. We expect that the effect of one injection will last for up to two months, providing a prolonged therapeutic benefit.

2. KEYWORDS

Polycystic kidney disease
Mammalian target of rapamycin
Kidney
Renal Function
Nanoparticles
Chronic Kidney Disease

3. ACCOMPLISHMENTS

- What are the major goals of the project?

Major Task 1: Mesoscale Nanoparticle synthesis: 1-4 months (partially completed)
Major Task 2: In Vivo Efficacy of ASO against mTOR: 6-13 months (partially completed)
Major Task 3: Efficacy of oligonucleotides against miR-17: 15-23 months (not completed)

- What was accomplished under these goals?

Mesoscale nanoparticles (MNP) have been synthesized and encapsulated with the mTOR inhibitor sirolimus. Encapsulation with oligonucleotides against mTOR has shown to be more challenging than anticipated and as result we are trying a different nanoparticle formulation that has high affinity against galectin and that we hope will be easier to encapsulate with oligonucleotides.

Since we had difficulties encapsulating oligonucleotides, we did perform in vivo experiments in PCK-*Pkhdpck*^{pck} rats utilizing nanoparticles encapsulated with the mTOR inhibitor sirolimus. These studies showed that the particles were safe and well tolerated and resulted in a significant reduction in cyst formation as assessed by MRI.

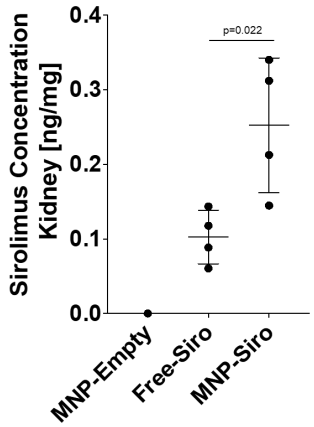

Figure 1. MNP sirolimus-encapsulated (MNP-Siro) target to kidneys more efficiently vs free sirolimus (Free-Siro) after an intravenous administration to *Pkhd1*$^{PCK/PCK}$ rats. The sirolimus (rapamycin) concentration in kidneys was determined with mass spectrometry. Empty MNPs without sirolimus (MNP-Empty) were used as negative control.

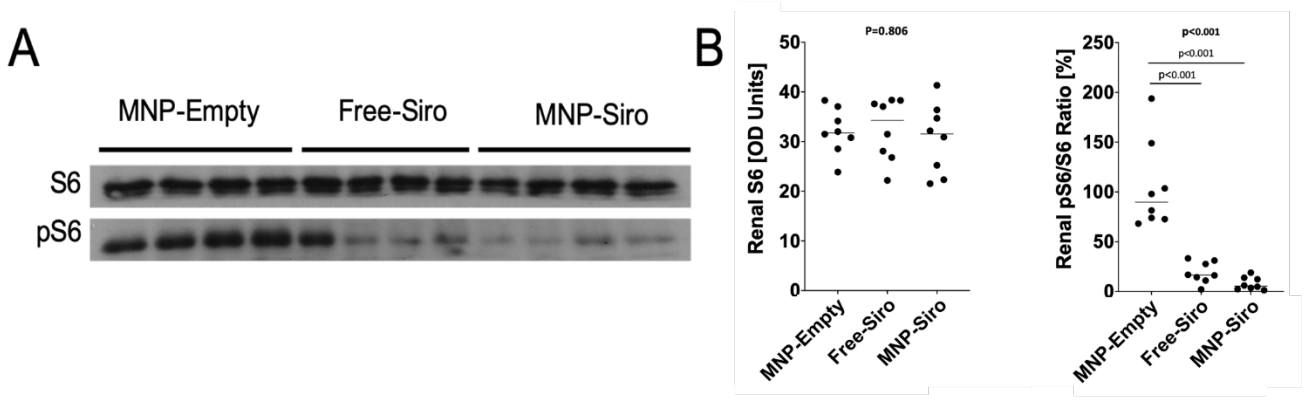

Figure 2. MNP sirolimus-encapsulated (MNP-Siro) inhibits mTOR pathway activity in *Pkhd1*$^{PCK/PCK}$ rat kidneys. Panel a: Representative example of an immunoblot of S6 and pS6 comparing the renal effect of MNP-Siro vs free sirolimus (Free-Siro) vs MNPs without sirolimus (MNP-Empty) administration. **Panel b:** While the S6 levels did not change (left panel), the pS6 levels and the pS6/S6 ratios the averages were reduced more efficiently by the MNP-Siro (vs Free-Siro) administration.

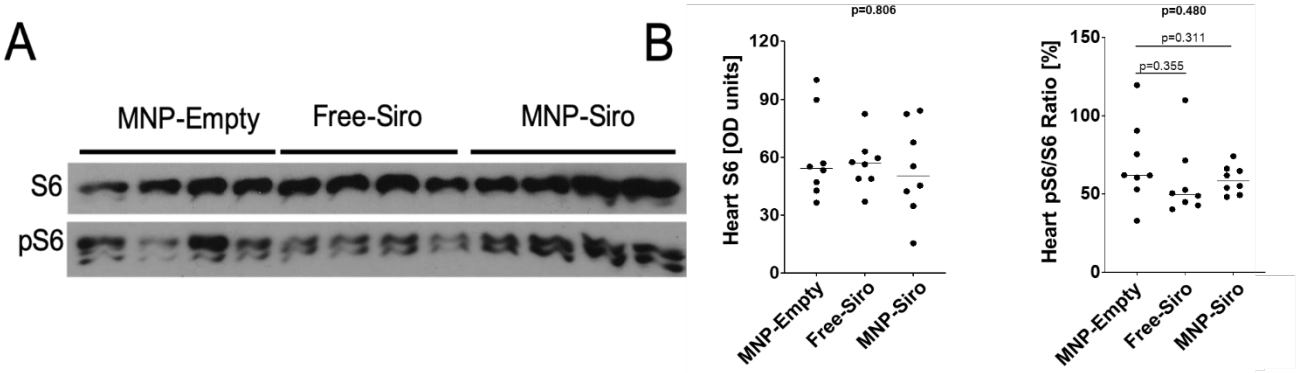

Figure 3. MNP sirolimus-encapsulated (MNP-Siro) effects on mTOR pathway activity in *Pkhd1*$^{PCK/PCK}$ rat hearts are similar to those of MNPs without sirolimus (MNP-Empty). We examined the cardiac mTOR activity because free sirolimus (Free-Siro) administration was associated with focal myocardia necrosis in outbred Sprague-Dawley rats (PMID: 1871790). **Panel a:** Representative example of an immunoblot of S6 and pS6 comparing the effect of MNP-Siro vs Free-Siro vs MNP-Empty. **Panel b:** The mean pS6 levels and the pS6/S6 ratios are similar after MNP-Siro and MNP-Empty administration; however, they are numerically lower after Free-Siro (pointing to a protective effect of MNP-Siro encapsulation).

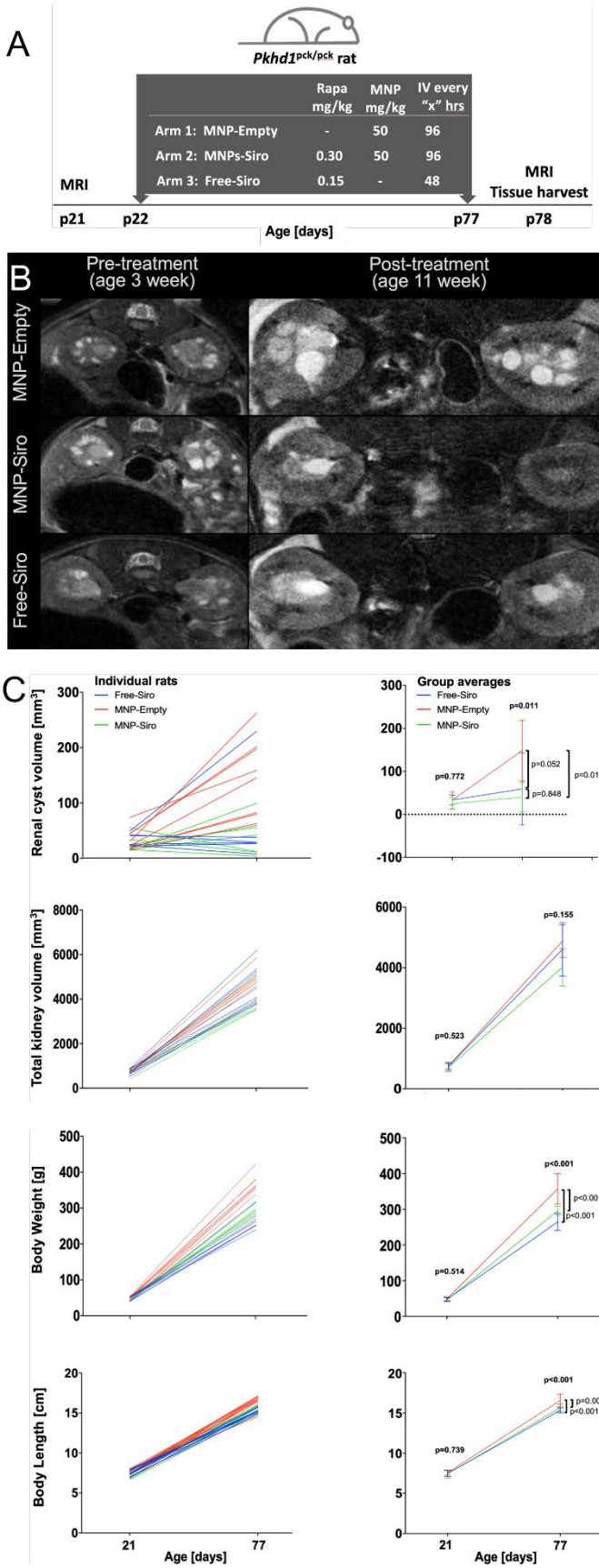

Figure 4. MNP-Siro (green lines) has stronger renal cystogenesis-inhibiting effects in *Pkhd1*$^{PCK/PCK}$ rat and lower adverse effects when compared to Free-Siro (blue lines); MNP-Empty was used as a control. Panel a: Experiment design. **Panel b:** Representative MRI images in an individual rat from each treatment arm before treatment at postnatal day 21 (3 weeks) and after treatment at postnatal day 77 (11 weeks). **Panel c:** Effects of MNP-Siro (vs MNP-Empty and Free-Siro) on renal cyst and total kidney volumes, body weights and body lengths.

Based on these, we concluded:

1. MNP-Sirolimus particles target the kidneys more efficiently vs free Sirolimus
2. MNP-Sirolimus particles inhibit renal mTOR activation and potentially less so in hearts
3. MNP-Sirolimus renal cystogenic-inhibiting effects are stronger vs free-Siro but did not reach statistical significance
4. MNP-Sirolimus side effects are less prominent as compared to free-Sirolimus

Galectin 3 LNP

In order to improve the efficacy of our drug delivery system and to have better success with the loading of oligonucleotides we developed several new nanoformulations that localize therapeutics to the kidney. One of these new drug delivery nanoparticles is targeted to the β-galactoside-binding lectin galectin-3. In the kidney galectin-3 is expressed in the branching ureteric bud and its collecting duct derivatives during nephrogenesis. Previous studies have shown that galectin-3 is expressed in cysts of human ARPKD and is also highly expressed in the *cpk* mouse model of recessive PKD.[1, 2]. Exogenous galectin-3 has been shown to reduce cyst formation in vivo and in vitro suggesting that it may act as a natural brake for cyst formation in *cpk* mice. Given the potentially higher efficacy of these particles given the high expression of galectin-3 in the kidneys we performed biodistribution studies in *Pkhd1*$^{PCK/PCK}$ rats. These studies demonstrate that these particles have a predominant kidney biodistribution but also distribute to liver and lungs.

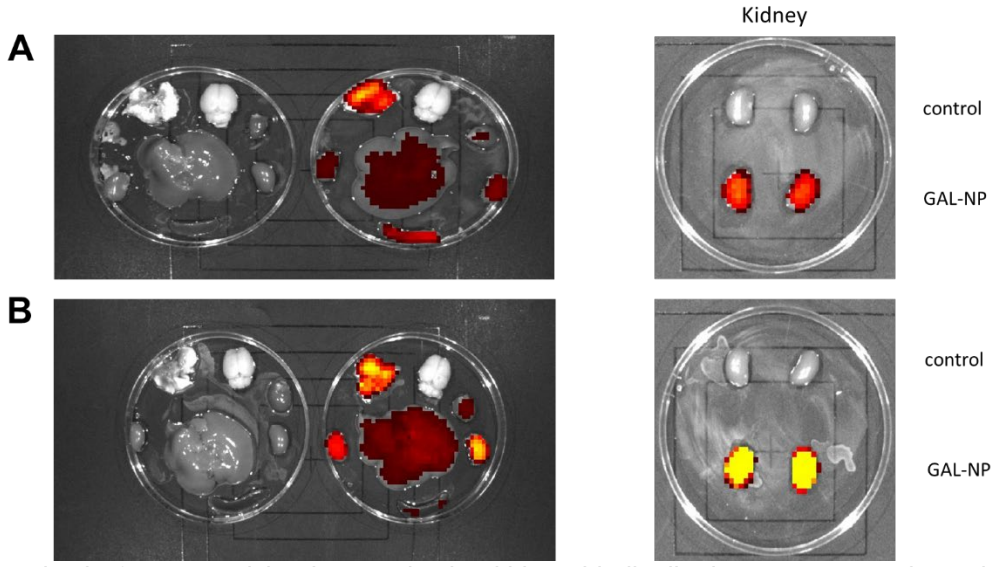

Figure 5. galectin 3 nanoparticles have selective kidney biodistribution as compared to other organs. Biodistribution is equivalent in male (A) as well as female animals (B)

In addition to these biodistribution studies we performed studies testing the inhibition of the mTOR pathway with the Galectin-3 nanoparticles encapsulating the mTOR inhibitor sirolimus. IV injections performed on 3-4 week old PCK/PCK rats, 4 total injections administered every 48 hours. Tissue collections 24 hours post last injection. As shown in Figure 6 Galectin 3-rapamycin NP produced a similar reduction in pS6 phosporylation as compared to free rapamycin.

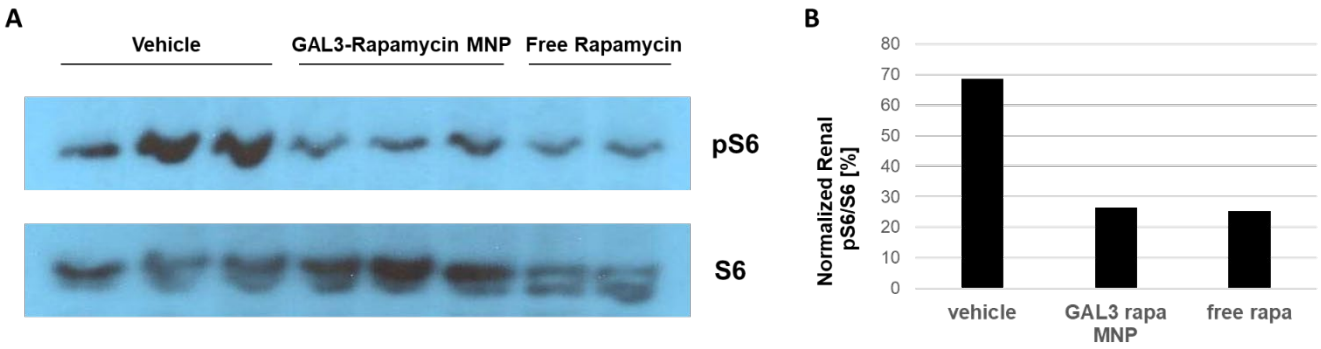

Figure 6. GAL3 Rapamycin MNPs inhibit mTOR pathway in kidneys of Pkhd1PCK/PCK male rats. A. Western blot of S6 and phosphorylated S6 after intra-venous (IV) administration of 0.15mg/kg rapamycin contained within GAL3 targeted MNPs or free rapamycin at equal dose. B. Normalized renal densitometry ratios of phosphorylated

- **What opportunities for training and professional development has the project provided?**

  Nothing to report

- **How were the results reported to communities of interest?**

  A poster with the above results was presented at the 2021 Kidney Week Meeting of the American Society of Nephrology and another one will be presented at the 2023 Kidney Week Meeting of the American Society of Nephrology

- **What do you plan to do during the next reporting period to accomplish the goals?**

We will continue the optimization of particles to allow efficient loading of oligonucleotides and perform experiments utilizing particles loaded with oligonucleotides against mTOR and miR-17. Our studies so far have shown that our system effectively blocks mTOR activation and reduces cyst formation as compared to free drug and therefore the main focus of the second year will be on blockade of miR-17 once we have the particle formulation to allow the efficient loading of oligonucleotides.

4. IMPACT

- **What was the impact on the development of the principal discipline(s) of the project?**

We anticipate that results from these studies will have a significant impact in the treatment of polycystic kidney disease and we expect that after full completion of this study clinical trials utilizing our technology will be planned and executed.

- **What was the impact on other disciplines?**

Nothing to report

- **What was the impact on technology transfer?**

Nothing to report

- **What was the impact on society beyond science and technology?**

Nothing to report

5. CHANGES/PROBLEMS

- **Changes in approach and reasons for change**

We had technical difficulties encapsulating oligonucleotides in our nanoparticles and therefore we used the small molecule mTOR inhibitor sirolimus as a payload. The scope of the study however remains unchanged. We are optimizing our particles to allow the use of oligonucleotides as a payload that we expect to accomplish during the second year of funding.

- **Actual or anticipated problems or delays and actions or plans to resolve them**

We had delays in the approval of our IACUC protocol which delayed the start of our studies.

- **Changes that had a significant impact on expenditures**

The changes we had to implement had no significant impact on expenditures

- **Significant changes in use or care of human subjects**

  Not applicable

- **Significant changes in use or care of vertebrate animals.**

  No changes

- **Significant changes in use of biohazards and/or select agents**

  Not applicable

6. PRODUCTS:

- **Publications, conference papers, and presentations**
  - **Journal publications.**

    Nothing to report

  - **Books or other non-periodical, one-time publications.**

    Nothing to report

  - **Other publications, conference papers, and presentations**
    - Poster presentation at ASN Kidney Week 2021: Efficacy and Adverse Effects of a Novel Mesoscale Nanoparticle-Guided Sirolimus Delivery Strategy in a *Pkhd1*^{PCK} Rat Model. Michal Mrug, Chintan H. Kapadia, Phillip Chumley, Gabriel Rezonzew, Janki Shah, Sean Mullen, Ronald Roye, Juling Zhou, Arthur Klausner, Daniel Heller, Edgar A. Jaimes.

- **Website(s) or other Internet site(s)**

  Nothing to report

- **Technologies or techniques**

  Nothing to report

- **Inventions, patent applications, and/or license**

  Nothing to report

- **Other Products**

  Nothing to report

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

- **What individuals have worked on the project?**

Name:	*Edgar A. Jaimes*
Project Role:	*PI*
Researcher Identifier:	
Nearest person month worked:	*1.0*
Contribution to project:	*Dr. Jaimes is in charge of the overall direction and supervision of this project*
Funding Support:	*Dr. Jaimes is funded by DOD and NIH.*

Name:	*Daniel Heller*
Project Role:	*Co-Investigator*
Researcher Identifier:	
Nearest person month worked:	*1.0*
Contribution to project:	*Dr. Heller is in charge of the design and supervision related to the nanoparticle formulations utilized in this project*
Funding Support:	

Name:	*Janki Shah*
Project Role:	*Research Assistant*
Researcher Identifier:	
Nearest person month worked:	*1.0*
Contribution to project:	*Mrs. Shah is in charge of the preparation and manufacture of the different formulations used in this study.*
Funding Support:	

Name:	*Michal Mrug*
Project Role:	*Co-Investigator*
Researcher Identifier:	
Nearest person month worked:	*1.0*
Contribution to project:	*Dr. Mrug is in charge of supervision of the experiments done at the PKD Core at the U of Alabama at Birmingham.*

Funding Support:	

- **Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?**

  Yes. See Other Supports attached.

- **What other organizations were involved as partners?**

  - **Organization Name:** University of Alabama at Birmingham
  - **Location of Organization:** Birmingham, Alabama
  - **Partner's contribution to the projec**t
    - **Facilities** The Cystic Diseases Core at UAB is used for the execution of the animal studies in this project
    - **Collaboration** Personnel from UAB works in collaboration with the MSKCC team in the execution of the experiments included in this study

8. SPECIAL REPORTING REQUIREMENTS

Not applicable

9. APPENDICES

1. Desmedt, V., et al., *Galectin-3 in renal pathology: more than just an innocent bystander?* 2016. **43**(5): p. 305-317.
2. Henderson, N.C., et al., *Galectin-3 expression and secretion links macrophages to the promotion of renal fibrosis.* 2008. **172**(2): p. 288-298.