AWARD NUMBER: HT9425-23-1-0104

TITLE: Systems Pharmacology Model of Heart Failure with Preserved Ejection Fraction

PRINCIPAL INVESTIGATOR: Sang Ging Ong

CONTRACTING ORGANIZATION: University of Illinois at Chicago

REPORT DATE: March 2024

TYPE OF REPORT: Annual

PREPARED FOR: U.S. Army Medical Research and Development Command
Fort Detrick, Maryland 21702-5012

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE	2. REPORT TYPE	3. DATES COVERED
MARCH 2024	Annual	1FEB2023 - 31JAN2024

4. TITLE AND SUBTITLE

Systems Pharmacology Model of Heart Failure with Preserved Ejection Fraction

5a. CONTRACT NUMBER
HT9425-23-1-0104

5b. GRANT NUMBER

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)
Sang Ging Ong

E-Mail: sangging@uic.edu

5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

THE BOARD OF TRUSTEES OF THE
UNIVERSITY OF ILLINOIS
809 S MARSHFIELD RM 520
CHICAGO IL 60612-4305

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)

U.S. Army Medical Research and Development Command
Fort Detrick, Maryland 21702-5012

10. SPONSOR/MONITOR'S ACRONYM(S)

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION / AVAILABILITY STATEMENT

Approved for Public Release; Distribution Unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT
Heart failure with preserved ejection fraction (HFpEF) is a significant cause of morbidity and mortality in the US. An excessive burden of comorbidities characterizes the clinical profile of this patient population. These comorbidities are typically more numerous and heterogeneous than that which is observed amongst individuals who are diagnosed with heart failure with reduced ejection fraction (HFrEF). In HFpEF, cardiac dysfunction is thought to arise from the systemic inflammatory conditions brought upon by these comorbidities. This may explain why existing cardio-centric therapies have been unable to benefit the HFpEF population. Herein, we have embraced the multi-factorial nature of HFpEF and employed a systems pharmacology approach that transcends conventional target-based discovery. The integration of multiple HFpEF RNA-sequencing datasets across different species was processed and analyzed through a drug-repurposing pipeline. Glucocorticoid agonists were predicted to reverse these HFpEF signatures. We then utilized a recently developed chrono-pharmacologic scheduling protocol to administer prednisone in a two-hit mouse model of HFpEF. This was observed to significantly improve diastolic function following HFpEF treatment, as well as cardiomyocyte hypertrophy and vascular rarefication. Cardiomyocytes were then isolated from HFpEF mice treated with either vehicle or prednisone. These results indicated that prednisone treatment directly benefited contractile parameters in isolated cardiomyocytes. In summary, we have used an innovative drug-discovery approach that is particularly well suited to identifying compounds capable of targeting complex polygenic diseases such as HFpEF. Next, we demonstrated the utility of this approach by performing in vivo and in vitro studies, highlighting the use of intermittent prednisone as a potentially beneficial strategy in treating HFpEF. These findings use innovative drug-discovery approaches and drug-dosing protocols, resulting in the identification of an FDA-approved compound that can be rapidly implemented to address a major shortfall in the paucity of therapeutics available to treat HFpEF.

15. SUBJECT TERMS
HFpEF, drug repurposing, rodents, heart failure, diastolic dysfunction

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			19b. TELEPHONE NUMBER *(include area code)*
U	U	U	UU	18	

Standard Form 298 (Rev. 8-98)
Prescribed by ANSI Std. Z39.18

TABLE OF CONTENTS

1. INTRODUCTION:

Heart failure with preserved ejection fraction (HFpEF) is a leading cause of death and disability worldwide, and the incidence of HFpEF is rapidly increasing, such that it now exceeds that of heart failure with reduced ejection fraction (HFrEF). Although the mortality associated with HFpEF is similar to that of HFrEF, there are currently no therapies that improve the outcome of HFpEF with the exception of sacubitril/valsartan (an angiotensin receptor – neprilysin inhibitor), the first drug to receive FDA approval for HFpEF treatment in 2021. This is due, in part, to a lack of understanding of the pathogenesis of HFpEF. The syndrome of HFpEF is characterized by a complex clinical phenotype that stems from the presence of multiple comorbidities, including obesity, metabolic syndrome/diabetes, aging, and hypertension. Conventionally, investigations of heart failures have focused on individual intracellular molecular regulators, which we believe is a major reason why therapies specifically targeting HFpEF, a multi-factorial complex disease, remain elusive. We aim to overcome the past limitations of focusing on a single signaling molecule by employing a systems approach that considers the more extensive network of signaling interactions and in silico screening of drugs that are viable candidates for drug repurposing to simultaneously target multiple pathological processes responsible for the onset and progress of HFpEF.

2. KEYWORDS:

HFpEF, systems pharmacology, drug screening, hiPSC-CMs, diastolic dysfunction, echocardiography, rodents

3. ACCOMPLISHMENTS:

What were the major goals of the project?

Specific Aim 1: To obtain candidate compounds from an expanded screening of diverse heart failure with preserved ejection fraction (HFpEF) models.	**Timeline**	**Progress**
Major task – drug screening/repurposing of multiple HFpEF transcriptomic signatures	Months	
• Subtask 1 – Selection of publicly available RNA-sequencing (RNA-seq) datasets of HFpEF samples to generate an integrated signature of HFpEF for compound prediction.	1-2	100% 03/01/23
• Subtask 2 – Identification of compounds that may have the ability to reverse the resulting gene expression signature. Publicly available datasets (GSE153923 and GSE163665) will be used as input for the CMap and L1000CDS2 database to obtain a list of and visual representation of compounds associated with the inverse integrated signature of these datasets.	3-5	100% 05/27/23
• Subtask 3 – statistics: Connectivity scores (Cs) are derived from the Tau measurement and are automatically computed using the CMap user interface according to the algorithm listed below. $\tau_{q,r} = \mathrm{sgn}(ncs_{q,r})\frac{100}{N}\sum_{i=1}^{N}[\lvert ncs_{q,i}\rvert < \lvert ncs_{q,r}\rvert]$ where ncsq,r is the normalized connectivity score for signature r w.r.t query q, ncsi,r is the normalized connectivity score for signature r relative to the i-th query in Qref and N is the number of queries in Qref.	1-5	100% 05/27/23
Milestone(s) Achieved: Obtain a list of compounds that will be used in subsequent aims to test whether they can reverse diastolic dysfunction in vitro	Yes	Please see Figure 1
Major task – securing Institutional Animal Care and Use Committee (IACUC) review and approval	1-2	100%
Major task – securing Office of Human and Animal Research Oversight (OHARO) review and approval	2-3	100%
Specific Aim 2: Assessing the ability of predicted drug targets to reverse diastolic dysfunction in isolated adult cardiomyocytes and induced pluripotent stem cells (iPSC)-derived engineered heart tissues (EHTs).		
Major task – testing of predicted compounds in vitro	Months	

• Subtask 1 – Isolation of adult mouse ventricular cardiomyocytes (AVCM) from healthy and HFpEF mice/rats. A total of 231 C57BL/6 mice (purchased from JAX lab) and 154 ZSF1 rats (purchased from Charles River) will be needed. AVCMs will be isolated from mice/rats using a Langendorff apparatus (excised hearts will be perfused with collagenase II, dissociated, and seeded onto laminin-coated surfaces)	5-12	In progress (80% completed)
• Subtask 2 – Generation of human stem cells-derived engineered heart tissues (EHTs). We will use control/healthy human iPSCs that we have in-house. If necessary, we will purchase additional control iPSCs from WiCell (cat#: iPS-DF19-9-11T). EHTs will be by seeding iPSC-CMs mixed with matrices into PDMS molds (https://www.eht-technologies.com/products.html)	5-12	In progress (80% completed)
• Subtask 3 – Functional assessment of isolated AVCMs and EHTs using the IonOptix Contractility System with CytoMotion. AVCMs/EHTs will be loaded with calcium dyes, stimulated at 1 Hz, and changes of sarcomeric length and calcium transients will be measured with IonOptix system. CytoMotion script will be used to analyze the results.	5-15	In progress (80% completed)
• Subtask 4 – Presentation of initial in vitro screening results at local/national conferences	12-15	In progress (50% completed)
• Subtask 5 – statistics: Measurements from the IonOptix system will consist of at least three replicates and will evaluate how candidate compounds affect parameters of contractility in either healthy or HFpEF mice. Since the effects of the drug will be specific to a group, only one comparison will be made, thus, a student's t-test will be used with a significance threshold of $p>0.05$.	5-15	In progress (80% completed)
Milestones (s) Achieved: Narrow down initial list of predicted compounds to a final list of 3-5 compounds that show protection against diastolic dysfunction in both AVCMs and EHTs.		
Specific Aim 3: In vivo validation of drugs that reverse diastolic dysfunction in vitro.		
Major task – testing of compounds from Aim 2 in mice/rodents. A total of 528 C56BL/6 mice (purchased from JAX lab) will be needed.	Months	
• Subtask 1 – Hemodynamics to determine diastolic and systolic function will be performed using the VisualSonics Vevo 2100 system along with tissue Doppler. LVEF, LVFS, E/E' and E/A will be determined.	12-24	In progress (25% completed)
• Subtask 2 – Exercise testing. After acclimatization to treadmill exercise, animals will run uphill with increasing speed until the animal is exhausted. Running time and running distance will be determined.	12-24	Not yet started
• Subtask 3 – Noninvasive blood pressure. Systolic blood pressure will be measured noninvasively in conscious mice using the tail-cuff method and a CODA instrument (Kent Scientific).	12-24	In progress (25% completed)
• Subtask 4 – Submission of publications based on the results accumulated over years 1-2	18-24	Not yet started
• Subtask 5 – statistics: In vivo parameters measured by echocardiography, exercise treadmill, and noninvasive blood pressure recordings will seek to address how these drugs affect the cardiovascular function in either chow of HFD diet mice, thus multiple comparisons will be made. This will be done using a One-way ANOVA, followed by Tukey's multiple comparison test.	12-24	In progress (25% completed)
Milestone(s) Achieved: We hope that compounds identified from Aims 1 and 2 will prove to be protective in rodent models of HFpEF. A caveat may be that protection may not always be observed across all three parameters (hemodynamics, exercise resistance, and blood pressure). Optimization may be needed.	In progress	In progress

What was accomplished under these goals?

MAJOR ACTIVITIES

Contained within this report are the findings obtained from our investigations which sought to address shortcomings in cardiovascular medicine as they relate to HFpEF, a condition which lacks generally effective therapies. Our primary objective is to identify and bring forward candidate compounds which might be beneficial in therapeutic strategies to treat HFpEF. To accomplish this, we devised a research strategy which consists of the three major tasks outlined in the above SOW which has been updated with our current progress towards completing these tasks.

In our first major task, **drug screening/repurposing of multiple HFpEF transcriptomic signatures**, we are pleased to report that the results have been completed in their entirety. In this task we were able to leverage insights obtained from drug/ compound expression signature libraries which allowed us to determine compounds whose signatures were inversely matched to HFpEF signatures. This resulted in the identification of glucocorticoid agonist as among the most significant hits obtained across multiple data sets and species. We then approached our second major task – **testing predicted compounds in vitro**. To determine which hits obtained from our in-silico screen would be most effective we tested a variety of compounds on isolated cardiomyocytes while measuring contractile and relaxation parameters using the IonOptix system. From these experiments we were able to determine that the effects of prednisone on relaxation parameters obtained from animal models of HFpEF were robust and significant in their ability to protect cardiomyocyte function against HFpEF related dysfunction. With an ideal candidate compound selected we were then allowed to advance towards our third and final major objective - **testing of compounds from Aim 2 in mice/rodents**. It is well recognized that corticosteroids, such as prednisone, can have adverse metabolic effects, which initially caused us to consider if this compound would be appropriate to utilize in our third and final objective. It has however recently been shown that when administered intermittently, and according to a prescribed circadian rhythm that the effects of this drug could be optimized to mitigate these adverse effects [[1]]. Moreover, the use of prednisone in this way was also shown that skeletal, cardiac, and metabolic dysfunction could be significantly improved. Thus, we decided to adopt this schedule for dosing and evaluate the effects of intermittent prednisone in vivo using animal models of HFpEF. To evaluate this, we performed transthoracic echocardiography and histological analysis which revealed that the use of intermittent prednisone offered significant protection against diastolic dysfunction in HFpEF as well as cardiomyocyte hypertrophy and vascular rarefication.

SPECIFIC OBJECTIVES

Specific Aim 1: To obtain candidate compounds from an expanded screening of diverse HFpEF models. Multiple publicly available RNA-seq data sets were first analyzed to obtaine gene expression signatures which consisted of the most significant genes that were differentially expressed in these experimental models of HFpEF. We were then able to use these signatures as input signatures which were compared to Connectivity Map (CMap) signatures as well as L1000CDS^2 (**Figure 1A, B**). These repositories are housed within the NIH library of integrated network-based cellular signatures (LINCS) allowed us to compare our HFpEF signatures to ~1.4 million unique gene expression profiles induced by more than 20,000 compounds. We then filtered our results and collected the most significant signatures predicted to mimic or reverse our HFpEF signatures (**Figure 1C, D**). Criteria used to determine the most significant signatures consisted of a combination of the raw connectivity score, FDR normalized significance values, and the percentage of hits contained within our input signatures whose expression connected to the signature contained within the LINCS databases.

Conclusions: This approach allowed us to identify multiple potential target compounds, with glucocorticoid receptor agonists as being among the most significant and reproducible findings obtained across multiple data sets.

Specific Aim 2: Assessing the ability of predicted drug targets to reverse diastolic dysfunction in isolated adult cardiomyocytes and iPSC-derived engineered heart tissues (EHTs). In these experiments we sought to evaluate multiple compounds obtained from our in-silico screen and test these compounds on cardiomyocytes isolated from both rat and mouse models of HFpEF and also EHTs. In screening multiple compounds, we observed that some of those drugs had little or no effect (data not shown). From these experiments though we

were able to determine that intermittent prednisone was effective in mitigating the pathological manifestations of HFpEF in both models, as indicated by the improved contractile and relaxation parameters (**Figure 2 A, B**), namely maximal systolic and diastolic velocities as well as the corresponding times to maximal velocities.
Conclusions: This approach allowed us to recognize intermittent prednisone as a promising candidate compound that has great potential to improve cardiac function in vivo.
Goals not yet met: We are currently in the process of completing and finalizing data completion (including statistical analysis) on different candidates identified in Specific Aim 1 using both rodent myocytes and EHTs. There was a slight delay in generating adequate EHTs as we had several batches of production that ran into contamination issues. This has now been addressed and we should be able to complete the remaining parts of this aim within the next 12 months.

Specific Aim 3: Testing of compounds from Aim 2 in mice/rodents. We are ahead of schedule in this aim (initially proposed for months 12 – 24) as prednisone appears to be very promising in our preliminary data. Upon determining that the use of intermittent AM prednisone might be a suitable candidate compound identified in our screen we next sought to evaluate these effects in vivo. To accomplish this transthoracic echocardiography was performed which determined that the hallmark features of diastolic dysfunction. These experiments were done using a two-hit mouse model of HFpEF induced by HFD feeding and L-NAME containing water (0.5g/L) buffered to pH 7.4 (**Figure 3A**). Here we were able to determine that the administration of once weekly prednisone (1mg/kg) at the start of the light cycle – termed AM prednisone – had therapeutic actions which opposed the effects of HFpEF. We first noticed that metabolic improvement as indicated by weight gain and LV weight were mitigated (**Figure 3B**). Echocardiographic measurements of left ventricle ejection fraction (LVEF%) and fractional shortening (FS%) remained unchanged across all groups (**Figure 3C**), while the hallmark features of HFpEF indicative of diastolic dysfunction E/E' were significantly improved in AM prednisone treated mice (**Figure 3D**). Additionally, we observed that AM prednisone injections also protected against cardiomyocyte hypertrophy and vascular rarefication (**Figure 3E**) – two additional disease features of HFpEF reported to occur in this model. When administered once weekly at the start of the dark phase, however, no such protection was achieved against HFpEF induced dysfunction. These effects appeared to be independent of any significant changes in blood pressure (**Figure 4A**).
Conclusions: This approach allowed us to provide strong evidence which suggests that the use of intermittent prednisone may be beneficial in the context of HFpEF where metabolic and hypertensive stress are factors in the underlying condition. Interestingly, the absence of protection observed with PM administration of prednisone suggests that our findings have uncovered a previously underappreciated biological aspect of cardiac dysfunction related to the circadian cycle which appears to play a significant role in HFpEF. These findings which identify the therapeutic potential of an FDA approved compound are highly relevant given the current lack of generally effective therapies for HFpEF. One advantage of this approach is that translational approaches could be more rapidly appreciated since this drug is already commonly utilized in the clinical setting.
Goals not yet met: To complete our major activities for this aim we will seek to complete the exercise treadmill experiments described in our original proposal. We have just recently finished optimizing settings and a protocol which will allow us to accomplish this goal and expect to obtain results promptly. Additionally, as other candidates from Aims 1 and 2 emerge, we will test these compounds *in vivo*.

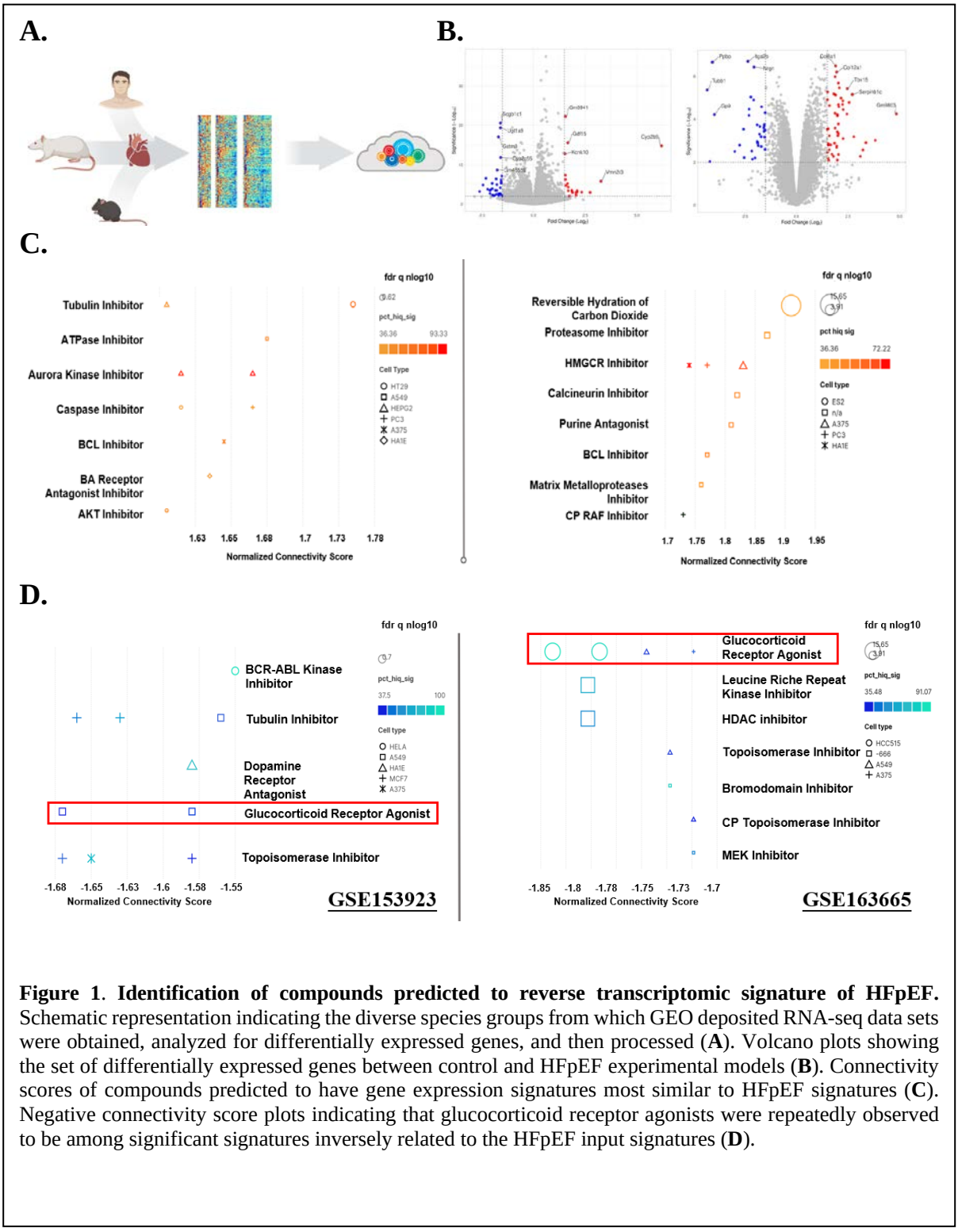

Figure 1. **Identification of compounds predicted to reverse transcriptomic signature of HFpEF.** Schematic representation indicating the diverse species groups from which GEO deposited RNA-seq data sets were obtained, analyzed for differentially expressed genes, and then processed (**A**). Volcano plots showing the set of differentially expressed genes between control and HFpEF experimental models (**B**). Connectivity scores of compounds predicted to have gene expression signatures most similar to HFpEF signatures (**C**). Negative connectivity score plots indicating that glucocorticoid receptor agonists were repeatedly observed to be among significant signatures inversely related to the HFpEF input signatures (**D**).

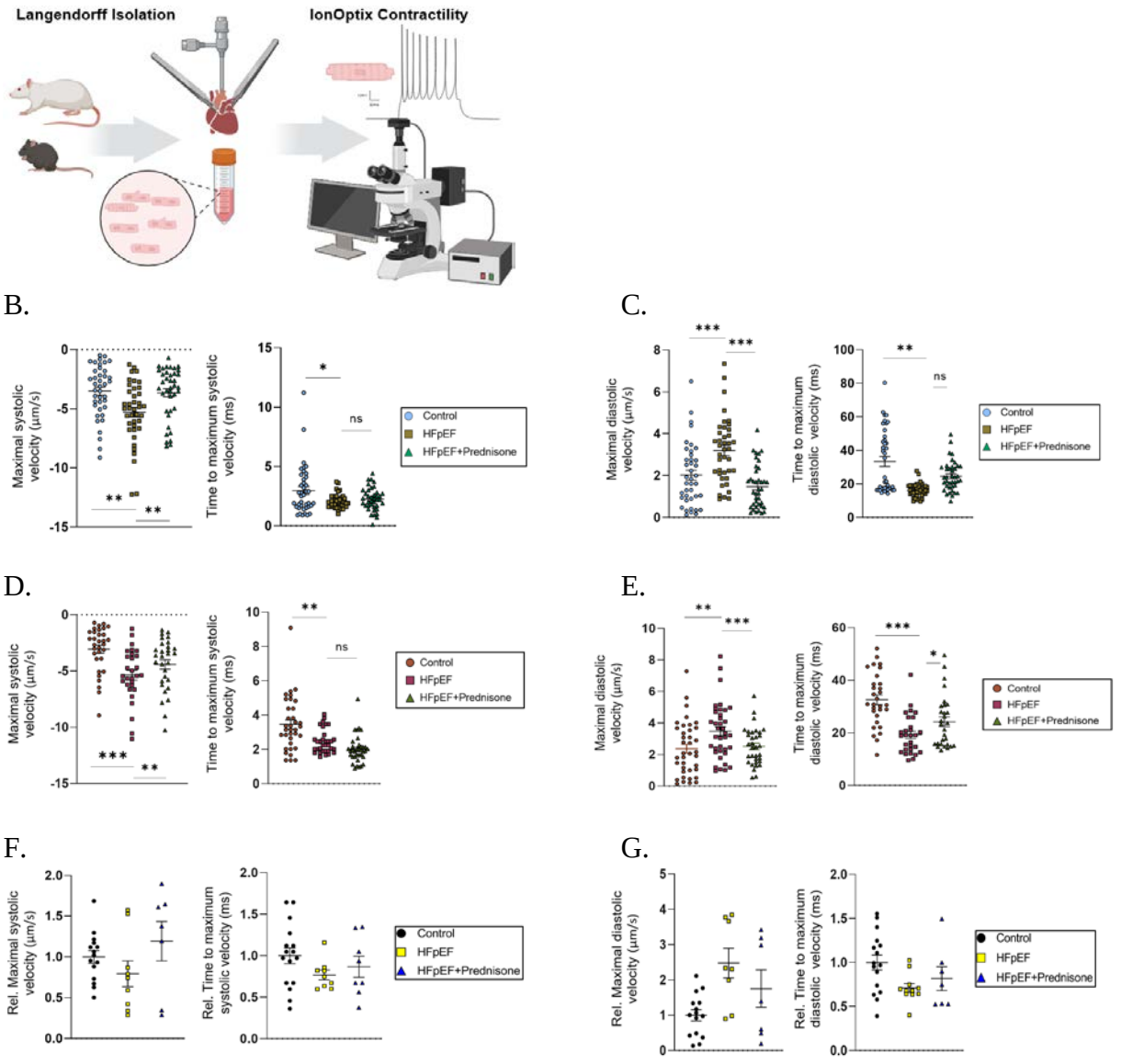

Figure 2. The effects of candidate compounds on contractility parameters measured on isolated cardiomyocytes and hiPSC derived EHTs. Schematic diagram showing the workflow for cardiomyocyte isolation followed by measurements recorded using the IonOptix system. Parameters of contractility in mice on HFpEF treatment and treated with prednisone during the light phase indicating how force generation and relaxation mechanisms are affected in isolated cardiomyocytes (**B, C**), as well as those parameters recorded in using the rat model (**D, E**) and hiPSC-EHT (**F, G**).

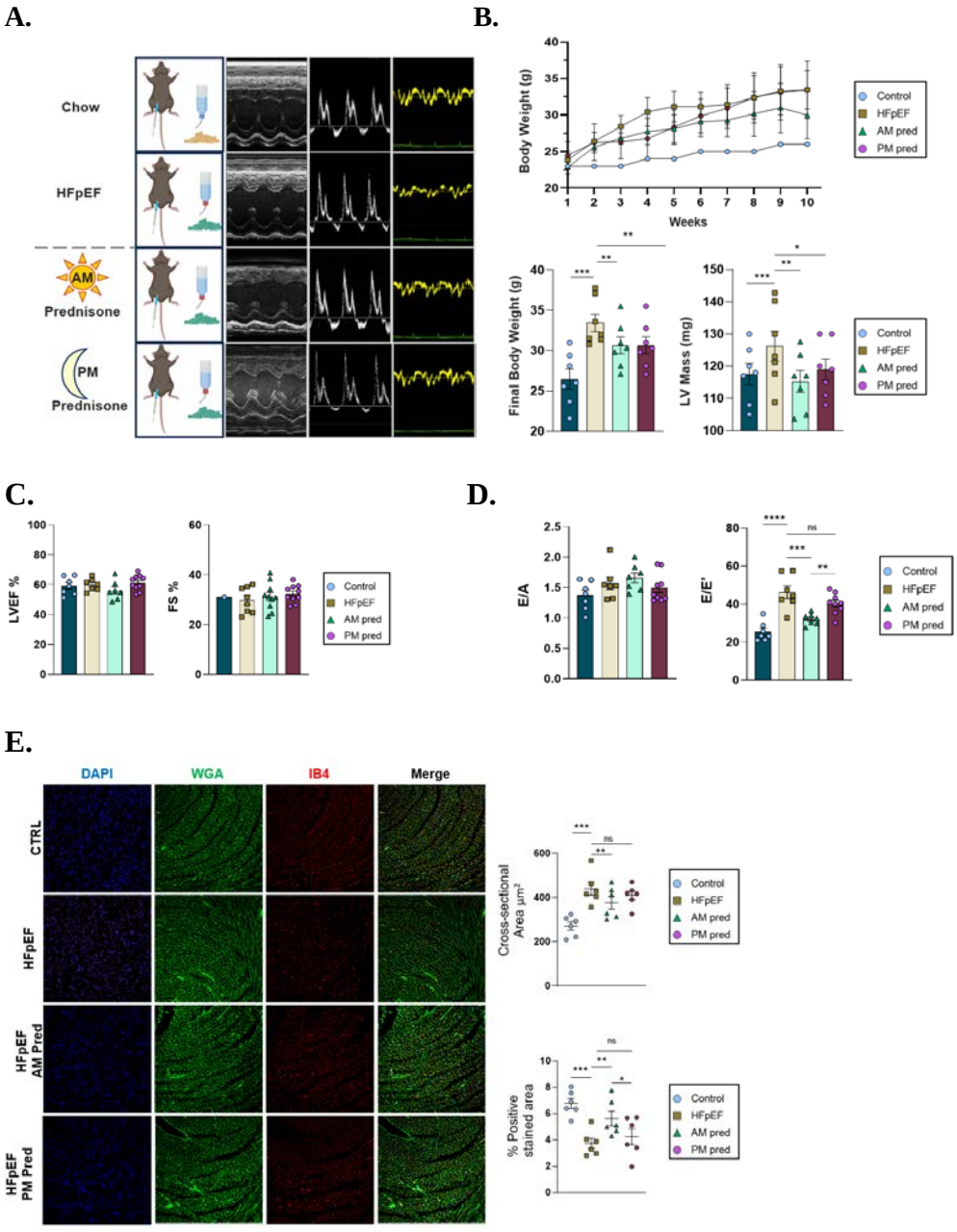

Figure 3. Schematic diagram showing indicated treatment groups and corresponding representative images of echocardiographic m-mode, pulsed-wave and tissue doppler measurements (**A**). Body weights recorded over treatment period, final body weight and LV mass (**B**). Measured parameters obtained from echocardiography show left ventricular ejection fraction (LVEF%) and fractional shortening (FS%) (**C**) E/A, and E/E' (**D**). Representative images from histological analysis of heart tissues stained with WGA and IB4 were used to measure cardiomyocyte cross-sectional area and vascular density respectively as shown in scatter plots (**E**).

A.

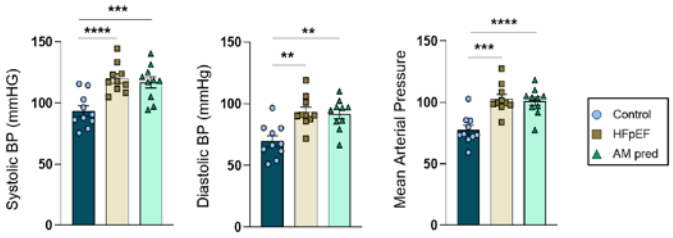

Figure 4. A tail-cuff monitoring system was used on acclimated mice. Each measurement indicates the mean value obtained in a session which included ten measurements. Final values represent the blood pressure recorded from a single animal obtained in a session and indicate the systolic, diastolic, and mean arterial pressures.

Unexpected outcomes and adaptations.

As described in our original proposal and indicated in the schematic shown in Figure 1A, we originally sought to obtain data sets from three species, analyze these, and then process them through previously described drug repurposing pipelines. This approach was successful in achieving its goal of identifying compounds that could be seen across multiple data sets that might reverse the gene expression signature obtained from HFpEF data sets, but it also presented new challenges. In this approach, we included only the top ten hits sorted by negative connectivity score for these data sets. Using this method, protein synthesis inhibitors were no longer included in the most significant hits, as we had previously observed. Instead, glucocorticoid receptor agonist was consistently observed among the most significant hits obtained in these data sets.

We were initially concerned that this might not be well suited for HFpEF, given that adverse effects of steroid use have been associated with weight gain, among other things. In reviewing the literature, though, we noted that the scheduling of corticoid steroid administration has recently been shown to improve muscle dystrophy[2, 3], metabolic dysfunction[1], and even cardiac dysfunction in the context of ischemic injury to a significant extent[4]. Interestingly though, these previously unrecognized benefits of prednisone were reported to be dependent on the timing and frequency of administration, which was only once a week and during the start of a light phase cycle. Recognizing that these findings were particularly relevant to HFpEF, we decided to adopt this dosing protocol and evaluate if prednisone, when administered intermittently by a circadian rhythm, might be beneficial in the context of HFpEF.

Summary/Conclusions

Thus far, our studies have identified candidate compounds capable of reversing aberrant gene expression signatures in HFpEF, as well as numerous in vivo and in vitro studies that provide evidence to support the use of intermittent prednisone as a potentially therapeutic agent. Following the selection of a candidate compound (**Figure 1 D**), we screened candidate compounds on isolated cardiomyocytes and hiPSC-EHT to determine which compound might have the greatest potential in alleviating relaxation parameters impaired by HFpEF (**Figure 2 B-G**). These experiments revealed that prednisone, when administered intermittently was the most suitable candidate to advance towards in vivo studies. We then made use of a two-hit mouse model and evaluated the broader functional consequences of intermittent prednisone treatment by performing echocardiography and histological analysis (**Figure 3C-E**). Here we revealed the hallmark features of HFpEF, namely an unchanged ejection fraction (EF) and an elevated E/E' were significantly improved in the AM prednisone treated group compared to vehicle treated mice on HFpEF.

Our prednisone treatment protocol was based on previously reported literature as discussed above, claimed that the beneficial effects on muscle and metabolic functions were dependent on circadian dosing. To determine if this might also be true for our experiments, we treated mice with a light-phase and dark-phase once-a-week injection of prednisone. From these experiments, we were able to determine that in the context of HFpEF, a similar dependency on the circadian period was required for prednisone to mediate beneficial effects. Measurements of blood pressure however showed that hypertensive stress was not attenuated by treatment with prednisone (**Figure 4A**).

PROTOCOL (1 of 1 total):

Protocol [ACURO Assigned Number]: PR220740.e001, [IACUC/ACC Protocol Number]: 22-166

Title: Systems Pharmacology Model of Heart Failure with Preserved Ejection Fraction

Target required for statistical significance: 690 mice; 140 rats.

Target approved for statistical significance: 690 mice; 140 rats.

Total subjects to date: 476 mice; 120 rats

SUBMITTED TO AND APPROVED BY:

ACURO approval date: 04/11/2023

IACUC/ACC Protocol#: 22-166 approval date: 01/30/2023.

STATUS:

HRPO (Not applicable/Nothing to Report)

			Enter information regarding number of subjects					
HRPO Protocol Number	Protocol PI Name	Organization (Site)	# Target	# Enrolled	# Completed	# Screened	# Recruited	Other

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

Nothing to Report

How were the results disseminated to communities of interest?

Nothing to Report for this particular reporting period. We plan to present our data at national conferences in the next performance period.

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

During the next year, we will attempt to achieve the unfinished goals described in the above SOW table and make efforts to present this work at either conferences or through the preparation of and submission of a manuscript. These goals are indicated by the percent completion described in the above provided, updated SOW. Some goals, such as exercise testing, which we have just recently begun to work towards completing, due to time required to optimize a reliable exercise protocol to measure exercise intolerance we anticipate will be completely promptly.

We will also complete data collection and analysis for additional in vitro targets using isolated rodent cardiomyocytes and iPSC-derived EHTs. Although our primary focus on the in vivo studies are on prednisone considering its promising effects so far, we will include additional testing of other candidate compounds in Aims 1 and 2 upon completion of in vitro data collection and analysis. Collectively, these results will provide valuable insights which speak to the translational potential of our findings thus far.

4. IMPACT:

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

We believe that the approach used in this proposal is impactful in terms of addressing how to treat HFpEF at a systemic level, focusing on the nature of HFpEF as a multifactorial disease involving various risk factors. Reflecting this paradigm, we utilized an approach of looking an broad gene signatures to identify compounds that can induce a broad reversal of these signatures, leading to the identification of intermittent prednisone dosing as a promising candidate to treat HFpEF. Our in vitro and in vivo data so far appears promising in supporting prednisone as a strong candidate.

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
Nothing to Report

Actual or anticipated problems or delays and actions or plans to resolve them
Nothing to Report

Changes that had a significant impact on expenditures
Nothing to Report

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
Nothing to Report

Significant changes in use or care of vertebrate animals
Nothing to Report

Significant changes in use of biohazards and/or select agents
Nothing to Report

6. PRODUCTS:

- **Publications, conference papers, and presentations.**
  Nothing to report.

- **Journal publications.**
  Nothing to report.

- **Books or other non-periodical, one-time publications.**
  Nothing to report.

- **Other publications, conference papers and presentations**.
  Nothing to report.

- **Website(s) or other Internet site(s).**
  Nothing to report.

- **Technologies or techniques.**
  Nothing to report.

- **Inventions, patent applications, and/or licenses.**
  Nothing to report.

- **Other Products.**
  Nothing to report.

PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?

Provide the following information for: (1) PDs/PIs; and (2) each person who has worked at least one person month per year on the project during the reporting period, regardless of the source of compensation (a person month equals approximately 160 hours of effort). If information is unchanged from a previous submission, provide the name only and indicate "no change".

Project Personnel	
Name:	**Sarath Babu Nukala**
Project Role:	Post-doc Researcher
Researcher Identifier (e.g., ORCID ID): N/a	N/A
Nearest person month worked:	1
Contribution to Project:	Dr. Nukala has performed assisted in the bioinformatic analysis, experimental methods, and data curation.
Funding Support:	Start-up funds
Name:	**Jordan Jousma**
Project Role:	Graduate Student
Researcher Identifier (e.g., ORCID ID):	0000-0001-5434-2573
Nearest person month worked:	6
Contribution to Project:	Mr. Jousma has assisted in echocardiography experiments, experimental methods, and data curation.
Funding Support:	NIHLBI T32 HL007829
Name:	**Zhenbo Han**
Project Role:	Post-doc Researcher
Researcher Identifier (e.g., ORCID ID): N/a	N/A
Nearest person month worked:	2
Contribution to Project:	Dr. Han has assisted in ipsc-cm differentiation, experimental methods, and data validation.
Funding Support:	AHA postdoc fellowship 917176/This grant
Name:	**Gege Yan**
Project Role:	Post-doc Researcher
Researcher Identifier (e.g., ORCID ID):	N/A
Nearest person month worked:	2
Contribution to Project:	Dr. Yan has assisted in experimental methods and data validation.
Funding Support:	AHA postdoc fellowship 23POST1029855
Name:	**Sang Ging Ong**
Project Role:	Principal Investigator
Researcher Identifier (e.g., ORCID ID): N/a	0000-0003-0182-8769
Nearest person month worked:	1
Contribution to Project:	Oversee the entire proposal's progress
Funding Support:	Institutional support/NIH grants

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?
Nothing to Report

What other organizations were involved as partners?
Nothing to Report.

SPECIAL REPORTING REQUIREMENTS

COLLABORATIVE AWARDS:
Nothing to Report.

QUAD CHARTS:
Nothing to Report.

7. APPENDICES:
Nothing to Report.

Reference Citations

1. Quattrocelli, M., et al., *Intermittent prednisone treatment in mice promotes exercise tolerance in obesity through adiponectin.* Journal of Experimental Medicine, 2022. **219**(5).
2. Quattrocelli, M., et al., *Intermittent glucocorticoid steroid dosing enhances muscle repair without eliciting muscle atrophy.* The Journal of Clinical Investigation, 2017. **127**(6): p. 2418-2432.
3. Quattrocelli, M., et al., *Muscle mitochondrial remodeling by intermittent glucocorticoid drugs requires an intact circadian clock and muscle PGC1α.* Science Advances, 2022. **8**(7): p. eabm1189.
4. Wintzinger, M., et al., *Impact of circadian time of dosing on cardiomyocyte-autonomous effects of glucocorticoids.* Molecular Metabolism, 2022. **62**: p. 101528.