

**APPLICATION FOR FEDERAL ASSISTANCE
SF 424 (R&R)**

		3. DATE RECEIVED BY STATE	State Application Identifier
1. TYPE OF SUBMISSION*		4.a. Federal Identifier	
<input type="radio"/> Pre-application <input checked="" type="radio"/> Application <input type="radio"/> Changed/Corrected Application		b. Agency Routing Number	
2. DATE SUBMITTED	Application Identifier	c. Previous Grants.gov Tracking Number	
5. APPLICANT INFORMATION			
Legal Name*: GLYSCEND, INC.			
Department:			
Division:			
Street1*:	101 W 39TH ST, STE C-2		
Street2:			
City*:	BALTIMORE		
County:			
State*:	MD: Maryland		
Province:			
Country*:	USA: UNITED STATES		
ZIP / Postal Code*:	212103164		
Person to be contacted on matters involving this application			
Prefix:	First Name*: Thomas	Middle Name: Henry	Last Name*: Jozefiak
Position/Title:	Suffix:		
Street1*:	Co-Founder & CSO		
Street2:	101 W 39TH ST, STE C-2		
City*:	BALTIMORE		
County:			
State*:	MD: Maryland		
Province:			
Country*:	USA: UNITED STATES		
ZIP / Postal Code*:	212103164		
Phone Number*:	617-275-1765	Fax Number:	Email: tjozefiak@glyscend.com
6. EMPLOYER IDENTIFICATION NUMBER (EIN) or (TIN)*		47-2030150	
7. TYPE OF APPLICANT*		R: Small Business	
Other (Specify):			
Small Business Organization Type		<input type="radio"/> Women Owned	<input type="radio"/> Socially and Economically Disadvantaged
8. TYPE OF APPLICATION*		If Revision, mark appropriate box(es).	
<input checked="" type="radio"/> New	<input type="radio"/> Resubmission	<input type="radio"/> A. Increase Award	<input type="radio"/> B. Decrease Award
<input type="radio"/> Renewal	<input type="radio"/> Continuation	<input type="radio"/> C. Increase Duration	<input type="radio"/> D. Decrease Duration
	<input type="radio"/> Revision	<input type="radio"/> E. Other (specify):	
Is this application being submitted to other agencies?*		<input type="radio"/> Yes	<input checked="" type="radio"/> No
What other Agencies?			
9. NAME OF FEDERAL AGENCY* National Institutes of Health		10. CATALOG OF FEDERAL DOMESTIC ASSISTANCE NUMBER TITLE:	
11. DESCRIPTIVE TITLE OF APPLICANT'S PROJECT* A novel oral enzyme therapy to treat enteric hyperoxaluria			
12. PROPOSED PROJECT Start Date* 12/01/2024		13. CONGRESSIONAL DISTRICTS OF APPLICANT Ending Date* 11/30/2025	
MD-007			

SF 424 (R&R) APPLICATION FOR FEDERAL ASSISTANCE**14. PROJECT DIRECTOR/PRINCIPAL INVESTIGATOR CONTACT INFORMATION**

Prefix: First Name*: Ashish Middle Name: Last Name*: Nimgaonkar Suffix:
 Position/Title: Co-Founder, Chief Medical Officer
 Organization Name*: GLYSCEND, INC.
 Department:
 Division:
 Street1*: 101 W 39TH ST, STE C-2
 Street2:
 City*: BALTIMORE
 County:
 State*: MD: Maryland
 Province:
 Country*: USA: UNITED STATES
 ZIP / Postal Code*: 212103164
 Phone Number*: 900-000-0000 Fax Number: Email*: animgaonkar@glyscend.com

15. ESTIMATE PROJECT FUNDING

a. Total Federal Funds Requested*	\$324,501.00
b. Total Non-Federal Funds*	\$0.00
c. Total Federal & Non-Federal Funds*	\$324,501.00
d. Estimated Program Income*	\$0.00

16. IS APPLICATION SUBJECT TO REVIEW BY STATE EXECUTIVE ORDER 12372 PROCESS?*

- a. YES THIS PREAPPLICATION/APPLICATION WAS MADE AVAILABLE TO THE STATE EXECUTIVE ORDER 12372 PROCESS FOR REVIEW ON:
 DATE:
- b. NO PROGRAM IS NOT COVERED BY E.O. 12372; OR
 PROGRAM HAS NOT BEEN SELECTED BY STATE FOR REVIEW

17. By signing this application, I certify (1) to the statements contained in the list of certifications* and (2) that the statements herein are true, complete and accurate to the best of my knowledge. I also provide the required assurances * and agree to comply with any resulting terms if I accept an award. I am aware that any false, fictitious, or fraudulent statements or claims may subject me to criminal, civil, or administrative penalties. (U.S. Code, Title 18, Section 1001)

I agree*

* The list of certifications and assurances, or an Internet site where you may obtain this list, is contained in the announcement or agency specific instructions.

18. SFLLL or OTHER EXPLANATORY DOCUMENTATION

File Name:

19. AUTHORIZED REPRESENTATIVE

Prefix: First Name*: Kevin Middle Name: Last Name*: Colbert Suffix:
 Position/Title*: Senior Director of Operations & Strategy
 Organization Name*: GLYSCEND, INC.
 Department:
 Division:
 Street1*: 101 W 39TH ST, STE C-2
 Street2:
 City*: BALTIMORE
 County:
 State*: MD: Maryland
 Province:
 Country*: USA: UNITED STATES
 ZIP / Postal Code*: 212103164
 Phone Number*: 913-314-1080 Fax Number: Email*: kcolbert@glyscend.com

Signature of Authorized Representative*

Completed on submission to Grants.gov

Date Signed*

03/26/2024

20. PRE-APPLICATION File Name:**21. COVER LETTER ATTACHMENT** File Name:

424 R&R and PHS-398 Specific

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Project/Performance Site Location(s)

Project/Performance Site Primary Location

I am submitting an application as an individual, and not on behalf of a company, state, local or tribal government, academia, or other type of organization.

Organization Name: GLYSCEND, INC.
UEI: CA69UUBUYMW7
Street1*: 101 W 39TH ST, STE C-2
Street2:
City*: BALTIMORE
County:
State*: MD: Maryland
Province:
Country*: USA: UNITED STATES
Zip / Postal Code*: 212103164
Project/Performance Site Congressional District*: MD-007

Additional Location(s)

File Name:

RESEARCH & RELATED Other Project Information**1. Are Human Subjects Involved?*** Yes No

1.a. If YES to Human Subjects

Is the Project Exempt from Federal regulations? Yes NoIf YES, check appropriate exemption number: 1 2 3 4 5 6 7 8If NO, is the IRB review Pending? Yes No

IRB Approval Date:

Human Subject Assurance Number

2. Are Vertebrate Animals Used?* Yes No

2.a. If YES to Vertebrate Animals

Is the IACUC review Pending? Yes No

IACUC Approval Date:

Animal Welfare Assurance Number none

3. Is proprietary/privileged information included in the application?* Yes No**4.a. Does this project have an actual or potential impact - positive or negative - on the environment?*** Yes No

4.b. If yes, please explain:

4.c. If this project has an actual or potential impact on the environment, has an exemption been authorized or an Yes No environmental assessment (EA) or environmental impact statement (EIS) been performed?

4.d. If yes, please explain:

5. Is the research performance site designated, or eligible to be designated, as a historic place?* Yes No

5.a. If yes, please explain:

6. Does this project involve activities outside the United States or partnership with international collaborators?* Yes No

6.a. If yes, identify countries:

6.b. Optional Explanation:

Filename

7. Project Summary/Abstract* Summary_Glyscend_20240328.pdf**8. Project Narrative*** Narrative_Glyscend_20240328.pdf**9. Bibliography & References Cited** References_Glyscend_20240328.pdf**10. Facilities & Other Resources** Facilities_Glyscend_20240320.pdf**11. Equipment** Equipment_Glyscend_20240320.pdf

PROJECT SUMMARY

Enteric hyperoxaluria (EH) is a dangerous and life-altering condition that typically occurs secondary to conditions or procedures that cause gastrointestinal (GI) malabsorption of nutrients - e.g. inflammatory bowel diseases and GI surgical procedures. EH leads to higher levels of oxalate excretion through urine, kidney stone formation, and often results in chronic- and end-stage kidney disease. Current treatment options are largely ineffective but include dietary changes to reduce oxalate intake, calcium supplements or other low-efficacy medications to bind oxalate prior to absorption, and hydration to dilute oxalate in the urine.

This project seeks to redress the high levels of oxalate absorption that occurs with EH. The work uses an enzyme that degrades oxalate in the gastrointestinal tract. This enzyme has gone through a Phase III trial where the performance fell short of expectations. The approach therefore is to combine the enzyme with a second drug, a mucus complexing polymer (MCP) that crosslinks to mucin at the lining of the gastrointestinal tract. The concept is that the second drug will prolong the retention time of the enzyme within the GI system, that this retention occurs at the epithelial interface where the MCP acts and where oxalate is absorbed, and oxalate binding is additionally increased by the MCP, which has been shown to reduce levels of oxalate in urine.

To demonstrate the potential for this drug combination, proposed are an in vitro, ex vivo, and in vivo study. The in vitro study will examine combinations of the enzyme and MCP candidates that maintain the enzymatic activity of the native enzyme. These combinations will then be tested for the retention of enzymatic activity in porcine intestinal tissues. The candidate combinations that again maintain the enzymatic activity of the native enzyme will then be tested in vivo to demonstrate proof of concept. In this latter effort the testing will initially explore the effect of dose timing and formulation strength on oxalate excretion, before further in vivo testing for rats on a high oxalate diet.

PROJECT NARRATIVE

Glyscend is developing a unique combination therapy for the treatment of enteric hyperoxaluria (EH). The approach combines an orally active oxalate decarboxylase enzyme and a mucus complexing polymer, with the promise of a best-in-class reduction in urine oxalate excretion. The development of this product creates treatment pathways for high oxalate conditions which can lead to kidney stones and chronic- and end-stage kidney disease.

FACILITIES AND OTHER RESOURCES- GLYSCEND

Glyscend's R&D team is based in Lowell, Massachusetts at a startup incubator facility affiliated with the University of Massachusetts at Lowell. The Massachusetts Medical Device Development Center (M2D2) is a state-sponsored initiative, committed to nurturing small and medium-sized enterprises, fostering innovation, job creation, and economic advancement.

Laboratory – Glyscend rents a 600 ft² private laboratory at M2D2's facility at 600 Suffolk Street. Our private lab is equipped with critical R&D tools owned by Glyscend (listed in the Equipment document). Our in-house capabilities enable Glyscend's R&D team to synthesize and purify polymers and small molecules, carry out various analytical procedures required for the development of our clinical candidate and characterization of new materials. In this regard, the team has generated a library of >135 Technical Memos and 19 Analytical Protocols pertaining to the development of our current clinical candidate.

Biohazards Handling and Disposal – N/A

Clinical – N/A

Animal – Glyscend is outsourcing animal studies to NeoSome Life Sciences, LLC.

Computer – Each employee has access to high-speed computers with all necessary software, for advanced imaging, statistics, etc. In addition, each employee is provided with site licenses for software for word processing, data analysis, image processing, statistical analysis, and others.

Office – Glyscend rents two spacious offices directly across the hall from our laboratory in M2D2.

IP – Glyscend acquired exclusive ownership of the large IP estate covering ALLN-177. Glyscend's IP estate covering the clinical candidate and a significant family of related MCP materials is large and secure. We are filing a provisional patent to secure priority date for IP protection of the novel formulations. Individually, the intellectual property describing each of the two agents is significant, describing both their compositions and methods of use in their primary indications.

Other – As members of the M2D2 Center, Glyscend also has access to shared equipment housed at M2D2's nearby facility at 110 Canal Street, Lowell, MA. The 11,000 sq. ft. center is made up of a fully-equipped, shared lab facility that can house 50 researchers and also includes plenty of co-working and meeting spaces. This shared laboratory facility provides Glyscend with direct, no cost access to a large collection of R&D tools (See list below). Canal Street also provides access to a BSL-2 lab with microbiology and cell culture tools at low cost.

EQUIPMENT- GLYSCEND

Glyscend has a suite of equipment owned by Glyscend, as well as access to additional equipment in the M2D2 (Massachusetts Medical Device Development Center) center where Glyscend is headquartered. The equipment and resources are listed below.

Glyscend Equipment

Instrument

- SPR System with autosampler, Biosensing Instrument, BI-4500A
- Automated titrator, Mettler-Toledo, G10S
- UV-Vis Spectrometer, ThermoFisher, Genesys 180
- Water purifier (Type-1), Sartorius, Arium Advance
- Water dispensing system, Sartorius, Arium Smart Station
- Incubating Mini Shaker, Ohaus
- Lab Pump (oil-free) (2), ThermoFisher, Max-Dry 20-75
- Lyophilizer, Labconco, Freezone-4.5L-105°C
- Lyo Pump, Edwards
- Centrifuge, Eppendorf, 5810 Benchtop Centrifuge
- Centrifuge Rotor, Eppendorf, FA-45-30-11 Rotor
- Centrifuge Rotor, Eppendorf, A-4-62 Rotor
- Centrifuge Accessories, Eppendorf, 2 Microplate Carriers, 4 Buckets
- Centrifuge Accessories, Eppendorf, 15ml/ 50ml Adaptors
- Flammables Cabinet, Eagle, 1932
- Incubators (2), Benchmark Sci., MYTEMP, H2200-H
- Plate reader, Molecular Dynamics, SpectraMax 384
- Top Loader (10 kg), VWR, E-Series10001
- Top Loader (4 kg), Ohaus, Voyager V0D120
- Analytical balance, VWR, VWR-164AC
- Analytical balance, Fisher Scientific
- UPC (2), APC, Smart-UPS 1500
- Refrigerator/freezer, Kenmore
- Freezer (-25), Fisher Scientific, IsoTemp
- pH meter, Fisher Scientific, Accumet AE150
- pH electrode (2), Fisher Scientific, 13-520-851
- pH meter, Hanna Instruments, HI2216
- pH electrode (2), Hanna Instruments, HI1131B
- Conductivity meter, Mettler-Toledo, Model S3 InLab® 73 Series
- UV lamp, UVP, UVGL-25
- temperature controller, J-Kem, Model 210 timer
- reaction block, STEM, RS1000A
- reaction block, React Array, GSIOC033A
- peristaltic pump for TFF, Cole-Palmer, 772000-62
- TFF filtration block, Pall Filtration, FS007X70
- TFF Reservoir, Pall Filtration, FS012K10

M2D2 110 Canal Street, Shared Equipment

As members of the M2D2 Center, Glyscend also has access to shared equipment housed at M2D2's nearby facility at 110 Canal Street, Lowell, MA. The 11,000 sq. ft. center is made up of a fully-equipped, shared lab facility that can house 50 researchers and also includes plenty of co-working and meeting spaces. This shared laboratory facility provides Glyscend with direct, no cost access to a large collection of R&D tools (See list below). Canal Street also provides access to a BSL-2 lab with microbiology and cell culture tools at low cost.

Genomics

- Eppendorf Pro S PCR
- G Storm PCR
- Applied Biosystems QuantStudio 5
- Applied Biosystems QuantStudio 3

Analytical

- Waters Acuity UPLC with SQD Mass Spec
- Waters Acuity UPLC HClass (PDA, RI)
- GE Akta Start FPLC with Frac30 fraction collector
- Biomate 3S UV/VIS Spectrophotometer
- Genesys 180 UV/VIS Spectrophotometer
- Thermo Electron Nicolet 6700 FT-IR
- Bioteck H1 Synergy Fluorescent microplate reader
- Bioteck Powerwave Plate Reader

Bio/Cellular Research

- Biosafety Cabinets (9)
- Laminar Flow Hood
- CO2 Incubators
- New Brunswick Innova 44R Shaker/Incubator
- New Brunswick Innova 4430R Shaker/Incubators
- Binder Refrigerated Incubator KB-115
- Binder Refrigerated Incubator KB-53
- Forma Scientific Water Jacketed CO2 Incubator
- Thermo Fisher 20-25 degree refrigerated Incubator
- CryoPlus One LN2 cell storage
- Fisher Scientific homogenizer

Specialized

- Labconco FreeZone Plus 6L Lyophilizer (-89°)
- Flexi-Dry Freeze Dryer (-89°)
- Harvest Right Shelf Freeze dryer
- Binder Humidity chamber KB-240
- Electrophoresis Power Supplies/Plates
- Beckman Coulter Z2 counter with Accucomp software
- Synbiosis Protocol 3 Automatic Colony Counter
- Protein Simple Fluorchem Gel Imager with Color Camera
- GXii lab chip DNA/RNA/ Protein reader
- FluorChem E Gel imager
- BioRad Western Blot transfer station
- Mettler Toledo HC-103 Moisture Analyzer
- TekTronix Oscilloscope
- Instron 5944 Materials Testing System
- Digital Melting point apparatus
- Brookfield CAP 200+ Viscometer
- Brookfield RSO Rheometer
- Beckman Coulter LS 13 320 Particle Size analyzer
- SFX-250 Branson Cell Disruptor/sonicator
- Silverson L5MA High Shear mixer
- Buchii Rotovap with vacuum pump
- Savant SpeedVac evaporator with vacuum pump

Microscopes

- Leica DMIL Fluorescent Inverted Microscope with Digital Camera

- Leica DM 750 Phase Contrast Microscope with Digital Camera
- Leica DM EZ4 Dissection Microscope with Digital Camera
- Zeiss Laboratory Microscope
- Leica Galen Laboratory Microscope
- VanGuard Inverted Laboratory Microscope

General

- Steris LV-250 Autoclave
- Tomy SX-700 Portable Autoclave
- Tomy SX-500 Portable Autoclaves
- Steris XL 500 Glassware Washer
- Beckman Coulter Avanti JE Floor Centrifuge
- Beckman Coulter JXN-26 Floor Centrifuge
- Beckman Coulter Optima L-100 XP Floor Ultracentrifuge
- Beckman Coulter TLX-120K Benchtop Ultracentrifuge
- Beckman Coulter Microfuge R Centrifuge
- Beckman Coulter X22 Centrifuge
- VWR Clinical centrifuge
- Eppendorf 5415 refrigerated microcentrifuge
- Freezers, -20C
- Freezers, -80C
- Refrigerators, 4C
- Fume Hoods
- Milli-Q Type 1 water systems
- DI Water throughout Lab
- Medical grade compressed air
- Vacuum at benchtop
- Analytical Balances
- Water Baths
- Dry Baths (Beads and blocks)
- Flammable Storage Cabinets
- Hoshizaki Ice maker
- 12 and 8 channel pipettors
- pH meters
- Hot Plates/Stir Plates
- Vacuum oven
- Muffle furnace

RESEARCH & RELATED Senior/Key Person Profile (Expanded)

PROFILE - Project Director/Principal Investigator						
Prefix:	First Name*:	Ashish	Middle Name	Last Name*:	Nimgaonkar	Suffix:
Position/Title*:	Co-Founder, Chief Medical Officer					
Organization Name*:	GLYSCEND, INC.					
Department:						
Division:						
Street1*:	101 W 39TH ST, STE C-2					
Street2:						
City*:	BALTIMORE					
County:						
State*:	MD: Maryland					
Province:						
Country*:	USA: UNITED STATES					
Zip / Postal Code*:	212103164					
Phone Number*:	00	00-0000	Fax Number:			
E-Mail*:						animgaonkar@glyscend.com
Credential, e.g., agency login:						ANIMGA01
Project Role*:	PD/PI					Other Project Role Category:
Degree Type:	MD, MTech, MSci					Degree Year: 1998, 2000, 2003
Attach Biographical Sketch*:	File Name:					1_Bio_Nimgaonkar_20240325.pdf
Attach Current & Pending Support:	File Name:					

PROFILE - Senior/Key Person					
Prefix:	First Name*:	Thomas	Middle Name	Henry	
Position/Title*:	Last Name*:				Jozefiak
Organization Name*:	Co-Founder & Chief Scientific Officer				
Department:	GLYSCEND, INC.				
Division:					
Street1*:	101 W 39TH ST, STE C-2				
Street2:					
City*:	BALTIMORE				
County:					
State*:	MD: Maryland				
Province:					
Country*:	USA: UNITED STATES				
Zip / Postal Code*:	212103164				
Phone Number*:	617-275-1765		Fax Number:		
E-Mail*:	tjozefiak@glyscend.com				
Credential, e.g., agency login: tjozefiak					
Project Role*:	Co-Investigator		Other Project Role Category:		
Degree Type:	PhD, BS		Degree Year: 1989, 1981		
Attach Biographical Sketch*:	File Name:		2_Bio_Jozefiak_20240327.pdf		
Attach Current & Pending Support:	File Name:				

PROFILE - Senior/Key Person					
Prefix:	First Name*:	Taylor	Middle Name	L	
Position/Title*:	Last Name*:				Carlson
Organization Name*:	Senior Scientist				
Department:	GLYSCEND, INC.				
Division:					
Street1*:	101 W 39TH ST, STE C-2				
Street2:					
City*:	BALTIMORE				
County:					
State*:	MD: Maryland				
Province:					
Country*:	USA: UNITED STATES				
Zip / Postal Code*:	212103164				
Phone Number*:	000		0-0000	Fax Number:	
E-Mail*:	tcarlson@glyscend.com				
Credential, e.g., agency login: TLCARLS					
Project Role*:	Co-Investigator		Other Project Role Category:		
Degree Type:	PhD, BS		Degree Year: 2018, 2012		
Attach Biographical Sketch*:	File Name:		3_Bio_Carlson_20240325.pdf		
Attach Current & Pending Support:	File Name:				

BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors.
Follow this format for each person. **DO NOT EXCEED FIVE PAGES.**

NAME: Ashish Nimgaonkar

ERA COMMONS USER NAME (credential, e.g., agency login): ANIMGAO1

POSITION TITLE: Co-Founder, Chief Medical Officer

EDUCATION/TRAINING (*Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable. Add/delete rows as necessary.*)

INSTITUTION AND LOCATION	DEGREE (if applicable)	Completion Date MM/YYYY	FIELD OF STUDY
NTR University of Health Sciences, India	MD	05/1998	Medicine
India Institute of Technology, Mumbai, India	M.Tech	01/2000	Biomedical Engineering
Harvard-MIT, Cambridge, MA	M.Sci	06/2003	Biomedical Informatics
Children's Hospital, Boston, MA	Postdoc	06/2005	Biomedical Informatics
Beth Israel Deaconess Med Ctr. Boston, MA	Residency	08/2008	Internal Medicine
Stanford Hospital, Palo Alto, CA	Fellowship	06/2012	Gastroenterology
Stanford University, Stanford, CA	Fellowship	06/2012	Biodesign (MedTech)

A. Personal Statement

My research focus has been to develop new technologies and therapies to address unmet clinical needs in GI and metabolic diseases. I am trained in clinical gastroenterology, biomedical sciences and translational technology development which has enabled this goal. I have led numerous translational research projects at Johns Hopkins with my primary research focus being on developing technologies to treat patients with metabolic diseases. I have been inventor on many technologies and a recipient of numerous awards including the AGA-Boston Scientific career development technology & innovation award and the CIMIT-Johnson & Johnson Young Clinician award. My work has been supported by grants from both foundational (AGA, CIMIT, NCIIA) and federal (NIH, NSF) agencies. For the last decade, my research has focused on developing oral therapies to mimic intestinal bypass (duodenal exclusion) physiology and investigating its metabolic effects. This led to formation of Glyscend Therapeutics, for which I am a co-founder and served as its CEO from 2016-2023 before transitioning to the Chief Medical Officer and Head of R&D role. Together, with my background and expertise, I believe that I am well equipped to lead this SBIR project as the Principal Investigator.

B. Positions, Scientific Appointments, and Honors**Positions**

2014 - present	Associate Medical Director, Center for Bioengineering Innovation and Design, Department of Biomedical Engineering, Johns Hopkins University
2013 - present	Assistant Professor, Division of Gastroenterology, Johns Hopkins Medicine
2012 - 2014	Visiting Assistant Professor, Division of Gastroenterology, Stanford Hospital
2012 - 2013	Staff Physician, Health-quest Medical Practice Lagrangeville, NY
2010 - 2012	Biodesign Fellowship, Stanford University, Stanford, CA
2009 - 2012	Clinical Fellowship, Gastroenterology, Stanford Hospital, Stanford, CA

2008 - 2009	Instructor, Department of Medicine, Beth Israel Deaconess Med Ctr, Boston, MA
2005 - 2008	Residency, Internal Medicine, Beth Israel Deaconess Medical Center, Boston, MA
2003 – 2005	Postdoctoral Research Fellow, Biomedical Informatics, Children's Hospital, Boston, MA
2000 - 2001	Research Assistant, Biomed Engineering, Indian Institute of Technology, Mumbai, India

Other Experience and Professional Memberships

2019 – present	Member, The Obesity Society
2018 - present	Member, American Diabetes Association
2009 - present	Member, American Society of Gastrointestinal Endoscopy
2009 - present	Member, American Gastroenterological Association

Honors

2014	AGA-Boston Scientific Career Development Technology and Innovation Award
2008	Winner of the Resident/Fellow Research award at Beth Israel Deaconess Medical Center/Harvard Medical School, Boston
2008	CIMIT-Johnson & Johnson Young Clinician Research Award
2001	Young Scientist Award and Hansraj-Nayyar Memorial Award for the best paper National Conference of Critical Care Medicine
1998	Dean's Honor list in medical school
1995	Distinction awarded in Pharmacology and Microbiology
1993	Best Medical Student Award
1992	National Merit Scholar
1990	National Talent Search Scholar

C. Contributions to Science

- 1. Metabolic Diseases (Obesity and Diabetes Therapies).** The primary focus of my work in this area has been around developing an oral approach to replicate the duodenal exclusion (DE) mechanism of Roux-en-Y (RYGB) surgery. DE is one of the key mechanisms in RYGB which results in significant improvements in various metabolic parameters (weight loss, blood glucose, lipids). The inspiration for an oral approach (pharmacologic duodenal exclusion) was driven by the fact that RYGB and endoscopic procedures targeting DE mechanism, while very effective for metabolic improvements, are not scalable and can have procedural risks associated with them. This work led to the formation of Glyscend Therapeutics, which is now advancing a drug (GLY-200) through clinical development. GLY-200 is a non-absorbed oral polymeric drug taken daily, which replicates the duodenal exclusion physiology. In phase 1 and phase 2a trial, this drug was found to be safe, tolerable, replicated the biomarker signature of duodenal exclusion and had robust effects on blood glucose and lipids with positive weight loss signal.
 - Nimgaonkar A. et al. 1209-P: Profound Metabolic Benefits Observed in an Eight-Week GK Rat Study of a Novel Orally Administered Polymeric Duodenal Exclusion Therapy: Implications for Type 2 Diabetes (T2D) Therapy. *Diabetes* 70, (2021).
 - Nimgaonkar, A. et al. 818-P: Chronic Oral Polymeric Duodenal Exclusion Therapy Has Profound Metabolic Benefits in a T2D Rat Model and Affects Duodenal Morphological Enteroplasticity Similar to RYGB surgery: Implications for T2D Therapy. *Diabetes* 71, (2022).
 - Fineman, M. S. et al. First-in-human study of a pharmacological duodenal exclusion therapy shows reduced postprandial glucose and insulin and increased bile acid and gut hormone concentrations. *Diabetes, Obes. Metab.* 25, 2447–2456 (2023).
 - Nimgaonkar, A. et al. GLY-200, a Pharmacologic Duodenal Exclusion Therapy, Improved Metabolic Parameters in the DIO Rat. *Diabetes* 72, (2023).

- 2. Early Detection of Colorectal Cancer.** As a practicing gastroenterologist, I recognized the need for having a blood-based biomarker (liquid biopsy) as an alternative to a stool-based test as a screening tool for colon cancer. I have been involved in guiding the development of a novel blood-based assay (FirstSight) as clinical investigator and advisor. The FirstSight assay is a multi-modal test that involves combining circulating epithelial cells (CECs) and somatic mutations of cell-free DNA for the detection of

colorectal advanced adenomas and cancer. This assay has also been expanded for early detection of prostate and breast cancer. By improving early detection methods, my goal is to facilitate timely interventions, which will ultimately reduce the morbidity and mortality associated with these cancers.

- a. Nimgaonkar, A. et al. A novel circulating tumor cell blood test for early detection of colorectal, prostate, and breast cancers: Results from 709 samples. *J. Clin. Oncol.* 36, e13549–e13549 (2018).
 - b. Tsai, W.-S. et al. Prospective clinical study of circulating tumor cells for colorectal cancer screening. *J. Clin. Oncol.* 36, 556–556 (2018).
 - c. Friedland, S. et al. A sensitive and quantitative multimodal blood test for the detection of colorectal adenomas and cancer: Correlation with size and number of polyps. *J. Clin. Oncol.* 38, 1555–1555 (2020).
 - d. Friedland, S. et al. Development and Clinical Validation of a Blood Test for Early Detection of Colorectal Adenomas and Cancer for Screening and Postpolypectomy Surveillance. *Gastro Hep Adv.* 1, 223–230 (2022).
3. **Innovative technologies in Gastroenterology and Hepatology.** In the field of gastrointestinal and liver diseases, my work has spanned from novel imaging technologies for the pancreas, exploration of gastrointestinal dysmotility, inventing a new device for management of refractory ascites and the development of new therapies for metabolic diseases. My research aims to push the boundaries of diagnostic and therapeutic approaches in gastroenterology, contributing to the future of the specialty and improving patient care.
 - a. Nimgaonkar, A. et al. Endoluminal MRI of the Pancreas: A Novel Imaging Technology. *Gastrointest. Endosc.* 67, AB132 (2008).
 - b. Nimgaonkar, A., Choi, J. W., Nguyen, L. & Triadafilopoulos, G. Gastrointestinal Dysmotility. *Dig. Dis. Sci.* 57, 1130–1133 (2012).
 - c. Kumbhari, V., Oberbach, A. & Nimgaonkar, A. Primary endoscopic therapies for obesity and metabolic diseases. *Curr. Opin. Gastroenterol.* 31, 351–8 (2015).
 - d. Jain, A. et al. PeriLeve: An Implantable Peritoneovesicular Biopowered Shunt to Manage Patients with Refractory Ascites. *Gastroenterology* 157, 21–22 (2019).
4. **Biomedical Informatics and Integrative Genomics.** As an informatician, my work in this area was focused on developing computational algorithms to solve problems in biology and medicine. My clinical informatics work was centered around intensive care medicine. I developed a machine learning mathematical model to predict the mortality of patients in the ICU - this model performed better than the APACHE (a standard model used in the ICU). My research in genomics was focused on building computational models from microarray gene expression data to understand reproducibility of gene expression across different microarray technologies as well as deciphering the regulatory mechanisms of gene expression under specific conditions. The overarching goal of this research was to gain insights into the genomic basis of diseases and their treatments, as well as reverse engineer molecular pathways involved in dysregulation.
 - a. Nimgaonkar A, et al: Prediction of mortality in an Indian intensive care unit: Comparison between APACHE II and Artificial Neural Networks. *Intensive Care Med.* 2004 Feb;30(2):248-53
 - b. Nimgaonkar A, et al: Reproducibility of gene expression across generations of Affymetrix microarrays. *BMC Bioinformatics.* 2003 Jun 25;4:27.
 - c. Rosalyn M. Adam, et al. Mechanical Stretch is a Highly Selective Regulator of Gene Expression in Bladder Smooth Muscle Cells. *Physiol Genomics.* 2004 Dec 15;20(1):36-44. 2004 Oct 5

BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors.
Follow this format for each person. **DO NOT EXCEED FIVE PAGES.**

NAME: Jozefiak, Thomas Henry

ERA COMMONS USER NAME (credential, e.g., agency login): tjozefiak

POSITION TITLE: Co-Founder and Chief ScientificS Officer

EDUCATION/TRAINING (*Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable. Add/delete rows as necessary.*)

INSTITUTION AND LOCATION	DEGREE (if applicable)	Completion Date MM/YYYY	FIELD OF STUDY
University of Massachusetts at Amherst	B.S	05/1981	Chemistry
University of Minnesota, Twin Cities	Ph.D.	12/1989	Organic Chemistry
California Institute of Technology, Pasadena	Postdoctoral	03/1992	Organic Chemistry, Materials Science

A. Personal Statement

Formally trained as an organic chemist, I am an experienced R&D leader who has built a distinguished and diverse industrial career in the areas of drug discovery, medical devices, biomaterials and drug delivery. I am passionate about the translation of discovery science into commercial products. I also place high value on relationship building, external outreach, and collaboration in industrial research and product development.

I received a Ph.D. degree in Organic Chemistry from the University of Minnesota in 1989, where my Doctoral Thesis described the synthesis of novel polyacene quinones and the study of their electrical and optical properties. Upon graduation, I joined the R&D lab at General Electric Plastics as Product Development Chemist at working on process chemistry for a new high temperature polyetherimide (next generation Ultem®). After 1.5 years I sought-out more a basic research experience and returned to an academic setting in the research group of Prof. Robert Grubbs at Caltech. At Caltech I developed a new family of electrically conductive organic polymers derived from the ring-opening metathesis of cyclooctatetraene; work I communicated in numerous publications and presentations. I then joined the prestigious Corporate Research Laboratories at The Eastman Kodak Company in Rochester NY as Senior Research Scientist in the Imaging Mechanisms Laboratory where I was inventor on numerous patents in silver halide color imaging and color photographic developers.

Seeking to redirect my career toward biomedical materials, I took the position of Principal Scientist at GelTex Pharmaceuticals in 1997, where I directed a team of polymer synthetic chemists to discover polymeric drugs efficacious as intestinal sequestrants and anti-obesity agents. Again, valuable IP was generated and a clinical candidate for obesity was found (GT-389-255). When GelTex was acquired by Genzyme in 2001, I accepted a leadership role combining chemistry assets from the two companies to establish a new medicinal chemistry group. I held positions of increasing responsibility at Genzyme, functioning as the Medicinal Chemistry lead in a highly matrixed team environment with clinical, quality, regulatory and manufacturing groups. This work led to a clinical candidate for Gaucher's disease (Genz-112638) and valuable intellectual property.

In 2006, recognizing my achievements as a leader in the MedChem group, and capitalizing on my earlier contributions at GelTex, I was appointed leader of Genzyme's Biomaterials Science and Engineering Group. Here, I led scientific efforts supporting the Genzyme Biosurgery product line, including; Seprafilm® adhesion barrier, Synvisc® viscosupplement, LeGoo® vascular occlusion device. My product support role was complimented by new product development initiatives in areas critical to the business unit including: sealants, hemostats, and regenerative medicine scaffolds. I originated product concepts and novel assays to support new

product development in these strategic areas. I also played a significant role representing the Science Organization on diligence teams for the evaluation of in-license or acquisition opportunities. I established several key collaborations with academic groups and private companies.

Due to shifting priorities at Genzyme and the take-over by Sanofi in 2011, I accepted the position of VP, Discovery at Living Proof, Inc. in April 2013. Living Proof is an MIT start-up company seeking to use material science innovations to invent and commercialize hair and skin care products. At Living Proof, I continued my biomaterials focus establishing a new polymer synthesis program in the area of elastic polyurethanes, and silicones for dermatological applications. I led the R&D team responsible for the successful commercial launch of Neotensil®, a unique in-situ forming skin reshaping film product. Once again, I led outreach efforts to enlist external industrial and academic expertise to advise our R&D team in solving complex product development problems. I also initiated an alliance with an external company to develop one of their proprietary materials for use in Living Proof products.

In April 2015, I left Living Proof to follow my own ideas and interests in biomedical polymers and resorbable biomaterials-based medical devices. As Principal at Jozefiak Consulting, I offered my clients and collaborators the benefit of my experience in diverse areas of polymer chemistry, biomaterials, and product development. In addition to my consulting activities, I co-founded Glycologix LLC, in 2016. Glycologix seeks to advance a new proteoglycan-mimic chemistry I invented that same year (SuperGAG biopolymers). Presently I serve as Scientific Founder and Advisor to Glycologix, where our lead candidate for the treatment of Interstitial Cystitis, GLY-100 is currently in a pilot clinical trial.

I am also a co-founder at Glyscend, Inc.,

My career has led me to understand the tremendous potential for therapeutic biomaterials, and I am excited about the prospects for Glycologix becoming a successful therapeutic biomaterials enterprise. I look forward to playing a major advisory role at Glycologix as we seek funding from granting agencies and private investors to grow the company with an initial focus on interstitial cystitis, a very difficult bladder disease with few therapeutic solutions.

Recent support I'd like to highlight

SuperGAGs for Intravesicular Treatment of Interstitial Cystitis 2R44DK116356-02A1

This was a Phase II SBIR from the NIDDK that provided early development support for GLX-100, a clinical candidate for the treatment of Interstitial Cystitis. Costs, \$1,996,642, 07/01/2020 – 06/30/2022 Role: Co-investigator

B. Positions and Honors

Positions and Employment

2017 - Present	Chief Scientific Officer, Glyscend Inc., Lowell, MA
2016 - Present	Scientific Founder, Advisor, Glycologix Inc., Beverly, MA
2015 - 2019	Principal, Jozefiak Consulting, Belmont, MA
2013 - 2015	Vice President, Discovery, Living Proof, Inc., Cambridge, MA
2009 - 2013	Vice President, Biomaterials Science and Engineering, Genzyme Corp., Framingham MA
2007 - 2009	Senior Director, Biomaterials Science and Engineering, Genzyme Corp., Framingham MA
2004 - 2007	Senior Director, Med Chem, Drug Discovery and Development, Genzyme Corp., Waltham MA
2002 - 2004	Director, Med Chem, Drug Discovery and Development, Genzyme Corp., Waltham MA
2001 - 2002	Principal Scientist, Drug Discovery and Development, Genzyme Corp., Waltham MA
1999 - 2001	Principal Scientist-II, GelTex Pharmaceuticals, Inc., Waltham MA
1997 - 1999	Principal Scientist-I, GelTex Pharmaceuticals, Inc., Waltham MA
1992 - 1997	Senior Research Chemist, Imaging Mechanisms Laboratory, Eastman Kodak Co, Rochester NY
1990 - 1992	Research Fellow, Robert H Grubbs Group, California Institute of Technology, Pasadena, CA
1989 - 1990	Prod. Dev. Chemist, High Performance Polymer Group, General Electric Plastics, Pittsfield,

MA

Other Experience and Professional Memberships

2015 - 2018	Associate Bioengineer, Brigham and Women's Hospital, Harvard Medical School, Boston, MA
2014 - Present	Science Advisory Board, Alafair Biosciences, Austin, TX
2012 - 2020	External Advisory Board, College of Natural Sciences, University of Massachusetts at Amherst
2012 - 2014	External Advisory Board, Department of Biomedical Engineering, University of Texas, Austin TX
2010 - 2012	Thesis Review Committee, Ph.D. Candidate Sarah Mayes, Department of Biomedical Engineering, University of Texas, Austin TX
2010 - 2012	Thesis Review Committee, Ph.D. Candidate Xiaoshu Dai, Department of Biomedical Engineering, Worcester Polytechnic Institute, Worcester MA
2007 - Present	Member, Controlled Release Society
2007 - Present	Member, Society for Biomaterials
2007 - 2010	Genzyme Representative, BEMA (Biomed. Eng. & Materials Applications) Roundtable, National Academy of Sciences
1980 - Present	Member, American Chemical Society

Honors

1985	Procter and Gamble Graduate Fellowship, University of Minnesota
1980	NSF Summer Research Fellowship, Polytechnic Institute of Brooklyn

C. Contribution to Science

1. Postsurgical adhesion formation is a recognized consequence of major abdominopelvic surgery, with an incidence estimated at over 93% in certain procedures. Adhesions commonly occur not only at the site of surgical intervention (incision lines, resections, tissue de-bulking) but also at neighboring sites due to tissue desiccation, handling, or abrasion. Adhesions cause serious patient morbidity such as bowel obstruction, female infertility, and pelvic pain. These consequences result in a significant economic burden due to hospital readmissions, prolonged lengths of stay, difficult reoperations, and litigation. The annual cost of abdominal adhesiolysis operations alone accounts for over \$1.1 billion dollars in the US. Thus, the prevention of postsurgical adhesions is of both clinical and economic importance to the healthcare system. During my years at Genzyme I established a research program investigating the biology of adhesion formation and applied several different biomaterials platform technologies to develop an easy to use in-situ forming adhesion barrier gel for laparoscopic surgeries. During this time, I also played a leadership role in providing technical support to Seprafilm, our market-leading anti-adhesion film.
 - a. Greenawalt, K.E., Colt, M.J., Corazzini, R.L., Syrkina, O.L., Jozefiak, T.H. (2011). Remote Efficacy for Two Different Forms of Hyaluronate-Based Adhesion Barriers. *Journal of Investigative Surgery*, 25(3), 174-180.
 - b. Dai, X., Chen, X., Yang, L., Foster, S., Coury, A.J., Jozefiak, T.H. (2011). Free Radical Polymerization of PEG-Diacrylate Macromers: Impact of Macromer Hydrophobicity and Initiator Chemistry on Polymerization Efficiency. *Acta Biomaterialia*, 7(5), 1965-1972.
 - c. Ruiz-Esparza, G.U., Wang, X., Zhang, X., Jimenez-Vazquez, S., Diaz-Gomez, L., Lavoie, A., Afewerki, S., Fuentes-Baldemar, A.A., Parra-Saldivar, R., Jiang, N., Annabi, N., Saleh, B., Yetisen, A.K., Sheikhi, A., Jozefiak, T.H., Shin, S.R., Dong, D., Khademhosseini, A (2021). The Nanoengineered Shear-thinning Hydrogel Barrier for Preventing Postoperative Adhesions, *Nano-Micro Letters*, 13, 212.
 - d. WO2019/231985 A1 Anti-adhesive shear thinning hydrogels, Ali Khademhosseini, Herrera, Guillermo Ulises Ruiz Esparza; Wang, Xichi; Shin, Su Ryon, Thomas Jozefiak (The Brigham and Women's Hospital, Inc.) Published May 29, 2019.

2. Human obesity is a recognized health crisis with greater than 160 million people considered clinically overweight in the United States. The accumulation or maintenance of body fat bears a direct relationship to caloric intake. Therefore, one of the most common methods for weight control to combat obesity is the use of relatively low-fat, low calorie diets, that is, diets containing less fat and calories than a "normal diet" or that

amount generally consumed by the patient. During my years at GelTex Pharmaceuticals, I had the opportunity to study how non absorbed polymers administered to the GI tract could interrupt the digestion of dietary triglyceride fat. I was particularly interested in the combination of polymeric fat-binding drugs with inhibitors of pancreatic lipase. This work was eventually out licensed to small company and advanced to human clinical trials.

- a. Dhal, P.K., Holmes-Farley, S.R., Huval, C., Jozefiak, T.H. (2006). Polymers as Drugs. *Advances in Polymer Science*, 192 (Polymer Therapeutics I), 9-58.
 - b. Jozefiak, T.H., Mandeville, W.H., Holmes-Farley, S.R., Arbeeny, C., Huval, C., Sacchiero, R., Concagh, D., Yang, K., Maloney, C. (2001) Synthetic Polymers for The Binding Of Fat In The Intestinal Tract. *Polymer Preprints (American Chemical Society, Division of Polymer Chemistry)* 2001, 42(2), 98.
 - c. US Patent 7,048,917. Fat-Binding Acrylamide Derivative Polymers. Thomas H. Jozefiak, Stephen Randall Holmes-Farley, W. Harry Mandeville III, Chad Cori Huval, Venkata R. Garigapati, Keith K. Shackett, Danny Concagh (GelTex Pharmaceuticals, Inc.), Issued May 23, 2006.
3. End-stage renal failure patients often have hyperphosphatemia, or elevated serum phosphate levels. Over extended periods of time, hyperphosphatemia leads to severe abnormalities in calcium and phosphorus metabolism, such as hyperparathyroidism, bone disease and calcification in joints, lungs, eyes and vasculature. For patients who exhibit renal insufficiency, elevation of serum phosphorus has been associated with progression of renal failure and increased risk of cardiovascular events. Calcium salts have been widely used to bind intestinal phosphate and prevent its absorption, however, hypercalcemia often results, causing serious side effects such as cardiac arrhythmias, renal failure, and skin and visceral calcification. During my years at GelTex Pharmaceuticals, I led a team of polymer and medicinal chemists in a project to identify molecules capable of inhibiting the active uptake of phosphate from the GI tract via the sodium-dependent phosphate co-transporter, NaPi-2b. My work advanced this field and helped to propel continued efforts at Genzyme (and elsewhere) once I moved onto other assignments.
 - a. US Patent 7,119,120. Preparation of Biaryl Phosphate Transport Inhibitors. Thomas H. Jozefiak, Cecilia M. Bastos, Andrew T. Papoulis, Stephen Randall Holmes-Farley (Genzyme Corporation), Issued October 10, 2006.
 - b. US Patent 7,109,184. Preparation Of Phosphate Transport Inhibitors. Thomas H. Jozefiak, Cecilia M. Bastos, Chad C. Huval (Genzyme Corporation), Issued September 19, 2006.

BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors.
Follow this format for each person. **DO NOT EXCEED FIVE PAGES.**

NAME: Carlson, Taylor L

ERA COMMONS USER NAME (credential, e.g., agency login): TLCARLS

POSITION TITLE: Senior Scientist

EDUCATION/TRAINING (*Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable. Add/delete rows as necessary.*)

INSTITUTION AND LOCATION	DEGREE (if applicable)	Completion Date MM/YYYY	FIELD OF STUDY
University of Nebraska, Kearney	BS	05/2012	Chemistry
Northeastern University	PhD	05/2018	Chemical Engineering

A. Personal Statement

As a Senior Scientist in the R&D department at Glyscend, Inc., I laid the groundwork for the proposed research by developing an *in vivo* model to quantify changes in urinary oxalate following a therapeutic intervention. In addition, I have an in-depth understanding of the mucus complexing polymers that will be tested in this application, built from developing *in vitro*, *ex vivo*, and *in vivo* assays to evaluate the behavior of the compounds in the intestinal environment. I have a broad background in mucosal biology, with specific training and expertise in the mucosal barrier and the impact of external stimuli and disease on mucosal biology. The current application utilizes the knowledge and insight from my prior and current work. In summary, I have the training, expertise, and motivation necessary to successfully carry out the proposed research project.

B. Positions, Scientific Appointments, and Honors

2022 - Present Senior Scientist, Glyscend, Inc, Lowell, MA
2020 - 2022 Research Scientist, Glyscend, Inc, Lowell, MA

Honors

2017 American Institute of Chemists Student Award
2017 Northeastern University - Dissertation Completion Fellowship

C. Contributions to Science

1. My early work focused on developing methods to detect analytes in runoff water and human serum. This work built an understanding of analytical chemistry and contributed to a book chapter.
 - a. Moser AM and **Carlson TL**, (2014), "General principles of immunoassays," Novel Approaches in Immunoassays Future Medicine Ltd, 6-19.
2. In addition to the contribution listed above, I evaluated the impact of external stimuli and disease on the integrity of the mucosal barrier. My work directly addressed the importance of mucosal biology in health and disease. These publications document this emerging area and help guide potential therapeutic interventions.
 - a. Lock JY, **Carlson TL**, Carrier RL, (2019) "Impact of Developmental Age, Necrotizing Enterocolitis Associated Stress, and Oral Therapeutic Intervention on Mucus Barrier Properties," Scientific Reports *under review*.

- b. **Carlson TL**, Yildiz H, Dar Z, Lock JY, Carrier RL, (2018) "Lipids alter microbial transport through intestinal mucus," *PLOS ONE* 13(12).
 - c. Lock JY, **Carlson TL**, Wang CM, Chen A, Carrier, RL, (2018) "Acute Exposure to Commonly Ingested Emulsifiers Alters Intestinal Mucus Structure and Transport Properties," *Scientific Reports* 8(1).
 - d. **Carlson TL**, Lock JY, Carrier RL, (2018) "Engineering the Mucus Barrier," *Annual Review of Biomedical Engineering* 20:1.
3. My current work has evaluated a novel mucus complexing polymer used in the treatment of metabolic disease. This work has brought new insight into the physiology behind duodenal exclusion and the potential of harnessing the mucosal barrier in the treatment of disease. These conference posters document this novel therapeutic and the mechanism behind treating metabolic disease.
- a. Nimgaonkar, A., Bryant, C., Carlson, T., Guerina, T., Colbert, K., Polomoscanik, S., Petersen, J., Jozefiak, T., Fineman, M. (2023, June 23-26th) *Duodenal Targeting by Oral Pharmacologic Duodenal Exclusion Therapy for Treatment of Type 2 Diabetes*. ADA 83rd Scientific Session, San Diego, CA, US
 - b. Carlson, T., Bryant, C., Colbert, K., Polomoscanik, S., Petersen, J., Jozefiak, T., Fineman, M., Nimgaonkar, A. (2023, Oct 14-17th) *Intestinal Mucus Complexation by an Oral Duodenal Exclusion Investigational Drug (GLY-200) for T2DM and Obesity*. Obesity Week, Dallas, TX, US

RESEARCH & RELATED BUDGET - SECTION A & B, Budget Period 1

UEI*: CA69UUBUYMW7

Budget Type*: Project Subaward/Consortium

Enter name of Organization: GLYSCEND, INC.

Start Date*: 12-01-2024

End Date*: 11-30-2025

Budget Period: 1

A. Senior/Key Person												
Prefix	First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base	Calendar	Academic	Summer	Requested	Fringe	Funds Requested (\$)*
						Salary (\$)	Months	Months	Months	Salary (\$)*	Benefits (\$)*	
1.	Ashish		Nimgaonkar		PD/PI	221,000.00	1.2			22,100.00	5,525.00	27,625.00
2.	Thomas	Henry	Jozefiak		Co-Investigator	221,000.00	0.24			4,420.00	1,105.00	5,525.00
3.	Taylor	L	Carlson		Co-Investigator	100,000.00	0.6			5,000.00	1,250.00	6,250.00

Total Funds Requested for all Senior Key Persons in the attached file

Additional Senior Key Persons:	File Name:	Total Senior/Key Person	39,400.00
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B. Other Personnel						
Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary (\$)*	Fringe Benefits* Funds Requested (\$)*
Post Doctoral Associates						
Graduate Students						
Undergraduate Students						
Secretarial/Clerical						
0	Total Number Other Personnel				Total Other Personnel	0.00
					Total Salary, Wages and Fringe Benefits (A+B)	39,400.00

RESEARCH & RELATED Budget {A-B} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTION C, D, & E, Budget Period 1

UEI*: CA69UUBUYMW7

Budget Type*: Project Subaward/Consortium

Organization: GLYSCEND, INC.

Start Date*: 12-01-2024

End Date*: 11-30-2025

Budget Period: 1

C. Equipment Description

List items and dollar amount for each item exceeding \$5,000

Equipment Item

Funds Requested (\$)*

Total funds requested for all equipment listed in the attached file

Total Equipment 0.00

Additional Equipment: File Name:

D. Travel

Funds Requested (\$)*

1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)

2. Foreign Travel Costs

Total Travel Cost 0.00

E. Participant/Trainee Support Costs

Funds Requested (\$)*

1. Tuition/Fees/Health Insurance

2. Stipends

3. Travel

4. Subsistence

5. Other:

Number of Participants/Trainees

Total Participant Trainee Support Costs

0.00

RESEARCH & RELATED Budget {C-E} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTIONS F-K, Budget Period 1

UEI*: CA69UUBUYMW7

Budget Type*: Project Subaward/Consortium

Organization: GLYSCEND, INC.

Start Date*: 12-01-2024

End Date*: 11-30-2025

Budget Period: 1

F. Other Direct Costs		Funds Requested (\$)*
1. Materials and Supplies		8,500.00
2. Publication Costs		
3. Consultant Services		10,000.00
4. ADP/Computer Services		
5. Subawards/Consortium/Contractual Costs		
6. Equipment or Facility Rental/User Fees		
7. Alterations and Renovations		
8. Data Management and Sharing Costs		0.00
9. Labcorp Method Development		17,525.00
10. Labcorp Samples & Report		12,261.00
11. NeoSome - 1		117,400.00
12. NeoSome - 2		28,200.00
Total Other Direct Costs		193,886.00

G. Direct Costs		Funds Requested (\$)*
Total Direct Costs (A thru F)		233,286.00

H. Indirect Costs			
Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base (\$)	Funds Requested (\$)*
1 . MTDC	30.0	233,286.00	69,986.00
Total Indirect Costs			69,986.00
Cognizant Federal Agency			
(Agency Name, POC Name, and POC Phone Number)			

I. Total Direct and Indirect Costs		Funds Requested (\$)*
Total Direct and Indirect Institutional Costs (G + H)		303,272.00

J. Fee		Funds Requested (\$)*
		21,229.00

K. Total Costs and Fee		Funds Requested (\$)*
		324,501.00

L. Budget Justification*	File Name: BudJust_merged_2024.03.27.pdf
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RESEARCH & RELATED Budget {F-K} (Funds Requested)

BUDGET JUSTIFICATION

Upon careful review of the budget required to complete the Aims as outlined, and review of the eligible waiver topics, we respectfully request the following budget outlined. This document justifies the budget and provides a basis for inclusion of the waiver topics. On 5/26/2023, Health and Human Services issued a revised version of "Approved SBIR/STTR Topics for Awards over Statutory Budget Limitations." Under NIDDK the applicable budget waiver topic for this proposal is:

"A. Development or evaluation of pharmacological agents (i.e., drugs, therapeutics), gene therapies, novel formulations, cell-based or other biological technologies for intervention in or prevention of Diabetes and Digestive and Kidney Diseases."

As shown at the end of this budget justification, the total cost associated with this Phase I SBIR proposal for the requested period of performance is below the budget limit of \$325k.

A. KEY PERSONNEL

(Phase I Y1: \$39,400)

Ashish Nimgaonkar, MD, Chief Medical Officer, Head of R&D, and PI (1.2 months)

Dr. Nimgaonkar will serve as PI on this project. He will directly supervise all research activities, coordinate formulation and testing, manage in vitro and ex vivo experiments, provide data analysis support, and prepare final reports to the funding agency. Dr. Nimgaonkar is an expert in gastroenterology, and as co-Founder, is dedicated to the success of Glyscend.

Thomas Jozefiak, Ph.D., CSO, and Co-I (0.24 months)

Dr. Jozefiak is an expert in polymeric chemistry with a focus on creating mucus complexing polymers. He will oversee and advise on drug synthesis and in vitro and ex vivo testing of formulations.

Taylor Carlson, Ph.D., Senior Scientist at Glyscend and Co-I (0.6 months)

Dr. Carlson is a chemical engineer with an extensive background in mucosal biology, and will guide the formulations and testing for in vitro and ex vivo testing.

Fringe benefits at a rate of 25% are included in the salaries.

OTHER PERSONNEL (\$0)

N/A

B. EQUIPMENT (\$0)

N/A

C. TRAVEL (\$0)

N/A

D. PARTICIPANT/TRAINEE SUPPORT COSTS (\$0)

N/A

E. OTHER DIRECT COSTS

Materials and Supplies (\$8,500)

Assorted chemicals and reagents for synthesis and assays:

Synthesis chemicals	\$2,000
Assay (FDH/NAD) reagents	\$2,000
Trinity Kits (oxalate assay)	\$4,500

Consultant Services (\$10,000)

Dr. Danica Grujic will provide consultant services on optimizing ALLN-177. As the former lead pharmacologist at Allena, where ALLN-177 was developed, her work followed the drug from inception to Phase III trials. She is in a unique position to provide deep insight into the interactions and mechanisms of ALLN-177, and will consult for 40 hours at a rate of \$250/hr (quote provided in letter of support).

Data Management and Sharing (\$0)

N/A

Equipment or Facility Rental/User Fees (\$0)

N/A

Contract Research Organizations (\$175,386)

Neosome (\$145,600)

Neosome Life Sciences is a Contract Research Organization that will conduct two separate studies in Sprague-Dawley rats, the first for OTT in vivo challenge study (\$117,400) and the second for the in vivo hyperoxaluria model (\$28,200). See attached quotes.

Lab Corp (\$29,786)

Lab Corp is a global Contract Research Organization specializing that offers a wide range of services, from toxicology, to pharmacokinetic studies, vaccines and bio analytics. They will perform the non-GLP Method Development and Qualification for the Determination of Oxalate in Rat Urine by LC/MS/MS (\$17,525) and non -GLP Determination of Oxalate in Rat Urine Samples by LC/MS/MS in Support of Glyscend's Protocol (\$12,261). See attached quote.

G. INDIRECT COSTS

The indirect cost rate of 30% has been applied to all direct costs.

H. FEE

The fee of 7% is requested which we believe demonstrates a reasonable profit margin for for-profit organizations performing research and development work.

QUOTATION FROM LABCORP

Test Compound: Oxalate

Prepared for:

**Taylor Carlson
Glyscend, Inc.**

Labcorp Contacts:

Alexa Singleton
Senior Proposal Associate
+1 (317) 468-6695

Erika Pfaunmiller
Business Development Director
+1 (717) 874-1533

Issue Date: 31 October 2023
Labcorp Quote No.: 787041
Version No.: 1

Expiration Date: 30 December 2023

labcorp

Proposal Assumptions

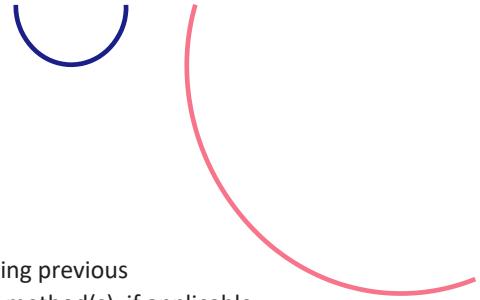
In the preparation of this quotation, certain assumptions have been made which are presented below:

- Estimated study start dates are based on current resources and schedule. No resources or schedule have been reserved at this time.
- The study information presented within this document is based on standard Labcorp practices and the study outline/assumptions included.
- Price estimate has been prepared according to the information provided in the study outline/assumptions listed.
- Price estimate is subject to change should there be any alteration to the study outline/assumptions or estimated start date.
- The final study price will be calculated on the authorized definitive protocol.
- All shipping is provided by a third party vendor. Costs will depend upon the type and volume of material shipped and these may vary. Any prices included within this quotation are an estimate and for budget purposes only. Labcorp reserves the right to charge the sponsor costs associated with shipping.
- Raw Data, unless otherwise agreed in the Study Plan, will be retained by Labcorp for one (1) year or inspection cycle from the date of the final report. The data archiving period can be extended at an additional charge. Storage/disposal/transfer charges will be identified at the time Archives makes contact, after completion of the one (1) year or inspection cycle.
- Study prices are based on current costs (as of the date of Proposal issue) for animals, materials or subcontracted services as applicable to the services provided. If any of these costs increase prior to study initiation Labcorp reserves the right to update these costs accordingly and the final quote will reflect the costs at the point of study initiation.
- An annual statement for tax purposes specifying the allocation between countries in which services were performed can be provided upon request.
- Additional costs for SEND data conversion may be incurred if TK is done outside of Labcorp and/or the TK lab selected is not 'SEND Ready' and able to provide a PC and PP domain for the respective BioA and TK phases of the study.
- If the test article is a potential biohazard, such as a genetically modified organism (GMO or GMO-derived), an infectious agent, bacterial toxin, or vaccine, is a stem cell preparation, or is radioactive, then a risk assessment will be necessary prior to receipt of the test articles. The resultant classification of the test article may have cost implications.

Bioanalytical Services

Quote No. 787041 Site: Madison, WI, USA Estimated Study Start: Q4 2023			
Non-GLP Method Development and Qualification for the Determination of Oxalate in Rat Urine by LC/MS/MS			
Product Description	Qty	Unit Price (USD)	Total Price (USD)
Non-Regulated Discovery Bioanalytical Method Development and Qualification (Days)	5	\$3,405	\$17,025
Supporting Items:			
Consumables Fee	1	\$500	\$500
Total Estimated Study Price			\$17,525
The number of days is only an estimate and we will only bill for days used.			
Assumptions:			
<input type="checkbox"/> Sponsor to provide Test Article and suitable internal standard <input type="checkbox"/> Target curve range of 0.3mM to 10mM			

Quote No. 787905 Site: Madison, WI, USA Estimated Study Start: Q4 2023/Q1 2024			
Non-GLP Determination of Oxalate in Rat Urine Samples by LC/MS/MS in Support of Client Protocol			
Product Description	Qty	Unit Price (USD)	Total Price (USD)
Non-GLP Toxicokinetic Sample Analysis (Tier II)	72	\$83	\$5,976
Non-GLP Budget for Reassays (Tier II)	15	\$83	\$1,245
Non-GLP Sample Analysis Report (Labcorp will provide one draft and one final report using a Labcorp template) (Tier II)	1	\$3,935	\$3,935
Sample Disposition (Final disposition of samples and/or retrieval of samples for shipment)	1	\$475	\$475
Sample Shipment (Post Study Disposition)	1	\$630	\$630
Sample Analysis – Sample Storage (Approximate monthly sample storage fee)	TBD	TBD	TBD
Back up Sample Storage (Approximate monthly sample storage fee)	TBD	TBD	TBD
Total Estimated Study Price			\$12,261
<input type="checkbox"/> Pricing excludes costs for TK reporting.			



Discovery General

- Sponsor will provide documentation and relevant historical data regarding previous development, known methodology, and/or general performance of the method(s), if applicable.
- The cost of special matrix, including tissues, and special columns may be charged to the sponsor. These are materials which, due to their unique nature, can contribute high expense to a study. The need to assign costs for these to the sponsor will be discussed with the sponsor prior to incurring the charges.
- Any sponsor-requested work adding to, or deviating from original study scope will be priced separately (e.g., unplanned development, additional troubleshooting, or decisions that alter the direction or scope of a study, including unexpected changes in sample number, receipt rate, or urgency of analysis may have a cost impact).
- This work will not be conducted per regulatory requirements and there will be no involvement from QA.
- Sponsor will provide sufficient quantity of reference standard and internal standard if applicable. Labcorp may procure or synthesize reference standard or internal standards (at additional cost) if required by the sponsor.
- The above prices include archival of all raw data, documentation, protocol, final report, or data report for the studies (except for the biological samples) for one (1) inspection cycle from the date of the final report. The archival period can be extended if required, at an additional charge. At the end of this time period, the Labcorp archives staff will contact the sponsor to determine disposition of those archived materials.

Discovery Method Development

- The number of days quoted is only an estimate, and we will only bill for the days used. Qualification of the method(s) is included in the number of method development days quoted unless specifically noted as a line item. If additional days are needed, we will request separate authorization before proceeding. Labcorp will make all efforts to design and execute method development activities to limit the cost for the sponsor. The additional time quoted is to allow us to resolve potential issues so we can develop an adequate method. No guarantees or warranties are implied as to when or if the development will be successfully completed. Should the development not be progressing effectively in the reasonable opinion of either party, then either party may terminate the development without any further obligation occurring.

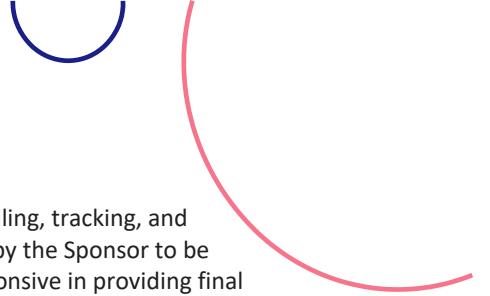
Discovery Method Qualification

- Tier II method qualification includes a precision and accuracy run containing a minimum of seven calibration standards (front/back, n=2) with acceptance criteria of $\pm 25\%$ ($\pm 30\%$ at LLOQ) for biofluids and $\pm 30\%$ for tissues, three QC concentrations (low/mid/high levels, n=2) with acceptance criteria of $\pm 25\%$ for biofluids and $\pm 30\%$ for tissues, carryover and specificity check, and a single reference material weighing. Data tables and reports would incur additional cost.

- If, by mutual agreement, the performance of the assay necessitates further investigations (such as more ruggedness or stability determinations), these will be charged, by agreement, in addition to the basic cost.
- If the qualification is unsuccessful, additional method development days will need to be awarded to move forward with the study.

Discovery Sample Analysis

- Tier II assay includes a minimum of seven calibration standards (front/back, n=2) with acceptance criteria of $\pm 25\%$ ($\pm 30\%$ at LLOQ) for biofluids and $\pm 30\%$ for tissues, three QC concentrations (low/mid/high levels, n=2) with acceptance criteria of $\pm 25\%$ for biofluids and $\pm 30\%$ for tissues, carryover and specificity check, and a single reference material weighing. Data are peer-reviewed and provided as an electronic summary of results at no additional cost. Full reports are available at additional cost.
- The price is offered on the assumption that the analytical method uses Protein Precipitation extraction and LC/MS/MS with a sample run time of 6 minutes or less for a single analyte. An additional \$8 per sample will be added to the sample analysis price for Liquid-Liquid (LLE) or Solid Phase extraction (SPE). More complex methodology or longer run times may be subject to additional charges.
- Non-GLP Sample Analysis minimum study fee or minimum batch fee are as follows: minimum study fee; Tiers II: 50 minimum batch fee for liquid or solid samples.
- Tier II quotes will include 20% reassays as the standard. The number of re assay samples (dilution repeats and sponsor-requested repeats) is an estimate for your budgetary purposes only. If dilution repeats are necessary, these will be charged at the per sample price. If sponsor-requested repeats are required, samples will be reanalyzed in duplicate at the per sample price. Reassays are charged at the per samples price apart from minimal batch fees outlined for original sample analysis.
- If applicable, the price for reports (one draft and final) is under the assumption that a Labcorp template will be used. Sponsor-specific report templates, provided by the sponsor, may also be used at an additional cost per report. Final bioanalytical reports will be provided as an electronic copy of the signed final report in fully text searchable Portable Document Format.
- Sample Storage / Sample Storage Fees:
 - Monthly storage charges may be incurred immediately for the handling, tracking, and storage of any samples received which are ultimately not required by the Sponsor to be analyzed as part of the services
 - Analyzed samples will be stored free of charge for 90 days following submission of first draft report and/or final data delivery, whichever comes later. Monthly charges will be incurred for samples stored beyond the complimentary storage period. In the event the sponsor is unresponsive in providing final disposition instructions, Labcorp will charge the sponsor for additional storage of samples in three month increments. If storage fees are unpaid for ninety (90) days from receipt of the first invoice, samples may be disposed of without sponsor approval. Samples may be shipped to you at an additional cost.

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- Monthly storage charges may be incurred immediately for the handling, tracking, and storage of any samples received which are ultimately not required by the Sponsor to be analyzed as part of the services. In the event the sponsor is unresponsive in providing final disposition instructions, Labcorp will charge the sponsor for additional storage of samples in three month increments. If storage fees are unpaid for ninety (90) days from receipt of the first invoice, samples may be disposed of without sponsor approval. Samples may be shipped to you at an additional cost.
 - 2023 Starts: \$1.16/sample for standard samples or \$3.14/sample for radioactive and tissue samples. All studies are subject to a minimum monthly storage charge of \$116.
 - 2024 Starts: \$1.28/sample for standard samples or \$3.45/sample for radioactive and tissue samples. All studies are subject to a minimum monthly storage charge of \$128.

Proposal Summary

Study Title	Price (USD)
Non-GLP Method Development and Qualification for the Determination of Oxalate in Rat Urine by LC/MS/MS	\$17,525
Non-GLP Determination of Oxalate in Rat Urine Samples by LC/MS/MS in Support of Client Protocol	\$12,261
Total Package Price	\$29,786



5 Fortune Dr. Billerica, MA 01821 Ph: 781-430-8558		QUOTATION Quote #: <u>NLS083-030824-01Q</u> Date: <u>03/08/2024</u> Valid until: <u>03/08/2025</u>			
TO	Taylor Carlson Glyscend, Inc. 110 Canal Street Lowell, MA 01852-4574 617.275.1765 Customer ID: <u>NLS-083</u>		Prepared by: <u>Sarah Di Croce</u>		
PAYMENT TERMS					
50% due prior to study initiation; 50% Due Upon Study Completion					
DESCRIPTION		TOTAL			
OTT challenge study: Three oOTT challenges					
1. Order special diet; base TD.06199, high fat 18%, low calcium 0.5% (2 boxes) 2. 12 SD-SAS Rats from CRL, approximately 250-275 grams (8-10 weeks), housing approximately 16 days 3. all animals put on special diet for 6 days 4. OTT Challenge <ul style="list-style-type: none"> a. D-6 Stabilize on diet 6 days, b. t-4: fast rats for 4 hours prior to TA gavage. c. t-1: Deliver vehicle or test article by oral gavage using 18g 3" gavage needle. d. t0: Deliver OTT by oral gavage (t 0h). e. After OTT challenge dosed animals are placed in metabolic caging, no food. Accumulated urine (overnight) will be collected t0-12 hours, press bladder to express urine. Precise volume is recorded, and samples are stored at -20C. Water consumption is recorded. f. rats will have food returned, but remain in metabolic caging for additional urine collection period t12-24 hours. 5. 2 nd challenge, return to cages with special diet for 2 days then repeat OTT challenge study (step 4.), challenge #2. 6. 3 rd challenge, repeat step 5. Two days on special diet, then OTT challenge #3. 7. Animals terminated at end of study		\$11,740.00			
		TOTAL COST FOR 10 STUDIES		\$117,400.00	

If you have any questions concerning this quotation, contact Roseanne Wexler phone#: 781-325-9024.

Signing this document verifies the acceptance of this quote for services to be provided at **NeoSome Life Sciences**.

Changes made to this study subsequent to the issue of this quotation may incur additional charges.

PRINT NAME	DATE	SIGNATURE

5 Fortune Dr. Billerica, MA 01821
 PHONE: 781-430-8558



5 Fortune Dr. Billerica, MA 01821 Ph: 781-430-8558		QUOTATION Quote #: <u>NLS083-032024-01Q</u> Date: <u>03/20/2024</u> Valid until: <u>03/20/2025</u>
TO	Taylor Carlson Glyscend, Inc. 110 Canal Street Lowell, MA 01852-4574 617.275.1765 Customer ID: <u>NLS-083</u>	Prepared by: <u>Sarah Di Croce</u>
PAYMENT TERMS		
50% due prior to study initiation; 50% Due Upon Study Completion		
DESCRIPTION		TOTAL
Diet Induced Enteric Hyperoxaluria Model		
1. Special diet; base TD.06199, high fat 18%, low calcium 0.5% spiked with 1.1% Oxalate (2 boxes) provided by sponsor 2. 12 SD-SAS Rats from CRL, approximately 250-275 grams (8-10 weeks), housing approximately 12 days 3. all animals put on special diet for 4 days 4. Therapeutic treatment administered orally for 4 days <ol style="list-style-type: none"> t-4: fast rats for 4 hours prior to TA gavage. t-1: Deliver vehicle or test article by oral gavage using 18g 3" gavage needle. t0: Return food to all rats 5. 24 hr. urine collections on day 3 (pre-treatment) and day 7 (post 4 days of treatment) <ol style="list-style-type: none"> Animals are placed in metabolic caging, no food. Accumulated urine (overnight) will be collected t0-12 hours, press bladder to express urine. Precise volume is recorded, and samples are stored at -20C. Water consumption is recorded. rats will have food returned, but remain in metabolic caging for additional urine collection period t12-24 hours. 6. Animals terminated at end of study	\$9,400.00	
TOTAL COST FOR 3 STUDIES		\$28,200.00

If you have any questions concerning this quotation, contact Roseanne Wexler phone#: 781-325-9024.

Signing this document verifies the acceptance of this quote for services to be provided at **NeoSome Life Sciences**.

Changes made to this study subsequent to the issue of this quotation may incur additional charges.

PRINT NAME	DATE	SIGNATURE
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RESEARCH & RELATED BUDGET - Cumulative Budget

	Totals (\$)
Section A, Senior/Key Person	39,400.00
Section B, Other Personnel	0.00
Total Number Other Personnel	0
Total Salary, Wages and Fringe Benefits (A+B)	39,400.00
Section C, Equipment	0.00
Section D, Travel	0.00
1. Domestic	0.00
2. Foreign	0.00
Section E, Participant/Trainee Support Costs	0.00
1. Tuition/Fees/Health Insurance	0.00
2. Stipends	0.00
3. Travel	0.00
4. Subsistence	0.00
5. Other	0.00
6. Number of Participants/Trainees	0
Section F, Other Direct Costs	193,886.00
1. Materials and Supplies	8,500.00
2. Publication Costs	0.00
3. Consultant Services	10,000.00
4. ADP/Computer Services	0.00
5. Subawards/Consortium/Contractual Costs	0.00
6. Equipment or Facility Rental/User Fees	0.00
7. Alterations and Renovations	0.00
8. Other 1	0.00
9. Other 2	17,525.00
10. Other 3	12,261.00
11. Other 4	117,400.00
12. Other 5	28,200.00
13. Other 6	0.00
14. Other 7	0.00
15. Other 8	0.00
16. Other 9	0.00
17. Other 10	0.00
Section G, Direct Costs (A thru F)	233,286.00
Section H, Indirect Costs	69,986.00

Section I, Total Direct and Indirect Costs (G + H)	303,272.00
Section J, Fee	21,229.00
Section K, Total Costs and Fee (I + J)	324,501.00

SBIR/STTR Information

Agency to which you are applying (select only one)*

 DOE HHS USDA Other:

SBC Control ID:*

000691459

Program Type (select only one)*

 SBIR STTR Both (See agency-specific instructions to determine whether a particular agency allows a single submission for both SBIR and STTR)

Application Type (select only one)*

 Phase I Phase II Fast-Track Direct Phase II Phase IIA Phase IIB Phase IIC Commercialization Readiness Program (See agency-specific instructions to determine application type participation.)

Phase I Letter of Intent Number:

* Agency Topic/Subtopic:

Questions 1-8 must be completed by all SBIR and STTR Applicants:

1a. Do you certify that at the time of award your organization will meet the eligibility criteria for a small business as defined in the funding opportunity announcement?* Yes No1b. Anticipated Number of personnel to be employed at your organization at the time of award.* 1c. Is your small business majority owned by venture capital operating companies, hedge funds, or private equity firms?*  Yes No1d. Is your small business a Faculty or Student-Owned entity?* Yes No2. Does this application include subcontracts with Federal laboratories or any other Federal Government agencies?* Yes No

If yes, insert the names of the Federal laboratories/agencies:*

3. Are you located in a HUBZone? To find out if your business is in a HUBZone, use the mapping utility provided by the Small Business Administration at its web site: <http://www.sba.gov> * Yes No4. Will all research and development on the project be performed in its entirety in the United States?* Yes No

If no, provide an explanation in an attached file.

Explanation:*

5. Has the applicant and/or Program Director/Principal Investigator submitted proposals for essentially equivalent work under other Federal program solicitations or received other Federal awards for essentially equivalent work?* Yes No

If yes, insert the names of the other Federal agencies:*

6. Disclosure Permission Statement: If this application does not result in an award, is the Government permitted to disclose the title of your proposed project, and the name, address, telephone number and email address of the official signing for the applicant organization to state-level economic development organizations that may be interested in contacting you for further information (e.g., possible collaborations, investment)?* Yes No7. Does the application include a request of SBIR or STTR funds for Technical and Business Assistance (TABA)? If yes, please follow the agency specific instructions to provide the budget request and justification. (Please answer no if you plan to use the agency TABA vendor, which does not require you to include a request for TABA funds in your application.)* Yes No

8. Commercialization Plan: The following applications require a Commercialization Plan: Phase I (DOE only), Phase II (all agencies), Phase I/II Fast-Track (all agencies). Include a Commercialization Plan in accordance with the agency announcement and/or agency-specific instructions.*

Attach File:*

SBIR/STTR Information

SBIR-Specific Questions:

Questions 9 and 10 apply only to SBIR applications. If you are submitting ONLY an STTR application, leave questions 9 and 10 blank and proceed to question 11.

9. Have you received SBIR Phase II awards from the Federal Government? If yes, provide a company commercialization history in accordance with agency-specific instructions using this attachment.*

Yes No

Attach File:*

10. Will the Project Director/Principal Investigator have his/her primary employment with the small business at the time of award?*

Yes No

STTR-Specific Questions:

Questions 11 - 13 apply only to STTR applications. If you are submitting ONLY an SBIR application, leave questions 11 - 13 blank.

11. Please indicate whether the answer to BOTH of the following questions is TRUE:*

Yes No

(1) Does the Project Director/Principal Investigator have a formal appointment or commitment either with the small business directly (as an employee or a contractor) OR as an employee of the Research Institution, which in turn has made a commitment to the small business through the STTR application process; AND

(2) Will the Project Director/Principal Investigator devote at least 10% effort to the proposed project?

12. In the joint research and development proposed in this project, does the small business perform at least 40% of the work and the research institution named in the application perform at least 30% of the work?*

Yes No

13. Provide UEI of non-profit research partner for STTR.*

PHS 398 Cover Page Supplement

1. Vertebrate Animals Section

Are vertebrate animals euthanized? Yes No

If "Yes" to euthanasia

Is the method consistent with American Veterinary Medical Association (AVMA) guidelines?

Yes No

If "No" to AVMA guidelines, describe method and provide scientific justification

.....

2. *Program Income Section

*Is program income anticipated during the periods for which the grant support is requested?

Yes No

If you checked "yes" above (indicating that program income is anticipated), then use the format below to reflect the amount and source(s). Otherwise, leave this section blank.

*Budget Period *Anticipated Amount (\$) *Source(s)

3. Human Embryonic Stem Cells Section

*Does the proposed project involve human embryonic stem cells? Yes No

If the proposed project involves human embryonic stem cells, list below the registration number of the specific cell line(s) from the following list: http://grants.nih.gov/stem_cells/registry/current.htm. Or, if a specific stem cell line cannot be referenced at this time, check the box indicating that one from the registry will be used:

Specific stem cell line cannot be referenced at this time. One from the registry will be used.

Cell Line(s) (Example: 0004):

4. Human Fetal Tissue Section

*Does the proposed project involve human fetal tissue obtained from elective abortions? Yes No

If "yes" then provide the HFT Compliance Assurance

If "yes" then provide the HFT Sample IRB Consent Form

5. Inventions and Patents Section (Renewal applications)

*Inventions and Patents: Yes No

If the answer is "Yes" then please answer the following:

*Previously Reported: Yes No

6. Change of Investigator/Change of Institution Section

Change of Project Director/Principal Investigator

Name of former Project Director/Principal Investigator

Prefix:

*First Name:

Middle Name:

*Last Name:

Suffix:

Change of Grantee Institution

*Name of former institution:

PHS 398 Research Plan

Introduction

1. Introduction to Application
(for Resubmission and Revision applications)

Research Plan Section

2. Specific Aims SA_Glyscend_20240327.pdf
3. Research Strategy* RS_Glyscend_20240328v2.pdf
4. Progress Report Publication List

Other Research Plan Section

5. Vertebrate Animals Vertebrate_Glyscend_20240326.pdf
6. Select Agent Research
7. Multiple PD/PI Leadership Plan
8. Consortium/Contractual Arrangements
9. Letters of Support LCO_Merged_20240328.pdf
10. Resource Sharing Plan(s) RSP_Glyscend_20240520.pdf
11. Other Plan(s) DMSP_Glyscend_20240326.pdf
12. Authentication of Key Biological and/or Chemical Resources

Appendix

13. Appendix

SPECIFIC AIMS

Glyscend Inc. is developing a unique combination therapy for the treatment of enteric hyperoxaluria (EH). The approach combines an orally active oxalate decarboxylase enzyme and a mucus complexing polymer, with the promise of a best-in-class reduction in urine oxalate excretion.

The Problem: Enteric hyperoxaluria (EH) has a prevalence of ~250,000 patients/year in the USA.¹ EH typically occurs secondary to conditions or procedures that cause gastrointestinal (GI) malabsorption of nutrients - e.g. inflammatory bowel diseases and GI surgical procedures. In these subpopulations, EH occurs in 5-24%² and 42-67%³ of patients, respectively. EH is dangerous and life-altering, leading to higher levels of oxalate excretion through urine (≥ 45 mg/24 h), kidney stone formation, and often results in chronic- and end-stage kidney disease.² Current treatment options are largely ineffective and include dietary changes to reduce oxalate intake, calcium supplements or other low-efficacy medications to bind oxalate prior to absorption, and hydration to dilute oxalate in the urine. There remains a need for a high-efficacy oral treatment option for EH patients.

Glyscend leverages its mucus complexing polymer (MCP) technology to prolong the exposure of the enzyme (ALLN-177) to oxalate. This proposal seeks to demonstrate that the combination increases enzyme residence time and availability, significantly enhancing breakdown of oxalate before absorption.

The Oxalate Enzyme (ALLN-177): ALLN-177 (ReloxaliaseTM) is an orally administered enzyme that has shown clinical promise for the degradation of dietary oxalate in the GI-tract.^{4,5} ALLN-177 degrades oxalate in the **upper GI tract**, decreasing oxalate available for absorption and lowering urine oxalate (UOx). ALLN-177 was proven safe and active across phase-2/3 clinical trials. The clinical endpoint of at least 20% reduction in 24-hour UOx from baseline to week 4 was achieved in 48.3% of the treatment group and 31.6% in the placebo group.⁶ This level of efficacy was promising, but insufficient for regulatory approval⁷, potentially due to the short residence time of ALLN-177 at the primary site of oxalate absorption. Glyscend recently acquired the asset because it is significantly derisked from a safety and regulatory perspective and has tremendous potential for best-in-class efficacy if residence time in the upper GI can be improved.

The Mucus Complexing Polymer (MCP): Glyscend has developed proprietary synthetic polymers capable of complexing mucin glycoproteins comprising the lining of mucosal membranes of the body. Upon oral administration, certain MCP polymers have been shown to condense with the mucus lining of the small intestine and create a temporary coating on the intestinal wall. This coating results in downstream neurohormonal signaling and changes in the GI transit time, including slowing of gastric emptying. The first clinical instantiation of an MCP material, GLY-200, has been shown to be safe in phase-1 clinical trials⁸ and is currently in phase-2 clinical trials for the treatment of type 2 diabetes and obesity.⁹

The Solution: Glyscend proposes a co-formulation approach combining ALLN-177 with an optimized MCP to enhance its efficacy in the treatment of EH. The postulated mechanism is increased enzyme activity due to longer residence time in the **upper GI tract**. To demonstrate proof-of-concept for the approach, we propose:

Aim 1: In vitro optimization. Benchtop studies will measure the activity of ALLN-177 alone and in combination with select MCP candidates. Oxalate activity will be measured at gastric and intestinal pHs with and without mucin. Evaluation of additional formulation components, (e.g., salts, polymers) with the potential to further enhance enzyme activity will be considered. Success Criteria: Demonstrate equivalent or maximum enzyme activity for at least 3 enzyme-MCP formulations compared to native ALLN-177.

Aim 2: Ex vivo optimization. The adherence of select enzyme-MCP formulations and retention of enzyme activity on the mucosal surface of excised porcine intestinal tissue will be assessed. Enzyme activity will be measured after serial rinsing of the tissue as a means of confirming activity retained on the tissue surface. Success criteria: Demonstration of retained ALLN-177 enzymatic activity unaffected by serial rinsing (enzymatic activity +/-20% of the baseline activity of ALLN-177 following rinsing).

Aim 3: In vivo Proof of Concept. Top formulations from Aims 1 and 2 will be tested in two rat models and compared to native ALLN-177 to evaluate the efficiency for reducing UOx excretion. 1) Candidate formulations will be administered to normal, fasted rats by oral gavage along with an orally administered oxalate challenge. UOx (24h) will be quantified with an HPLC/ion-exchange bioanalysis method. This work will inform dose timing, formulation strength, and oxalate excretion profile. 2) Rats will be placed on a high oxalate diet for 4 days to induce EH, followed by 4-day treatment with the formulation identified from Aim 3.1. Success Criteria: a target 2x reduction in UOx relative to native ALLN-177. This reduction is clinically meaningful and provides assurance that the co-formulation approach would be worthy of clinical development.

RESEARCH STRATEGY

SIGNIFICANCE

Glyscend, Inc. is developing an effective treatment for enteric hyperoxaluria (EH) that combines an orally active oxalate decarboxylase enzyme (ALLN-177) and a mucus complexing polymer (MCP). In this proposal we describe our plan to optimize the two-drug combination *in vitro* (Specific Aim 1), *ex vivo* (SA2), and establish proof of concept in a rat model of EH (SA3).

Enteric hyperoxaluria. Patients with enteric hyperoxaluria (EH) exhibit increased intestinal oxalate absorption, leading to kidney stones, chronic kidney disease (CKD), and ultimately kidney failure.⁶ EH is a dangerous and life-altering condition impacting ~ 250,000 patients per year in the US.¹ EH generally occurs as a secondary presentation to conditions or procedures that cause gastrointestinal malabsorption of nutrients - e.g. inflammatory bowel diseases (Crohn's disease and ulcerative colitis), gastrointestinal procedures (Roux-en-Y gastric bypass surgery), exocrine pancreatic insufficiency (e.g., Cystic Fibrosis), or short bowel syndrome following ileal resection.^{10,11} In addition to causing nephrolithiasis and nephrocalcinosis,^{12,13} EH is increasingly recognized as contributing to both acute and chronic kidney disease, resulting in end-stage renal disease as a consequence of oxalate nephropathy.^{2,10} As kidney function decreases, plasma oxalate levels can increase, potentially leading to systemic oxalosis. The mechanisms potentially contributing to excessive gastrointestinal (GI) absorption of oxalate and subsequent excess urinary oxalate (UOx) excretion in these settings include fat malabsorption (unabsorbed fat and bile acids form calcium salts in the intestinal lumen, limiting the amount of calcium available to bind oxalate, thereby increasing free absorbable oxalate), and increased GI permeability.¹⁴

Current treatments for EH. There is no FDA-approved pharmacologic therapy to reduce the absorption of oxalate from the GI tract. Current management for EH consists of recommendations to reduce dietary oxalate intake, increase fluid intake, and introduce calcium and citrate supplementation and thiazide diuretics. These interventions are aimed at decreasing the oxalate absorbed from food and decreasing UOx excretion, thereby minimizing the subsequent risks of kidney stone formation and/or oxalate nephropathy.¹⁵ Patients struggle to follow the strict fluid intake and dietary requirements and the management therapies are largely ineffective. Therefore, there remains a significant unmet need for an approved EH pharmacologic therapy.

Rigor of previous work. The safety and efficacy of the compounds proposed for combination in this proposal has been shown individually. Firstly, **the enzyme ALLN-177**, was a phase III clinical asset, with demonstrated safety, tolerability, and reduced UOx. However, the improvement in UOx was insufficient to justify advancing the program, potentially due to the short residence time of ALLN-177 at the primary site of enzymatic action in the stomach and **upper GI tract**. Secondly, **the MCP platform** is a family of proprietary non absorbed synthetic polymers exemplified by GLY-200 which is currently in phase-2 clinical trials for the treatment of type 2 diabetes and obesity. Clinical trials to-date have shown safety, tolerability, and robust activity, including favorable changes in glucose, insulin, bile acids, incretin, and gut hormones following 14-days of dosing. Postulating that the combination of ALLN-177 and an MCP (GLY-200 itself will not be used) could lengthen the residence time of ALLN-177 in the **upper GI tract**, we conducted *in vitro* studies confirming oxalate decarboxylase activity in the presence of MCP. In this proposal, we further examine combinations of select MCPs engineered at Glyscend and ALLN-177 to maximize enzyme activity, residence time, and demonstrate feasibility for treating EH.

Path to commercialization. Glyscend will confirm a **fixed-dose combination** regulatory pathway with the FDA requiring IND-filing, phase I and II clinical trials following the successful execution of nonclinical studies, including studies described in this proposal. For commercial launch, the company intends to partner the product. There is significant pharma interest in the space as EH is expected to be an \$8.3B market by 2028¹⁶ with no currently approved drugs.

Strength and success (significance) of the team

Ashish Nimgaonkar, MD, Chief Medical Officer, Head of R&D, Glyscend Co-Founder, and PI on this proposal. He is a gastroenterologist and scientist by training. He co-founded Glyscend where he served as Chief Executive Officer for 7 years and now is its Chief Medical Officer. In addition to his role at Glyscend, he is an Asst Prof of Medicine and Medical Director of the Ctr for Bioengineering Innovation & Design at Johns Hopkins University. He completed his clinical training in internal medicine from BIDMC/Harvard Med School and in gastroenterology from Stanford hospital.

Thomas Jozefiak, Ph.D., CSO, Co-Founder of Glyscend, and Co-I on this proposal, received his doctorate in Chemistry from the University of Minnesota. He is an experienced R&D leader with a distinguished 30-year

industrial career advancing polymers and biomaterials as drugs and medical devices. In 2006, he assumed leadership of Genzyme's Biomaterials Science and Engineering Group, where he led scientific efforts supporting the Genzyme Biosurgery product line, including Seprafilm® adhesion barrier, Synvisc® viscosupplement, LeGoo® vascular occlusion device. He then served as VP, Discovery at Living Proof, Inc, leading the commercial launch of Neotensil®, a unique in-situ forming skin reshaping film product. In 2016, he founded Glycologix Inc., to advance his inventive SuperGAG biopolymers for the treatment of interstitial cystitis. Presently, he serves as CSO at Glyscend, Inc., where he leads the scientific development of the MCP platform.

Taylor Carlson, Ph.D., Senior Scientist at Glyscend and Co-I on this proposal, received her Ph.D. in Chemical Engineering from Northeastern University where she built a broad background in mucosal biology, with specific training and expertise in the mucosal barrier and the impact of external stimuli and disease on mucosal biology. As a Senior Scientist in the R&D department at Glyscend, Inc., she laid the groundwork for the proposed research by developing an in vivo model to quantify changes in urinary oxalate following therapeutic intervention. In addition, she has in-depth understanding of MCP materials from developing in vitro, ex vivo, and in vivo assays to evaluate their behavior in the environment of the GI tract.

Glyscend also has a deep bench of experienced drug development experts on staff: **Mark Fineman, PhD**, Chief Development Officer and **Christine Bryant, PhD**, Sr. Director Clinical & Translational Science, who have extensive translational and clinical experience. **John Petersen, PhD**, VP CMC adds significant manufacturing and CMC scale-up expertise. Glyscend has also incorporated former Allena personnel as advisors: **Hugh Wight**, former Senior VP Technical Operations, **Danica Grujic, PhD**, former Executive Director of Pharmacology, and **Lou Brenner MD**, former President, and CEO of Allena (see letters of support).

INNOVATION

The Innovation leverages the mechanism of action of both drugs: the MCP retains ALLN-177 in the upper gastrointestinal tract prolonging its enzymatic activity and more efficiently reducing oxalate absorption.

The innovation is the synergistic co-formulation of ALLN-177 and a mucus complexing polymer (MCP). **ALLN-177** is a microcrystalline oxalate decarboxylase enzyme effective in the treatment of EH by degrading oxalate in the gastrointestinal tract. **MCPs**, meanwhile, are a novel class of synthetic polymers designed to crosslink mucin through covalent binding with sialic acid moieties. This in-situ mucin complexation results in a condensed mucus layer coating the surface of the intestinal wall and modulating mucus barrier function as in a temporary duodenal exclusion effect. We hypothesize that co-formulation of ALLN-177 + MCP would significantly improve the clinical benefit for patients with EH for three reasons:

- Increased Enzyme Retention Time in the Stomach:** MCPs have been shown to increase gastric retention by approximately 200% and 500% compared to controls at 1 and 4hr, respectively. These GI transit effects have been observed in multiple Glyscend nonclinical studies using: (a) phenol red dye as a transit marker, (b) IVIS imaging to monitor GI transit of MCPs conjugated to FITC, and (c) CT imaging to visualize GI retention of an MCP conjugated to the radiopaque agent Gastrograffin.¹⁷
- Enzyme Retention at the Epithelial Interface:** We propose that in-situ polymer-mucus complexation will entrap active ALLN-177 at the epithelial interface. Glyscend has successfully demonstrated this effect in ex vivo experiments using microspheres to model enzyme particles. We observed robust particle entrapment in the polymer-mucus complex, and resistance to particle removal on serial rinsing.
- Direct MCP-Oxalate Binding:** The cationic MCPs in their native form have the potential to lower urine oxalate via direct binding of GI luminal oxalate (as shown in Glyscend's phase-1 clinical study, where MCP GLY-200 reduced levels of 24-hr UOx).

APPROACH

ALLN-177 Preliminary Studies. ALLN-177 is a microcrystalline form of recombinant *Bacillus subtilis* oxalate decarboxylase (OxDC), an oxalate-specific enzyme expressed from *Escherichia coli*. ALLN-177 is an orally ingested, non-absorbed enzyme that acts locally within the GI tract to degrade oxalate into formate and carbon dioxide. Decreasing oxalate available for absorption into the systemic circulation directly reduces UOx excretion thereby preventing kidney stone formation, as well as possibly decreasing systemic (renal and extra-renal tissue) oxalate deposition.

Preclinical pharmacology and toxicology, ALLN-177. The preclinical studies of ALLN-177, summarized briefly here, successfully resulted in advancement of the program to late-stage clinical studies. These studies supported the concept that orally administered ALLN-177 can significantly reduce urinary and plasma oxalate (POx) levels

in rodent and porcine models of primary and secondary hyperoxaluria. Toxicology studies also revealed a very safe drug profile, including no related clinical or toxicological observations, and no pathology or histopathology findings in either rats or dogs. The no observed adverse effect level from a 26-week toxicology study revealed that oral administration of ALLN-177 was well tolerated at doses up to 11-fold higher than the highest planned human dose of 37,500 units/day based on a 60 kg individual, and 6-fold higher than the highest planned human dose for an adolescent or small adult based on the minimum permitted body weight of 35 kg.

Table 1. Summary of late clinical trials conducted for ALLN-177.

Clinical Trial	Study Design	Study Population	Dose, Regimen, Duration	Key Efficacy Results
Study 4, Phase II, US	Randomized, double-blind, multi-center, PBO controlled	EH and history of kidney stones	7,500 units or PBO, 3x/day with meals, 28 days	Mean (%) change in UOx from baseline to Week 4 on ALLN-177, vs. PBO, All: -6.35 mg/24 hr (-15.8%), Enteric: -16.45 mg/24 hr (-36.25%), Mean (%) change in UOx from baseline to TWA on ALLN-177, vs. PBO: All: -8.13 mg/24 hr (-14.2%), Enteric: -25.69 mg/24 hr (-39.15%)
Study 5, Phase II, Global	Open-label, multi-center, single-arm	Primary hyperoxaluria (PH), and EH with CKD and elevated POx	7,500 units, 5x/day with meals/snacks, 12 weeks	Enteric ESRD: Mean plasma oxalate (POx) change from baseline to Weeks 4-12 of -12.5 μ mol/L (-29.2%). Enteric CKD Stage 3b: Mean POx change from baseline to Weeks 4-12 of -2.1 mol/L (-28.7%). Mean UOx change from baseline to Weeks 4-12 of -44.7 mg/24 hr, (-34.4%). PH, No substantive change from Baseline in UOx or POx
Study 6, Phase III, Global	Randomized, double-blind, multicenter PBO-controlled	EH N=115, Male=60, Female=55 Age 28-79 years	7,500 units or PBO, 3-5x/day with meals/snacks, 28 days	UOx % change from Baseline to Wks 1-4, All: ALLN-177 -22.6%, PBO -9.7%; LS mean treatment difference -14.3% (p=0.004), Bariatric: ALLN-177 -21.2%, PBO -6.0%; LS mean treatment difference -16.19% (p=0.010), Proportion achieving \ge 20% reduction from baseline in UOx: All: ALLN-177 48.3%, PBO 31.6% (p=0.061), Bariatric: ALLN-177 50% vs. PBO 28.9% (p=0.036).

Clinical studies, ALLN-177. Six clinical studies have been completed globally for ALLN-177, the most recent three of those studies are summarized in **Table 1**. For brevity, only the results for the phase III trial, the most recent and largest of the clinical studies, are discussed below. **Phase III ALLN-177** used randomized 115 subjects: 58 to ALLN-177 and 57 to Placebo (PBO), of which 114 (99.1%) completed the study in its entirety. The study enrolled 78 (67.8%) subjects with a history of malabsorptive bariatric surgery and 37 (32.2%) with other enteric disorders, including inflammatory bowel disease, short bowel syndrome, exocrine pancreatic insufficiency, and other malabsorption conditions. The mean baseline UOx was 87.3 mg/24 hr and 91.1 mg/24 hr in the ALLN-177 and PBO groups, respectively.

The study achieved its primary endpoint, with a mean reduction in 24-hour UOx from baseline to the average of Weeks 1-4 of 22.6% on ALLN-177, compared to 9.7% on PBO; the least square (LS) mean treatment difference was -14.3% (p=0.004). In the secondary endpoint examining the subgroup of bariatric surgery patients, mean reduction in 24-hour UOx was -21.2% on ALLN-177 and 6.0% on PBO (LS mean treatment difference -16.19%, p=0.010). The week-to-week results showed that ALLN-177 manifests immediately on 24-hour UOx excretion. The proportion of subjects achieving a \ge 20% reduction from baseline in 24-hour UOx excretion was 48.3% on ALLN-177, compared to 31.6% on PBO (p=0.06). These results demonstrated the efficacy of ALLN-177 but failed to support advancement of this formulation of native ALLN-177.

MCP Preliminary Studies. Glycend has developed a series of proprietary synthetic polymers (MCPs) that crosslink with mucin, a major component of the mucus layer that lines the gastrointestinal tract (**Figure 1**). The most clinically advanced of these MCPs, GLY-200, has completed phase I and IIa clinical trials demonstrating a postprandial glucose biomarker signature that is

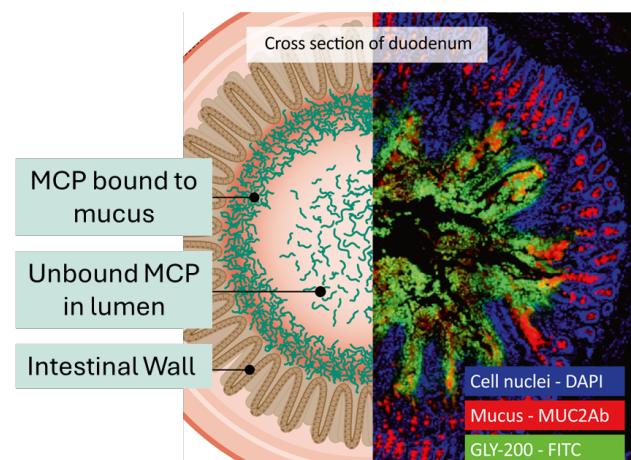


Figure 1: (left) cartoon of GLY-200 complexing with mucin on a cross-section of the intestine, (right) representative histology image of a GLY-200 coating is shown two hours after dosing in a rat model.

directionally consistent with a duodenal exclusion mechanism of action (MOA). This result positions GLY-200 as a noninvasive alternative to metabolic surgery or duodenal exclusion devices. GLY-200 dissolves rapidly in the low pH environment of the stomach (pH < 5.5). As the dissolved polymer passes through the pylorus, the higher pH environment of the duodenum (pH > 5.5) facilitates rapid crosslinking of the polymer with endogenous mucin. This in-situ complexation with mucin alters the mucus barrier in the duodenum to achieve temporary pharmacological duodenal exclusion and slows gastric emptying.

The Phase I Clinical study established the safety and tolerability of GLY-200 after single- and multiple-ascending oral doses in healthy volunteers. In Part 1 (single ascending dose, SAD) 32 subjects were enrolled and received single doses of up to 6 g. GLY-200 was found to be generally well tolerated. In Part 2 (multi-ascending dose, MAD), GLY-200 was administered on 5 consecutive days at various doses. A small dose-dependent decrease in weight was observed over time for the GLY-200 treated groups. The placebo groups had a slight weight gain over this time, with a mean weight increase of 0.5 kg (1.1) on Day 6. The data suggested that 2x/day administration was better tolerated than 3x/day. The study demonstrated that the serum biomarker signature of duodenal exclusion was reproduced (↓ glucose, insulin with ↑ GLP1, PYY, Bile acids) and that there were dose-dependent reductions in blood glucose with progressive weight loss.

The Phase IIa Clinical study investigated the efficacy of GLY-200 in type 2 diabetes patients, showing proof of the duodenal exclusion MOA. GLY-200 dosed at 0.5g BID, 1.0g BID or 2.0g BID for 14-days was found to be well tolerated. There were no serious or severe adverse events, and 97% of adverse events were mild. Constipation, diarrhea, and nausea (all mild) were the most common adverse events, and incidences of nausea were reduced over time. Through Day 14, there was a dose-dependent decrease in fasting and post-prandial blood glucose and weight observed.

Preliminary MCP and ALLN-177 Studies. To demonstrate the viability of our co-formulation approach, a) the adhesion/entrapment capability of the polymer-mucus complex, b) proximal gut transit time, and c) the activity of OxDC in the presence of MCP were examined. Adhesion and retention of the microcrystalline ALLN-177 were modeled using fresh porcine duodenal tissue and polystyrene microspheres. When applied to mucosal tissue in the presence of MCP, the microspheres were found to be evenly distributed and robustly retained on the tissue surface, and resistant to rinsing. In contrast, the microspheres were minimally retained and easily cleared by rinsing when applied in the absence of the MCP. GI transit of a non-absorbed dye probe was evaluated in rats after treatment with GLY-200 or a noninteractive control polymer (Dextran70). The dye was retained in stomach and duodenum for at least 4-hours longer with GLY-200 than control (Figure 2). To confirm the enzyme activity is not negatively impacted in the presence of MCP, a commercially available kit was used to measure the OxDC enzyme activity in the presence and absence of GLY-200. The combination of GLY-200 with the positive control (commercially available OxDC) did not result in precipitation or visible incompatibility. This experiment showed that the activity of OxDC was not affected by the presence of MCP.

The combined weight of these studies indicates the strong potential for an MCP-based formulation to lengthen the residence time of ALLN-177 in the gastrointestinal tract, allowing for the realization of more complete enzymatic oxalate degradation. The premise of this phase I project will be to rigorously test this approach and optimize a candidate ALLN-177/MCP formulation for clinical development.

Aim 1: In vitro formulation optimization.

Table 2: Phase I, Aim 1, In-Vitro Milestones

Milestone	Quantitative Metric
1.1 ALLN-177 activity with MCP	At least 5 different MCP/ALLN-177 combinations with enzymatic activity similar to that of baseline (maximum) ALLN-177 activity (minimal-to-no activity loss)
1.2 Impact of mucin on ALLN-177/MCP	At least one MCP/ALLN-177 combination with activity $\geq 50\%$ of baseline (maximum) ALLN-177 activity after immobilization in MCP-mucin complex (minimal activity loss)
1.3 Impact of pH	Determine pH dependence for the lead ALLN-177 activity in MCP-mucin complex (pH 2-8)

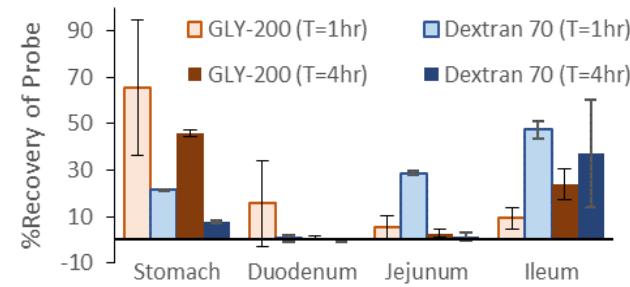


Figure 2: Results from GLY-200 preclinical trial

Rationale: While preliminary results have shown that OxDC remains active in the presence of MCPs, the choice of a lead MCP and the resulting activity of ALLN-177 in the presence of mucin have not been optimized. In this aim we will quantify the enzymatic activity of ALLN-177 in various candidate MCP formulations. This Aim will examine the base (maximum) activity of ALLN-177 in buffer alone, and then again in the presence of various MCPs to confirm that MCPs do not diminish enzyme activity. Then mucin will be added forming the MCP-mucin complex and entrapping the enzyme. The intent of this aim is to optimize the formulation to maintain maximal activity for the enzyme retained within the mucin-MCP complex prior to testing in a simulated GI environment.

Milestone 1.1 ALLN-177 activity with MCP. ALLN-177 activity will first be assessed to calibrate its baseline activity in an oxalate decarboxylase activity assay. Enzyme activity will be measured using the commercially available oxalate decarboxylase activity assay. In this 96-well plate-based assay, oxalate levels are determined as a function of time and the extent of oxalate degradation by ALLN-177 is measured spectrophotometrically. The assay will be run in triplicate for each condition and formulation. Conveniently, MCPs themselves have shown a capacity for binding oxalate. Therefore, the impact of MCP oxalate binding on the activity assay will also be determined. We will then assess ALLN-177 activity in the presence of at least 5 MCPs of interest to confirm our preliminary result (described above) that ALLN-177 enzymatic activity is not altered in the presence of MCPs. **Success criterion:** Identify at least 5 different MCP/ALLN-177 combinations providing enzymatic activity nearly equivalent to the baseline (maximum) activity of ALLN-177 alone.

Milestone 1.2 Impact of mucin on ALLN-177/MCP. The next iteration will test the ALLN-177 activity of the top 3 MCP/ALLN-177 combinations from Milestone 1.1 in the presence of mucin. This step is intended to represent the actual intestinal environment in which the MCP-mucin complex will form and immobilize the microparticles of ALLN-177. The goal of this work is to determine the impact of immobilization on the enzymatic activity of ALLN-177 bound within this complex. Enzymatic activity will be measured according to the protocol outlined in Milestone 1.1. **Success criteria:** at least one MCP/ALLN-177 combination that provides enzymatic activity $\geq 50\%$ of the baseline activity of ALLN-177.

Milestone 1.3 Impact of pH. For the lead MCP-ALLN-177 formulation from milestone 1.2, the effect of pH on enzyme activity will be assessed in increments of 1 pH unit from 2 to 8. ALLN-177 at a baseline is inactive at a pH of 7 and above, and it is expected that this step will provide a rate curve over different pHs. This information is invaluable in terms of assessing additives that could accelerate the activity of the retained ALLN-177. **Success criteria:** Generation of a rate curve of ALLN-177 in the lead MCP-mucin complex for pH 2-8.

Expected outcomes, pitfalls, and alternative approaches. It is possible that the MCP-mucin complex could limit the access of oxalate to ALLN-177, attenuating enzyme activity. In this event, Glyscend is prepared to explore additional formulation components to enhance diffusion in the MCP-mucin complex, such as water-soluble polymers (polyacrylic acid, polyethylene glycol, polyvinyl pyrrolidone).

Aim 2: Ex vivo optimization.

Table 3: Phase I, Aim 2 Milestones

Milestone	Quantitative Metric
2.1 Demonstrate ex vivo retained enzymatic activity on tissue	Enzymatic activity of ALLN-177 immobilized on intestinal tissue is retained despite serial rinsing (enzymatic activity +/-20% of the baseline activity of ALLN-177 following rinsing).

Rationale: To further advance the understanding of in vivo performance, Aim 2 will test the lead formulation from Aim 1 for retention of ALLN-177 enzymatic activity on intestinal mucosa. This aim is a critical step that demonstrates longer retention time of ALLN-177 and prolonged activity due to immobilization in the MCP, the theoretical basis for this proposal. The work will use porcine tissue to mimic the gastrointestinal tract lined with mucus as the testing environment for the lead formulation(s) from Milestone 1.2. This demonstration provides insight into the optimization parameters critical to maximize the time and distribution of ALLN-177 in the gut.

Milestone 2.1 Analysis enzymatic activity ex vivo. Porcine duodenum will be obtained from a local abattoir within 2 hrs. of harvest (Research 87, Boylston, MA). Sections of fresh porcine duodenum (6 cm long) will be isolated for ex vivo testing. Porcine tissue pouches (N=3) will be formed and filled with lead MCP/ALLN-177 formulation(s) from Milestone 1.2. The pouches will be spiked with oxalate and incubated. Of the (minimum) of 3 pouches per formulation: one pouch will have a sample taken to assess the enzyme activity using the assay outlined in Milestone 1.1; the second pouch will be rinsed twice with 10 mL of buffer, followed by oxalate activity analysis; and the third pouch will undergo 3 sets of rinsing (10 mL of buffer a total of 3 times) followed by oxalate activity analysis. **Success criteria:** Demonstration of retained ALLN-177 enzymatic activity unaffected by serial rinsing (enzymatic activity +/-20% of the baseline activity of ALLN-177 following rinsing).

Expected outcomes, pitfalls, and alternative approaches. If a significant reduction in enzymatic activity is observed due to serial rinsing, additional formulation optimization work will be undertaken with the goal of improving immobilization of ALLN-177 particles. This may include optimizing the ratio of formulation components or evaluating additives to impact adherence of the MCP-mucin complex to ALLN-177 particles.

Aim 3: In vivo Proof of Concept.

Table 4: Phase I, Aim 3 Milestones	
Milestone	Quantitative Metric
3.1 UOx reduction – fasted, oOTT	At least one formulation with a 2x reduction in UOx relative to ALLN-177 alone
3.2 UOx reduction – high oxalate diet	At least one formulation with a 2x reduction in UOx relative to ALLN-177 alone

Rationale: The lead formulations from Aims 1 and 2 will be tested in vivo for their impact on UOx excretion in rat models of EH, and their efficacy will be compared to that of native ALLN-177. CRO NeoSome Life Sciences (Billerica, MA) will conduct rat studies. UOx will be measured in response to administration of an oxalate challenge by oral gavage, in a manner similar to an oral oxalate tolerance test (oOTT) in fasted rats. This model, developed by Glyscend, evaluates the efficacy of candidate therapeutics for the treatment of EH in the fasted state. The most promising formulation will then undergo testing using rats with EH induced by a 4-day high oxalate diet. Again, UOX will be measured after administration of the test formulation. This model mimics delivery of oxalate in the diet, adding nutrient flow as a factor. The overall goal of SA3 is to identify potential candidates to advance to clinical-stage development.

Milestone 3.1 Fasted oOTT. Candidate formulations will be first evaluated in normal rats (Sprague Dawley, 6 male, 6 female, 8-10 weeks old) in a fasted state using an acute rat model capable of measuring a UOx signal in response to a gavaged oxalate challenge (with & without a meal). All animals will receive an oxalate-free, high-fat (17%), low-calcium (0.5%) synthetic diet (TD. 06199, Research Diets, New Brunswick, NJ) for the duration of the acclimation and study periods. This model will be performed by CRO NeoSome to quantify UOx excretion over 24 hours for rats treated with ALLN-177 alone and with candidate formulations containing MCP polymers and ALLN-177. Oxalate will be measured with an HPLC/ion-exchange bioanalytical method. This model will inform dose timing, formulation strength, and oxalate excretion profile. Success criteria: at least one formulation with a 2x target reduction in UOx relative to ALLN-177 alone.

Milestone 3.2 High Oxalate Diet. Normal rats (Sprague Dawley, 6 male, 6 female, 8-10 weeks old) will be placed on a high oxalate diet for 4 days to induce enteric hyperoxaluria. Rats on the high oxalate diet will be treated with the selected formulation identified from Aim 3.1 for a 4-day treatment regimen and UOx will be quantified over 24 hours. Oxalate will be measured with an HPLC/ion-exchange bioanalytical method. Success criteria: a target 2x reduction in UOx relative to ALLN-177 alone.

Expected outcomes, pitfalls, and alternative approaches. Our approach aims to illustrate translation of in vitro activity to the rat model both in the absence or presence of nutrient flow in the gut. This approach helps us understand the impact of nutrient flow in the gut and gives us the best chance of success. Failure to observe translation of in vitro activity will be addressed by iterative modifications of the formulations and interrogating in vivo testing parameters (e.g. total dose, dose timing) to help assess the reasons for failure to achieve efficacy.

Table 5. Aims Summary and Timeline		Month											
Aim	Description	1	2	3	4	5	6	7	8	9	10	11	12
1.1	ALLN-177 activity with MCP												
1.2	Impact of mucin on ALLN-177/MCP												
1.3	Impact of pH												
2.1	Analysis enzymatic activity ex vivo												
3.1	Fasted oOTT												
3.2	High oxalate diet												

Summary: This phase I SBIR project will determine whether Glyscend's hypothesis for utilizing its novel MCP platform to enhance a promising enzyme therapy for EH has practical merit. If successful, follow-on efforts during a potential phase II project will continue development work to ready the therapy for IND filing and clinical evaluation. The phase II project will likely include dose finding studies to reduce potential GI side effects while remaining effective, a deeper exploration of treatment timing relative to meals, and an examination of nonclinical safety and efficacy on longer-term dosing.

PHS Human Subjects and Clinical Trials Information

OMB Number: 0925-0001

Expiration Date: 01/31/2026

Use of Human Specimens and/or Data

Does any of the proposed research in the application involve human specimens and/or data *

Yes No

Provide an explanation for any use of human specimens and/or data not considered to be human subjects research.

Are Human Subjects Involved Yes No

Is the Project Exempt from Federal regulations? Yes No

Exemption Number 1 2 3 4 5 6 7 8

Other Requested Information

Delayed Onset Studies

Delayed Onset Study#	Study Title	Anticipated Clinical Trial?	Justification
The form does not have any delayed onset studies			

VERTEBRATE ANIMALS SECTION

1. Description of Procedures

The first test will evaluate oOTT in normal, fasting rats (6 male, 6 female Sprague Dawley rats (8-10 weeks) per group) in a fasted state using an acute rat model capable of measuring a UOx signal in response to a gavaged oxalate challenge. All animals will receive an oxalate-free, high-fat (17%), low-calcium (0.5%) synthetic diet (TD. 06199, Research Diets, New Brunswick, NJ) for the duration of the acclimation and study periods. This model will be performed by CRO NeoSome to quantify UOx excretion over 24 hours for rats treated with ALLN-177 alone and with candidate formulations containing MCP polymers and ALLN-177. Oxalate will be measured with an HPLC/ion-exchange analysis.

In the second test rats (6 male and 6 female Sprague Dawley rats ((8-10 weeks) per group) will be placed on a high oxalate diet for ~~8~~ days to induce enteric hyperoxaluria. Rats on the high oxalate diet will be treated with the selected oOTT for a 4-day treatment regime and UOx will be quantified over 24 hours. Oxalate will be measured with an HPLC/ion-exchange analysis.

2. Justifications

Sprague Dawley rats are commonly chosen for biomedical research due to their genetic uniformity, well-characterized physiology, and relevance to human disease model. Sprague Dawley rats have been used extensively in models of kidney stones and oxalate metabolism, making them a relevant choice for studying EH. Their physiological responses to high oxalate diets and their ability to mimic aspects of human oxalate metabolism enhance the translational value of the research. In addition, the use of Sprague Dawley rats in similar contexts allows for direct comparison with historical data and benchmarking against existing ALLN-177 data. This comparative aspect is crucial for assessing the relative efficacy of new formulations.

3. Minimization of Pain and Distress

NeoSome Life Sciences maintains and operates a 7,500 square foot purpose built animal facility. The facility is designed specifically for the housing of mice, rats, hamsters, gerbils, guinea pigs and rabbits. The facility has 10 animal holding rooms for housing animals by species, a dedicated room for housing immune compromised mice, and two dedicated rooms equipped for performing small animal surgery. The cage wash area contains a rack style passthrough washer and double door autoclave. The facility also has 7 procedure rooms to perform animal manipulations in. The IVIS in-life imager is also placed in one of these procedure rooms. NeoSome maintains a USDA certification for housing mice, rats, hamsters, guinea pigs and gerbils (USDA #14-R-0215). Our daily average animal inventory is: 1,734 mice and 105 rats. Other covered species are housed infrequently.

NeoSome has an established animal care and use program which follows the National Research Council's Guide for the Care and Use of Laboratory Animals. The NeoSome IACUC consists of 7 members which reviews and approves all animal protocols prior to any study being conducted. NeoSome utilizes an Attending Veterinarian who is available 24/7 via mobile phone including plans in place for a backup veterinarian if needed. NeoSome's Animal Welfare Assurance Number is: D16-00934. Animals with significant signs of pain/distress will be observed more often and, if need, given sufficient concentration of suitable analgesic by subcutaneous or IM injection; analgesic will be readministered, if need, at appropriate frequency. Animals with signs of severe pain/distress considered irreversible will be humanely euthanized, per the decision of the Attending Veterinarian.

4. Euthanasia

All animals surviving to termination will be euthanized by CO₂ overdose.

REFERENCES

1. Witting C, Langman CB, Assimos D, Baum MA, Kausz A, Milliner D, Tasian G, Worcester E, Allain M, West M, Knauf F, Lieske JC. Pathophysiology and Treatment of Enteric Hyperoxaluria. *Clin J Soc Nephrol*. 2021;16(3):487-495.
2. Nazzal L, Puri S, Goldfarb DS. Enteric hyperoxaluria: an important cause of end-stage kidney disease. *Nephrol Dial Transplant*. 2016;31(3):375-382. doi:10.1093/ndt/gfv005
3. Agrawal V. Chapter 40 - Enteric Hyperoxaluria, Calcium Oxalate Nephrolithiasis, and Oxalate Nephropathy After Roux-en-Y Gastric Bypass. In: Rajendram R, Martin CR, Preedy VR, eds. *Metabolism and Pathophysiology of Bariatric Surgery*. Academic Press; 2017:361-370. doi:10.1016/B978-0-12-804011-9.00044-3
4. Langman C, Grujic D, Pease R, et al. A Double-Blind, Placebo Controlled, Randomized Phase 1 Cross-Over Study with ALLN-177, an Orally Administered Oxalate Degrading Enzyme. *Am J Nephrol*. 2016;44:150-158.
5. Lingeman JE, Pareek G, Easter L, et al. ALLN-177, oral enzyme therapy for hyperoxaluria. *Int Urol Nephrol*. 2019;51(4):601-608.
6. Lieske JC et al. Randomized Placebo-Controlled Trial of Reloxaliase in Enteric Hyperoxaluria. *NEJM Evid*. 2022;1(7).
7. Enteric Hyperoxaluria: Understanding the Disease: Episode 7. Phase 3 URIROX-1 Trial for Enteric Hyperoxaluria. *Urol Times*. Published online January 1, 2022.
8. Fineman MS, Bryant CLN, Colbert K, et al. First-in-human study of a pharmacological duodenal exclusion therapy shows reduced postprandial glucose and insulin and increased bile acid and gut hormone concentrations. *Diabetes Obes Metab*. 2023;25(9):2447-2456.
9. Glyscend. A Study Evaluating the Safety, Tolerability, and Pharmacodynamic Effects of GLY-200 in Type 2 Diabetic Patients. *Phase II Interv Clin Trial*. Published online March 14, 2023.
10. Nelson WK, Houghton SG, Milliner DS, Lieske JC, Sarr MG. Enteric hyperoxaluria, nephrolithiasis, and oxalate nephropathy: potentially serious and unappreciated complications of Roux-en-Y gastric bypass. *Surg Obes Relat Dis*. 2005;1(5):481-485.
11. Sinha MK, Collazo-Clavell ML, Rule A, Milliner DS, Nelson W, Sarr MG, et al. Hyperoxaluric nephrolithiasis is a complication of Roux-en-Y gastric bypass surgery. *Kidney Int*. 2007;72(1):199-7.
12. Canales BK, Gonzalez RD. Kidney stone risk following Roux-en-Y gastric bypass surgery. *Transl Androl Urol*. 2014;3(3):242-249.
13. Canales BK, Hatch M. Kidney stone incidence and metabolic urinary changes after modern bariatric surgery: review of clinical studies, experimental models, and prevention strategies. *Surg Obes Relat Dis*. 2014;10(4):734-742.
14. Kumar R, Lieske JC, Collazo-Clavell ML, Sarr MG, Olson ER, Vrtiska TJ, et al. Fat malabsorption and increased intestinal oxalate absorption are common after Roux-en-Y gastric bypass surgery. *Surgery*. 2011;149(5):654-661.
15. Hoppe B, Leumann E, von Unruh G, Laube N, Hesse A. Diagnostic and therapeutic approaches in patients with secondary hyperoxaluria. *Front Biosci*. 2003;8:1-3.

16. North America Secondary Hyperoxaluria Drug Market – Industry Trends and Forecast to 2028. *Data Bridge Mark Res.* Published online February 2021.
17. A. Nimgaonkar, C . Bryant, T. Carlson, T. Guerina, K. Colbert, S. Polomoscanik, J. Petersen, T. Jozefiak, M. Fineman. Poster: Duodenal Targeting by Oral Pharmacologic Duodenal Exclusion Therapy for Treatment of Type 2 Diabetes. *ADA 83rd Sci Sess San Diego*.



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March 20, 2024

To: National Institutes of Health Grant Committee

On behalf of the Oxalosis & Hyperoxaluria Foundation (OHF), I would like to express our support for Glyscend's National Institute of Health (NIH) grant to find a treatment for Enteric Hyperoxaluria.

OHF has been a trailblazer in the fight against Hyperoxaluria for over 35 years, achieving significant milestones through collaborative efforts with industry stakeholders. Notably, our collaborative endeavors have been instrumental in obtaining FDA approval for two medications, Oxlumo and Rivfloza, targeting Primary Hyperoxaluria Type 1. However, it is crucial to note that there are presently no approved therapies for Enteric Hyperoxaluria.

Enteric Hyperoxaluria presents a formidable health obstacle, mirroring the challenges of Primary Hyperoxaluria. Individuals affected by this condition endure a demanding regimen of extensive daily fluid intake and numerous surgical interventions to combat the relentless formation of kidney stones.

These recurrent episodes not only diminish patients' quality of life but also increase the need for kidney transplantation as the only solution. However, this invasive procedure is fraught with substantial risks and demands lifelong adherence to immunosuppressive therapies, further complicating the already burdensome journey for those afflicted with Enteric Hyperoxaluria.

Therefore, we support the potential for an effective treatment proposed by Glyscend and look forward to working closely with their team.

Sincerely,

A handwritten signature in black ink, appearing to read 'K. Hollander'.

Kim Hollander
Executive Director

CHIRAG R PARIKH, MD, PhD
DIRECTOR, DIVISION OF NEPHROLOGY
RONALD PETERSON PROFESSOR OF MEDICINE
DEPARTMENT OF MEDICINE
JOHNS HOPKINS UNIVERSITY SCHOOL OF MEDICINE
1830 E. MONUMENT STREET, SUITE 416
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410-955-5268 (OFC) / 410-367-2258 (FAX)



March 24, 2024

RE: Letter of Support of Glycend Inc.'s NIH Proposal

Dear NIH Review Committee,

With great enthusiasm, I write this letter supporting Glycend's NIH Proposal: "A Novel Oral Enzyme Therapy To Treat Enteric Hyperoxaluria." I am intimately familiar with the Enteric Hyperoxaluria (EH) condition, given my current role as a Professor of Medicine and Director of the Division of Nephrology at Johns Hopkins University School of Medicine, and I frequently see patients with this condition as part of recurrent kidney stones or hyperoxaluria associated chronic kidney disease progression.

With this letter of recommendation, I would like to emphasize the Glycend team's dedication to engineering a solution that prioritizes the patient's needs. As a nephrologist, I see many patients who struggle with both primary and enteric hyperoxaluria. My focus is on employing strategies to prevent kidney stone formation and kidney damage, which most typically include:

- i. Dietary adjustments include increased hydration, avoiding foods high in oxalates such as spinach, nuts, and chocolate, and limiting the intake of salt and animal protein.
- ii. Attempts to bind oxalate in the gut via calcium and magnesium supplementation or the drug Cholestyramine to reduce oxalate absorption
- iii. Reduce kidney stone formation via administration of pentosan polysulfate or potassium citrate

These options are limited in effectiveness, and patients continue seeking a better alternative pharmaceutical option. My ideal treatment is a high-efficacy, orally-dosed drug that dramatically slows or altogether halts the development of hyperoxaluria and the progression of kidney disease.

It is, therefore, with great interest that I learned of Glycend. Their approach to combining an oral oxalate enzyme and mucus complexation polymer (MCP) is very compelling. The ALLN-177 enzyme has been previously shown quite effective in clinical studies, and should the MCP be able to prolong the duration of exposure for the enzyme in the stomach and duodenum, it should result in a profound and clinically relevant enhancement in efficacy. This would be a major step forward for the field and allow patients access to a first-in-class treatment for EH.

Sincerely,

A handwritten signature in black ink that reads "Chirag R. Parikh".

Chirag R. Parikh, MD, PhD
Director, Division of Nephrology
Ronald Peterson Professor of Medicine
Johns Hopkins University School of Medicine



25 March 2024

Dr. Ashish Nimgaonkar
Chief Medical Officer
Glyscend, Inc.
600 Suffolk Street, Suite 250
Lowell, MA, 01854

Dear Dr. Nimgoankar,

I am writing this letter to confirm my strong support and my active role as a project consultant in Glyscend's SBIR project titled: "A Novel Oral Enzyme Therapy To Treat Enteric Hyperoxaluria, to be submitted to the NIH in April 2024.

I am an accomplished biopharmaceutical scientist and leader, with over 20 years of direct research, people, and technical project management experience focused on discovery of novel therapeutics including oral and injectable biologics and small molecule therapies for chronic metabolic, gastrointestinal, and rare genetic diseases and cancer. Much of my career has been focused on translating human physiology and pathophysiology into pre-clinical models to enable successful transition from early discovery stage assets to clinical trials.

Most relevant to Glyscend's proposed work, I spent five years at Allena Pharmaceuticals as the Executive Director of Pharmacology. At Allena, I was Lead Pharmacologist on the oral biologics platform for treatment of metabolic diseases that included ALLN-177. My role encompassed concept initiation, molecular evolution, protein engineering and IND filing from Phase 1 through Phase 3 clinical trials. I am extremely proud to have helped develop and advance ALLN-177 to late-stage clinical trials. I continue to believe that the enzyme has tremendous potential for patients with enteric hyperoxaluria and am therefore fully supporting Glyscend's development efforts.

For the Glyscend NIH grant, I will help optimize experimental design and evaluate results across the three specific aims: (1) *in vitro* formulation optimization, (2), *ex vivo* characterization, and (3) *in vivo* demonstration of efficacy in rat models. My deep understanding of the disease space, and historical knowledge about how ALLN-177 alone performed in similar assays, should expedite and improve the development pathway for the enzyme + mucus complexing polymer therapy.

My agreed-upon hourly rate for the project is \$250/hour and we anticipate up to 40 hours of consulting work which would translate to the budgeted \$10,000 in total consulting compensation for the duration of the proposal.

I fully support Glyscend's endeavors to provide an improved therapy for patients with Enteric Hyperoxaluria. Best of luck in securing funding for this important work.

Sincerely,

Danica Grujic
Independent Consultant

To whom it may concern:

March 20, 2024

It is with great pleasure that I write in support of Glyscend's Phase I NIH Proposal: "A Novel Oral Enzyme Therapy to Treat Enteric Hyperoxaluria". Given my previous role as President and CEO of Allena Pharmaceuticals, and as a trained and practicing nephrologist, I am intimately familiar with the opportunities and challenges that developing a treatment for Enteric Hyperoxaluria (EH) presents.

I led Allena as President then also CEO from a private, early-stage to a public, late-stage biotech company with multiple homegrown assets in Phase 2 and Phase 3 development, including ALLN-177 (Reloxaliase™) for the treatment of EH. We raised more than \$200M for the company and completed multiple financings including a crossover round, an IPO and multiple secondary offerings. I am proud to have built a fantastic team at Allena and have led the development of ALLN-177 which was a promising clinical candidate in a space that demands greater innovation.

In leading Allena, I also benefited from my clinical training as a nephrologist. I completed my residency in internal medicine at Brigham and Women's Hospital and my fellowship in nephrology at Brigham and Women's Hospital and Massachusetts General Hospital, and now hold appointments at Brigham and Women's Hospital and Dana Farber Cancer Institute.

I believe Glyscend's approach to combine ALLN-177 and their MCP platform is highly innovative and has a strong likelihood of success. ALLN-177 is an orally administered crystalline form of oxalate decarboxylase. It was developed as an oral enzyme therapy that specifically degrades oxalate within the gastrointestinal tract. Based on the pH profile of the enzyme, and experiments performed in the porcine model of diet-induced hyperoxaluria¹, the primary site of action of ALLN-177 is thought to be in the upper GI tract, primarily in the stomach. This portends well for combination with Glyscend's MCP, which is known to extend residence time in the upper GI tract. Although the native ALLN-177 oral enzyme therapy did not reach the efficacy threshold required for regulatory approval, it is exciting to consider the synergistic combination of ALLN-177 with the MCP platform as the next generation formulation. Having reviewed the proposal's Specific Aims, I believe the team is focused on the correct nonclinical development milestones including demonstrating that the enzyme-MCP combination remains enzymatically active in vitro (SA1) and ex vivo (SA2) and then quantifying the reduction of urine oxalate (UOx) excretion in the relevant hyperoxaluric rat model (SA3).

The Glyscend team has proposed a solution which truly has the potential to alter the hyperoxaluria treatment landscape. Should it prove effective, it would provide a significant value to patients and providers in need. I am also confident that the clinical and technical network they've established will allow the team to quickly achieve developmental milestones as results are gathered from further testing over the coming months.

As this technology has the potential to impact the lives of EH patients in the US and around the world, I fully endorse the Glyscend team in this endeavor.

¹ Grujic D, Brettman L, Langman C, Fedkiv O, Goncharova K, Kardas M, et al. PD31-11 ALLN-177 reduces Hyperoxaluria in a porcine model of secondary hyperoxaluria (2[°]HO) induced by a human-like oxalate rich diet. *J Urol.* 2016;195(4):e721.

Sincerely,



Louis Brenner, MD, MBA
CEO at Hopewell Therapeutics
Ex-President and CEO at Allena Therapeutics

27 March 2024

Dr. Ashish Nimgaonkar
Chief Medical Officer & Head of R&D
Glyscend, Inc.
600 Suffolk Street, Suite 250
Lowell, MA, 01854

Dear Dr. Nimgaonkar,

I am delighted to have the opportunity to provide a letter of support for your SBIR proposal entitled: "A Novel Oral Enzyme Therapy to Treat Enteric Hyperoxaluria". Having developed ALLN-177 through phase III clinical trials, my insight on this important therapeutic compound and the broader field of hyperoxaluria is uniquely deep and enabling. I am excited to have the opportunity to use this insight to help advise your team on your creative new approach for treating hyperoxaluria.

I am in a strong position to assist Glyscend on this ambitious project, having built a diverse career spanning several companies in the biotechnology and pharmaceutical industry. I currently serve as Chief Corporate Development Officer at Anagram Therapeutics, Inc. where I drive the long-range corporate strategy for product development and financing. Of course, prior to joining Anagram I was Senior Vice President of Technical Operations at Allena Pharmaceuticals where I led the manufacturing and clinical development of both ALLN-177 and ALLN-346. Prior to Allena, I held key operating positions at Alcresta, Inc., Alnara Pharmaceuticals, Inc., Altus Pharmaceuticals, Inc., Alkermes, Inc., InSite Vision, Inc., and Genetics Institute, Inc. In these positions, I led various teams, managed manufacturing operations, and oversaw product development and regulatory interactions.

While the termination of our program at Allena was more than disappointing, I am heartened at the possibility of a new start for ALLN-177 in the hands of your capable team. My interactions with Glyscend have been both rewarding and encouraging. Glyscend brings great enthusiasm and energy to this new project. The capabilities of your team are impressive, having successfully developed multiple therapeutic agents over their accumulated years of experience.

I am confident that my wealth of experience drug development and my familiarity in using ALLN-177 in treating this difficult disease will make me a valuable asset in advising your team. I fully support Glyscend's program for treating Enteric Hyperoxaluria. Best of luck in securing support for this important proposal.

Sincerely,



Hugh Wight
Chief Corporate Development Officer
Anagram Therapeutics, Inc.



March 20, 2024

Ashish Nimaonkar, MD
Glyscend Therapeutics
600 Suffolk Street, Suite 250
Lowell, MA 01854-3643

Dear Dr. Nimgaonkar,

NeoSome Life Sciences, LLC. is a contract research organization skilled at providing services in several pharmacological therapeutic areas including metabolic disease research. NeoSome Life Sciences will undertake supportive activities required to complete the attached Glyscend Therapeutics SBIR grant proposal: A Novel Oral Enzyme Therapy To Treat Enteric Hyperoxaluria.

The NeoSome group, with a total staff of 15 people, has ten-degree level staff members dedicated to *in vivo* modeling of inflammatory diseases, infectious diseases, oncology, as well as general pharmacology and safety studies. The guidelines established by NeoSome Life Sciences for the ethical care and treatment of laboratory animals are strictly adhered to. All regulatory approvals within our facility conform to established NIH animal research guidelines. The animal protocols will be approved by the NeoSome IACUC board prior to the purchase of any animals. The vivarium director, veterinarian, and staff provide excellent veterinary care and have over 100 years of cumulative experience. The biological studies will be conducted in our 15000 sq. ft. laboratory in Billerica, MA. Our facility consists of a purpose-built animal vivarium and adjacent laboratory space for conducting *in vitro* assays. The vivarium has a dedicated BSL-2 suite with proper environmental controls for maintaining containment of pathogens. The NeoSome Life Sciences PHS/ OLAW assurance number is D16-00934. Our USDA certification number is 14-R-0215.

NeoSome looks forward to supporting your grant submission in this critical need disease area.

Sincerely,

A handwritten signature in black ink, appearing to read "Tim Murphy".

Tim Murphy
Director, Preclinical Research
NeoSome Life Sciences
781-325-8895



March 19, 2024

On behalf of Santé Ventures, I am writing to endorse the Glyscend team for the NIH's Small Business Innovation Research grant. Sante Ventures is a life science venture capital group which collectively manages approximately \$800 million in assets. As a Partner & Chief Scientific Officer, I focus on early to growth-stage investment opportunities in the life sciences sector, including biopharmaceuticals, medical devices, and diagnostics.

Recently, there has been considerable interest and development in treatments for hyperoxaluria, which can cause oxalate nephropathy and result in acute kidney injury (AKI) and chronic kidney disease (CKD). The venture capital community recognizes this as a significant unmet need and are looking to capitalize on the market opportunity. The Glyscend solution to leverage its innovative mucin-complexing polymer (MCP) platform to enhance delivery of ALLN-177 offers a compelling value proposition in that its component parts are both quite advanced with Phase 2 and 3 clinical data, respectively. The development path is therefore substantially derisked from a safety and tolerability perspective which can often be the Achilles heel for novel approaches in the kidney space. Having read and discussed the grant's Specific Aims with the team, I believe there is a real opportunity to efficiently evaluate the potential efficacy of the approach and end the grant with a compelling package to support venture capital investment and continued clinical development.

Santé prioritizes investment in a competent team to drive a solution to commercial viability. The Glyscend team is led by Ashish Nimgaonkar, MD, a leading clinician and innovator in the gastroenterology space, and Thomas Jozefiak, PhD, an experienced chemist with drug development expert in the non-absorbed polymer space. The other members of the Glyscend team have technical backgrounds in chemistry and biomedical engineering, and relevant experience in clinical trial design and regulatory strategy. In my experience, multidisciplinary groups are often the most likely to bring disruptive innovations to market.

Being responsible for a diverse group of healthcare companies in Santé's portfolio, I would like to emphasize the clinical and commercial value proposition of the Glyscend solution. If Glyscend delivers on the milestones put forth in the SBIR grant, the team will be able to build compelling clinical evidence, attract serious consideration from venture firms, and receive competitive bids for investment.

Best Regards,

Casey Cunningham, MD
Partner & Chief Scientific Officer
Sante Ventures



Ashish Nimgaonkar, MD
Chief Medical Officer and Head of R&D
Glyscend, Inc.

March 25, 2024

Dear Ashish,

In my capacity as the Executive Director of Innovation at UMass Lowell and Director of Operations for The Massachusetts Medical Device Development Center (M2D2), I am delighted to extend this letter of support for your Phase 1 SBIR grant proposal entitled: "A Novel Oral Enzyme Therapy to Treat Enteric Hyperoxaluria". Your team's dedication and innovative approach to solving difficult problems in biomedicine are well known here at M2D2. I am excited to express our strong endorsement of your new endeavor.

M2D2 is a collaborative program between the University of Massachusetts Lowell and the University of Massachusetts Medical School, acting as a lifeline for the community of innovators and biomedical entrepreneurs here in Massachusetts. We offer coordinated access to world-class research facilities and resources at the university. By providing a comprehensive ecosystem, we enable inventors and executives to navigate the complex landscape of innovation, spanning clinician review, animal trials, clinical trials, business mentorship, and investor partnerships. Housed within our 11,000 square foot shared lab facility at 110 Canal Street, and our 14,000 square foot suite of private laboratories at the historic Wannalancit Mills Technology Center, M2D2 serves as a collaborative hub for over 50 researchers.

It has been exciting to witness Glyscend's rapid progress here at M2D2. You first arrived in 2017 taking a pair of lab benches in our shared lab facility and quickly made good use of all we have to offer here in our facility and at University core facilities in Lowell and the UMass Med school. You took advantage of our undergraduate internship program, even hiring one of our student interns into a full-time position. I am also proud to know that your current Phase-2 clinical candidate was invented right here in our facility. It has been fulfilling to watch Glyscend succeed and grow, now occupying one of our private laboratories at the Wannalancit Center.

With unwavering enthusiasm, I extend our best wishes for your new program tackling hyperoxaluria. Your pursuit holds promise for extending the reach of your technology and treating patients in desperate need.

Sincerely,

Mary Ann Picard
Mary Ann Picard, MBA, MS
Executive Director Innovation / Director of Operations
The Massachusetts Medical Device Development Center (M2D2)
110 Canal St, 4th floor
Lowell, MA 01852

RESOURCE SHARING PLAN

The proposed scope of work will not result in the production of (1) unique model organism research resources and/or (2) unique research tools as defined by the NIH in their guidance documents entitled "Model Organisms Sharing Policy" and "Research Tools Policy" available on the NIH website.

Consistent with Bayh-Dole Regulations, Glyscend is committed to the timely development of a commercial product with support from the NIH SBIR/STTR funding mechanism. Commercialization of a dual combination drug to treat enteric hyperoxaluria will lead to its broad dissemination and use by the research community and the general public, thereby enhancing the value of NIH-sponsored research.

INTELLECTUAL PROPERTY RIGHTS

The investigators will assert copyright in scientific and technical articles based on data produced under the grant where necessary, but we will also make every effort to keep technologies developed as a result of this research project widely available and accessible to the research community. If additional patents are filed and the technology licensed, we will only seek exclusivity in cases where this approach is determined to be the best route for successful development of the technology for public use and benefit.

NIH Generated message:

The Other Plan(s) attachment included with the application is not evaluated during the peer review process but will be evaluated prior to a funding decision. Although part of the official submission, the attachment is maintained as a separate document in eRA Commons viewable by authorized users and is not part of this assembled application.