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TITLE: A New Treatment for Heritable Pulmonary Artery Hypertension Caused by Nonsense Mutations

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14. ABSTRACT:

Heritable Pulmonary Arterial Hypertension (hPAH), caused by a nonsense mutation [premature termination codon (PTC)] in the *BMPR2 (Bone Morphogenetic Protein Receptor type II)* gene, is a progressive fatal disease. We have identified Small Molecule Readthrough (SMRT) compounds that can readthrough PTCs in genes with nonsense mutations. Using specific genetic mouse models (***Bmpr2***$^{+/R899X}$ **and** ***Bmpr2***$^{+/R584X}$ **mice**) of hPAH, we propose to test the efficacy of GJ103, our lead SMRT molecule in preventing hPAH and determine its efficacy in combination with nonsense mediated decay (NMD) suppression in preventing the hPAH development in our mouse models. We have genetic models in hand, approval to conduct the proposed work, which is going on. However, due to Covid 19-related severe restrictions and the complete shutting down of all non-essential animal studies from April 2020 to April 2021, progress on proposed aims was severely limited during the last year. On a positive note, however, with the gradual removal of the imposed restrictions, the animal work has re-started since last month, allowing us to be confident on making significant progress by the next reporting period.

15. SUBJECT TERMS
None listed.

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- **INTRODUCTION:**

Pulmonary arterial hypertension (PAH), a condition characterized by progressively elevated pressure in the pulmonary arteries is often transmitted genetically and carries a 5-year mortality rate of 65%. An underlying genetic mutation called a nonsense mutation that encodes a premature termination codon in *bone morphogenetic protein receptor 2 (Bmpr2)* often leads to the formation of either no protein or an abnormal protein, which otherwise is essential for normal pulmonary vessel formation and function. This proposal tests the efficacy of a compound (GJ103) discovered by us that can rescue expression of the protein and potentially solve the underlying problem of genetically-caused unrelenting pulmonary hypertension and premature death. Using animal models, we have proposed two specific aims: **1.** Using specific genetic mouse models (***Bmpr2*$^{+/R899X}$ and *Bmpr2*$^{+/R584X}$ mice**) of heritable pulmonary hypertension **(hPAH)** caused by human relevant mutations we plan to test the efficacy of GJ103 in preventing hPAH. **2.** Determine the efficacy of readthrough treatment with GJ103 combined with nonsense mediated decay (NMD) suppression in preventing the development of hPAH in *Bmpr*$^{2+/R899X}$ and *Bmpr2*$^{+/R584X}$ mice. We expect the proposed studies to lead to a breakthrough in treating patients with hPAH.

- **KEYWORDS:**

BMPR2: Bone morphogenetic protein receptor type II
BMPs: Bone morphogenetic proteins
hPAH: heritable pulmonary artery hypertension
NMD: Nonsense mediated mRNA decay
PAH: Pulmonary artery hypertension
PTC: Premature termination codon
WT: Wild type

- **ACCOMPLISHMENTS:**

  - **What were the major goals of the project?**

    As explained below, due to factors beyond our control, i.e., shutting down of animal studies at both the Lundquist and UCLA (Westwood campuses) due to Covid 19 pandemic, not much progress could be made on both Aims and the corresponding subtasks. However, since last month, animal work has been gradually allowed and now studies proposed in Aim 1 are in progress.

  - **What was accomplished under these goals?**

    1) Major activities: Soon after we obtained the funding and ACURO's approval, all non-essential animal work at the Lundquist and UCLA Westwood campuses stopped due to Covid 19-related restrictions. This started at the beginning of April 2020 and this situation remained in effect for full one year, i.e., until April 2021, since when animal experiments have gradually begun. This significantly curtailed any meaningfulprogress on the aims proposed. However, during the closure we were able to maintain the mouse colonies (*Bmpr2*$^{+/R899}$ *and Bmpr2*$^{+/584X}$). We now expect that with the gradual opening up of animal experiments on both campuses, we will be able to significant progress in our goals by the time of next reporting.

    2) Specific objectives: The two Aims and the proposed approach to achieve these Aims is outlined next.

    AIM 1: Test efficacy of GJ103 in preventing development of hPAH in *Bmpr2+/R899X* and *Bmpr2+/R584X*mice. We aim to assess right heart hemodynamics via catheterization using a Millar catheter and cardiopulmonary remodeling using echocardiography in GJ103-treated vs.

vehicle-treated mouse models of hPAH (*Bmpr2+/R899X* and *Bmpr2+/R584X* mice) that express nonsense mutations in the *Bmpr2* gene.

AIM 2: Determine the efficacy of readthrough treatment with GJ103 combined with NMD suppression in preventing the development of hPAH in *Bmpr2+/R899X* and *Bmpr2+/R584X* mice. We aim to combine GJ103 readthrough therapy with NMD suppression strategies using NMDI-1 or NMDI-14 and determine how this combination therapy prevents the development of hPAH in *Bmpr2+/R899X* and *Bmpr2+/R584X* mice, assess right heart hemodynamics via catheterization using direct recording with a Millar catheter, and determine right heart structural changes using micro-echocardiography.

3) Significant results or key outcomes, including major findings, developments, or conclusions (both positive and negative). As alluded to above (#1), due to Covid 19-related shutting down of animal studies at both Lundquist and UCLA (Westwood) campuses, we are unable to report any major findings other than the fact that we have been able to maintain animal colonies exhibiting hPAH due to human relevant nonsense mutations to a much longer period than previously reported. Therefore, once we perform cardiac echo and cardiac catheterizations in these animals, we expect to describe the natural progression of PAH in these models, which is likely to be usefulness in future studies.

4) Other achievements. Please note that although unrelated directly to the Aims proposed, since the original submission of our grant, we have been able to determine the safety (lack of toxicity) of GJ103 following its chronic use, i.e., up to 1 year. Three-month old WT (C57BL/6J) mice were administered GJ103 (25 or 50 mg/kg) intraperitoneally (i.p.) 3 times/week for 12 months. Vehicle administered mice served as controls. GJ103 treated mice did not show any overt signs of toxicity (feeding, behavioral changes, changes in body weight, and breathing). Using VetScan Comprehensive Diagnostic and HM5 hematology systems (Abaxis, Inc), we performed **complete blood counts (data not shown)**, **basal metabolic profile** (serum electrolytes and glucose), **liver** [total protein, albumin, globulin, total bilirubin, serum alanine aminotransferase (ALT), serum aspartate transferase (AST), alkaline phosphatase (ALP), and serum amylase (AMY)], **renal** [blood urea nitrogen (BUN), and creatinine (CRE)], and **lipid panels** (cholesterol and triglyceride). We found no significant changes in any of the parameters determined, indicating GJ103's safety even after one year use at the doses specified.

Blood Biochemistry results in *Bmpr2* KI mice with 12 month GJ103 treatment

Treatment Group	Genotype	BUN mg/dL	CRE mg/dL	Cholesterol mg/dL	Triglyceride mg/dL	NA^+ mM	PHOS mg/dL	CA^+ mg/dL
Untreated	WT	27±2.55	0.3±0.06	<100	70±17.83	152±1.41	9.4±5.26	9.6±0.50
	Mutant	25±2.39	0.3±0.04	<100	70±15.02	154±3.32	9.7±1.74	9.4±0.34
Vehicle	WT	27±6.02	0.2±0.04	<100	67±5.91	154±3.96	10.8±1.07	9.2±0.79
	Mutant	25±2.41	0.3±0.04	<100	80±24.07	153±1.52	9.8±2.13	9.3±0.75
GJ103 25mg/kg	WT	28±5.18	0.2±0.09	<100	78±30.30	155±1.87	9.9±1.37	9.5±0.36
	Mutant	30±2.88	0.3±0.04	<100	61±8.06	154±2.17	10.5±1.70	9.4±0.22
GJ103 50mg/kg	WT	31±4.85	0.2±0.08	<100	67±18.02	158±4.49	10.6±1.99	9.3±0.59
	Mutant	27±5.13	0.3±0.04	<100	71±18.81	155±3.88	9.9±1.10	9.0±0.41

Treatment Group	Genotype	TP g/dL	ALB g/dL	ALP g/dL	ALT g/dL	TBIL mg/dL	GLOB mM
Untreated	WT	5.3±0.17	3.9±0.31	72±24.24	57±35.00	0.3±0.05	1.4±0.30
	Mutant	5.2±0.37	3.9±0.31	50±14.11	66±28.68	0.3±0.04	1.3±0.09
Vehicle	WT	5.4±0.32	3.9±0.22	66±25.82	82±69.80	0.3±0.05	1.5±0.25
	Mutant	5.2±0.29	3.8±0.08	59±13.21	56±22.87	0.3±0.04	1.3±0.21
GJ103 25mg/kg	WT	5.4±0.11	3.9±0.26	71±23.73	54±16.27	0.3±0.04	1.5±0.18
	Mutant	5.4±0.18	4.0±0.15	54±6.72	56±29.26	0.3±0.04	1.3±0.18
GJ103 50mg/kg	WT	5.4±0.21	3.9±0.23	64±17.43	44±21.73	0.3±0.04	1.5±0.14
	Mutant	5.4±0.21	4.0±0.31	53±21.52	73±34.18	0.3±0.04	1.4±0.19

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

- "Nothing to Report."
-

The project has allowed opportunity for Dr. Shomir Deshpande, a Neonatal-Perinatal Fellow (Harbor-UCLA Medical Center), to learn animal work and molecular techniques utilized in this proposal. As a result of his involvement, recently (May 12, 2021), he presented this proposal and the data we have so far in a joint (UCSD, UCI, Loma Linda and Harbor-UCLA) Neonatal-Perinatal Fellowship Conference held at UC San Diego.

- **How were the results disseminated to communities of interest?**
    - "Nothing to Report"
    - As alluded to above, the project and our data so far was presented at a regional conference.
- **What do you plan to do during the next reporting period to accomplish the goals?**
    - All of the work proposed to be completed during the first year of the project will be pursued and completed by the next reporting period.

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

"Nothing to Report."

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

"Nothing to Report."

- **What was the impact on technology transfer?**
    - *If there is nothing significant to report during this reporting period, state "Nothing to Report."*

- *Describe ways in which the project made an impact, or is likely to make an impact, on commercial technology or public use, including:*
  - *transfer of results to entities in government or industry;*
  - *instances where the research has led to the initiation of a start-up company; or*
  - *adoption of new practices.*

  "Nothing to Report."

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

  "Nothing to Report."

- **CHANGES/PROBLEMS:**

  - **Changes in approach and reasons for change**
    "Nothing to Report."

  - **Actual or anticipated problems or delays and actions or plans to resolve them**
    - Soon after the approval of the funding (March 2020) and the approval of IACUC (The Lundquist Institute and UCLA Westwood campus), and ultimately the ACURO forms, due to Covid 19-related restrictions, all non-essential animal work at the Lundquist and UCLA Westwood campuses stopped at the beginning of April 2021. This situation remained in effect for almost one year, i.e., until March 2021, when animal experiments have gradually begun. Of, note, even during the closure we had to maintain our genetic model colonies (*Bmpr2*$^{+/R899}$ *and Bmpr2*$^{+/584X}$). We expect that once the animal work restrictions are completely restricted, we will advance the goals of the proposed work.

  - **Changes that had a significant impact on expenditures**
    - As noted above, the maintenance of genetic mouse colonies and the research staff during the epidemic period, and the expiry of the previously purchased but unused molecular reagents have significantly added to the cost of the proposed work.

  - **Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents**
    -

"Nothing to Report."

- **Significant changes in use or care of human subjects** N/A
- **Significant changes in use or care of vertebrate animals.** Nothing to report
- **Significant changes in use of biohazards and/or select agents** N/A

- **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.**
      "Testing a novel approach to treat heritable pulmonary artery hypertension caused by nonsense mutations": Presented at the Joint Neonatal-Perinatal Joint Conference held at UC San Diego (May 2021).

- **PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS:**

  - **Participants/Personnel who worked on this on this project:**

    - **Virender K. Rehan, M.D.:** No Change
    - **Ying Wang, Ph.D.:** No Change
    - **Soban Umar, M.D., Ph.D.:** No Change

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

    - **New Grant Award for Dr. Rehan:**
      1R01HL151769-01 / NIH/NHLBI / 06/01/2020 – 05/31/2024
      E-Cigarette Vaping During Pregnancy and Lactation, Germ Cell Epigenetic Memory and Transgenerational Asthma

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

"Nothing to Report."

- **SPECIAL REPORTING REQUIREMENTS:**

"Nothing to Report."

- **APPENDICES**

"Nothing to Report."