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TITLE: ARF6 Inhibitor for Treatment of Acute Lung Injury/Acute Respiratory Distress

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CONTRACTING ORGANIZATION: Navigen, Inc.

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13. SUPPLEMENTARY NOTES

14. ABSTRACT

The goal of this project is to advance nonclinical development of A6-5188 and file an IND. Significant progress has been made against Specific Aims 1 (GMP manufacture of A6-5188 Drug Substance) and 2 (conduct of nonclinical studies to support IND filing). Progress has also been made against Specific Aims 3 (GMP manufacture of A6-5188 Drug Product) and 4 (filing of IND).

15. SUBJECT TERMS

Acute lung injury, acute respiratory distress syndrome

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TABLE OF CONTENTS

TABLE OF TABLES

TABLE OF FIGURES

1 INTRODUCTION

Acute lung injury (ALI) and acute respiratory distress syndrome (ARDS) can result from several causes, including but not limited to, pulmonary aspiration, severe lung infections, smoke or chemical inhalation, direct trauma to the chest, fat emboli, or multiple transfusions. There are an estimated 190,000 cases of ALI/ARDS each year in the US, and ARDS is a major complication and cause of death with SARS-Cov-2 infection. Many chemical and biological weapons cause ALI/ARDS, and so the absence of an effective therapy makes Warfighters and the general population extremely vulnerable to such attacks. Some degree of ARDS occurs in between 26-33% of combat casualties. Mortality rates in patients with ALI/ARDS remain at approximately 40% even with current advances in critical care. Management of ALI/ARDS is challenging because care is limited to supportive measures, which are often inadequate. These measures consist of mechanical ventilation, fluid management, and, where possible, treatment of the underlying cause. There are no specific pharmacotherapies for prevention or treatment of ARDS. Our small-molecule ARF6 inhibitor, A6-5188, is pathogen-agnostic in that it reduces vascular permeability induced by several inflammatory cytokines and growth factors as well as several bacterial infections, both Gram-negative and Gram-positive. We envision that A6-5188 will be used as first-line therapy for patients with ARDS or at risk of developing ARDS regardless of the causative agent(s), and adjunctive to targeted antibacterial therapy initiated subsequent to pathogen identification. This grant is focused on nonclinical development of A6-5188 through IND filing.

Proprietary information is highlighted in light gray.

2 KEY WORDS

acute lung injury (ALI); acute respiratory distress syndrome (ARDS); ARF6; Good Laboratory Practice (GLP); Good Manufacturing Practice (GMP); Internation Conference on Harmonization (ICH); Investigational New Drug Application (IND); nonclinical development; pharmacokinetics (PK); safety pharmacology; toxicokinetics (TK); vascular leak

3 ACCOMPLISHMENTS

3.1 Aim 1: GMP manufacture of A6-5188 Drug Substance

The stated milestone was the GMP manufacture of A6-5188 Drug Substance, including starting an ICH stability study, to support GLP-compliant nonclinical studies and first-in-human clinical trials, with a completion date by the end of Month 6. As described below, we made slight revisions to this plan to manufacture a non-GMP engineering batch of A6-5188 (98.7% purity) to support the nonclinical studies, and this goal was accomplished before the end of Month 12. We elected to delay the GMP manufacture of A6-5188 Drug Substance in order to delay the start of a 36-month ICH stability study and so have a longer timeline for use of GMP Drug Substance in human clinical trials.

3.1.1 Aim 1 Major activities and accomplishments

We contracted with WuXi STA Pharmaceuticals (STA) for accomplishment of this goal. Our laboratory methods for synthesis of A6-5188 as the disodium salt (A6-5188-diNa), including analytical methods, were transferred to STA and their early development team became familiar with the synthetic scheme and suggested modifications and improvements. STA conducted analytical chemistry and stability studies on A6-5188-diNa and determined that the compound was a noncrystalline, amorphous substance with relatively poor stability. Therefore, a salt and polymorph screening effort was carried out by STA. It was determined that A6-5188 as an L-proline co-crystal

form (A6-5188-LP) was preferred as it is highly crystalline and stable. A non-GMP engineering batch of A6-5188-LP was manufactured by STA (542.7 g, 98.7% purity) to support nonclinical studies. A 12-month stability study of the engineering batch sufficient to support the nonclinical studies will begin in 3Q2021.

A6-5188 will be administered to humans as an intravenous (IV) infusion. Therefore, A6-5188 Drug Product will be A6-5188-LP as a sterile solution. STA subsequently conducted a preformulation/formulation campaign to determine the appropriate vehicle/buffer for preparation of A6-5188 Drug Product. It was determined that a 20 mM histidine buffer with glucose to adjust osmolarity, pH 7.4, led to high solubility of A6-5188-LP (up to 30 mg/mL) as well as high stability. This formulation will be used in conduct of the GLP-compliant nonclinical studies described in Section 3.2.1 below as well as in subsequent human clinical trials described in Section 3.7.

STA also synthesized a 60 mg batch of stable label ^{13}C isotope of A6-5188 for use as an internal standard in bioanalytical assays.

In summary, significant progress was made against Specific Aim 1. Salt and polymorph screening campaigns identified A6-5188-LP as a preferred crystalline and stable Drug Substance. A non-GMP engineering batch of A6-5188-LP was manufactured in sufficient quantity and purity to support nonclinical studies and a 12-month stability study will begin soon. Preformulation/formulation studies identified a histidine buffer for manufacture of A6-5188-LP Drug Product in solution for use in nonclinical and early clinical studies. We purposefully delayed the GMP manufacture of A6-5188-LP in order to have A6-5188-LP Drug Substance available longer into the future to support clinical trials. STA is available and ready to carry out the GMP manufacturing campaign at the 1.5 kg scale. Our current plan is to conduct this GMP manufacturing campaign in 4Q2021 (Months 17-19).

3.2 Aim 2: Conduct GLP-compliant Nonclinical Toxicology and Safety Pharmacology Studies to Support IND Filing

The stated milestone was completion of a GLP-compliant nonclinical package for IND filing by the end of Month 24. As described below, significant progress has been made on this goal and we estimate to have the nonclinical studies completed, including delivery of final study reports, before the end of Month 18.

3.2.1 Aim 2 Major activities and accomplishments

We contracted with WuXi AppTec (WuXi) for accomplishment of this goal. Our laboratory analytical and bioanalytical methods were transferred to WuXi and they became familiar with these methods. Analytical methods were developed and validated at WuXi to support nonclinical studies. Similarly, bioanalytical methods sufficient to detect A6-5188 and its metabolite A6-5171 in rat and monkey plasma were developed and validated at WuXi to support conduct of GLP-compliant nonclinical studies.

Several exploratory nonclinical studies were conducted at WuXi using A6-5188-diNa which Navigen provided early in this program, as described below.

In vitro plasma protein binding studies were conducted on both A6-5188 and A6-5171. The results demonstrate that both A6-5188 and A6-5171 are highly protein bound in plasma from mice, rats, monkeys, and humans, with no significant differences among species.

A pharmacokinetic (PK) study of A6-5188-diNa was conducted in monkeys. ACURO approval for this study was received on November 12, 2020. In brief, three male monkeys were administered a single 10 mg/kg IV bolus of A6-5188-diNa in 0.9% sodium chloride solution. Approximately 0.5 mL of blood was collected via the peripheral vein in K_2EDTA tubes prior to dosing (pre-dose, 0 h) and 0.083, 0.25, 0.5, 1, 2, 4, 8, 24 and 48 hours post-dose.

Blood samples were processed to plasma, and concentrations of A6-5188 and its metabolite, A6-5171, were determined using an LC-MS/MS method with a lower limit of quantitation (LLOQ) of 100 ng/mL. The pharmacokinetic parameters were determined by non-compartmental analysis using WinNonlin.

Table 1. PK Parameters of A6-5188 and the Metabolite A6-5171 in Male Monkeys After IV Dose Administration

Analyte	A6-5188 (Active)		A6-5171 (Metabolite)	
PK Parameters	**Mean**	**SD**	**Mean**	**SD**
C_0 or C_{max} (ng/mL)	344000	54600	3880	644
T_{max} (h)	--	--	0.500	0.00
$T_{1/2}$ (h)	7.74	0.745	1.63	0.701
Vd_{ss} (L/kg)	0.0874	0.00733	--	--
Cl (mL/min/kg)	0.366	0.0512	--	--
$AUC_{0\text{-}last}$ (ng•h/mL)	460000	69000	6690	1210
$AUC_{0\text{-}inf}$ (ng•h/mL)	462000	69400	7070	1180
AUC (M:P) Ratio [a]	--	--	0.0147	0.00322

a: AUC (M:P) Ratio = Metabolite $AUC_{0\text{-}last}$ / Parent $AUC_{0\text{-}last}$; PK = Pharmacokinetic; "--" means not calculated. All data reported with absolute three significant figures.

All monkeys tolerated the single 10 mg/kg IV bolus of A6-5188 and there were no abnormal clinical observations during the course of the study.

Following a single 10 mg/kg A6-5188 IV bolus, A6-5188 plasma clearance (Cl) was 0.366±0.0512 mL/min/kg and the terminal half-life ($T_{1/2}$) was 7.74±0.745 h. The volume of distribution (Vd_{ss}) was 0.0874±0.00733 L/kg and the exposure (expressed as the area under the plasma concentration-time curve from time zero to the last quantifiable concentration [$AUC_{0\text{-}last}$] and the initial plasma concentration [C_0]) was 460,000±69,000 ng·h/mL and 344,000±54,600 ng/mL, respectively.

Following a single 10 mg/kg A6-5188 IV bolus, A6-5171 exposure (expressed as $AUC_{0\text{-}last}$ and the maximal plasma concentration [C_{max}]) was 6,690±1,210 ng·h/mL and 3,880±644 ng/mL, respectively while the time to reach C_{max} was 0.500±0.00 h. The metabolite to parent ratio of systemic exposure ($AUC_{0\text{-}last}$) was 0.0147±0.00322.

WuXi conducted a 2-phase non-GLP toxicity study of A6-5188 in monkeys. ACURO approval was first received on January 6, 2021, with subsequent ACURO approval of an amended study protocol received on March 22, 2021. In the maximum tolerated dose (MTD) phase, two monkeys (1/sex) were used to determine the toxicity of A6-5188 when administered by IV bolus in a dose escalating manner. Four (4) single, escalating IV bolus doses of A6-5188 (10, 30, 60, and 200 mg/kg) were administered to the monkeys. No adverse events or changes in clinical pathology parameters were noted through the 60 mg/kg dose level. Death occurred quickly after dosing (within 35-45 mins) at 200 mg/kg. In the dose range finding (DRF) phase, monkeys were administered A6-5188 once daily by IV bolus for 7 days at 10, 30, and 60 mg/kg/day. There were no test article-related clinical signs noted at 10 mg/kg. The main clinical signs at 30 and 60 mg/kg/day were swelling in the leg in the dosing site. All monkeys survived to the scheduled necropsy with gross lesions observed at dosing sites (thick skin). There were no test article-related changes in body weight and food consumption in 7 days. An increase in alanine aminotransferase (ALT), aspartate aminotransferase (AST), and white blood cell (WBC) count was observed at

30 and 60 mg/kg/day after necropsy without any related gross lesion in liver. Based on these results, dose levels for a GLP-compliant 14-day repeat dose toxicity study were selected at 0, 2.5, 5, and 10 mg/kg/day. The final study report for this 2-phase toxicity study is in progress.

Pilot studies were conducted by WuXi to evaluate the potential for A6-5188 and A6-5171 to inhibit cardiac hERG channels. The objective of these studies was to evaluate the *in vitro* concentration-response relationship of the effects of A6-5188 and A6-5171 on electrical current passing through hERG (human ether-à-go-go-related gene) potassium channels (a surrogate for IKr, the rapidly activating, delayed rectifier cardiac potassium current) stably expressed in a Chinese Hamster Ovary (CHO) cell line tested using manual patch clamp assay at physiological temperature. A6-5188 and A6-5171 were tested at nominal concentrations of 0, 0.3, 1.0, 3.0, and 30 µM in 0.1% (v/v) DMSO in extracellular solution. The IC_{50} values for A6-5188 and A6-5171 were > 30 µM under the experimental conditions. A positive control, terfenadine, was used and inhibited current within the historical range, indicating the test system was responsive and the results were valid.

The following nonclinical studies (Table 2) are in progress or will be conducted soon. ACURO approval has been received for all *in vivo* studies. The goal is to have all studies completed and preliminary data available to support a clinical trial grant submission to NIH/NHLBI by the submission deadline of October 2, 2021 (PAR-21-118: NHLBI Early Phase Clinical Trials for Therapeutics and/or Diagnostics, R33 Clinical Trial Required). Final study reports will be available before the end of 2021 (Month 19) to support an IND filing date around the end of February 2022 (Month 21).

Table 2. Nonclinical Studies in Progress or Planned

Study Title	In-life Start Date	Audited Draft Study Report Available
A6-5188: *In Vitro* Hemolysis Study	06/11/2021	08/12/2021
Bacterial Reverse Mutation Assay (Ames) (GLP)	06/07/2021	08/06/2021
In Vitro Chromosome Aberration Assay in CHO-WBL Cells (GLP)	06/14/2021	08/07/2021
In Vivo Peripheral Blood Micronucleus Assay in the Rat (GLP)	06/03/2021	09/01/2021
hERG Assay in CHO-hERG Cells in Manual Patch Clamp Platform (GLP)	06/07/2021	08/13/2021
Rat Respiratory Safety Pharmacology Study (GLP)	06/08/2021	08/06/2021
A6-5188: 14-day Intravenous Dose Toxicity and Toxicokinetic Study in Rats with a 14-day Recovery Period (GLP)	06/03/2021	09/10/2021
A6-5188: 14-day Intravenous Dose Toxicity and Toxicokinetic Study in Cynomolgus Monkeys with a 14-day Recovery Period (GLP)	06/17/2021	09/30/2021

3.3 Aim 3: Manufacture of A6-5188 Drug Product

The stated milestone was the GMP manufacture of A6-5188 Drug Product (A6-5188 in solution in vials), including conduct of an ICH stability study, to support first-in-human clinical trials, with a completion date by the end of Month 34.

3.3.1 Aim 3 Major activities and accomplishments

Early progress against this goal has been described in the preformulation and formulation results presented in Section 3.1.1 above. We have decided to manufacture lyophilized vials of A6-5188-LP Drug Substance for use as clinical trial material (CTM) for completion of this goal rather than manufacture of A6-5188-LP Drug Product in solution in vials. These single-use vials containing, for example, 20 mg of lyophilized Drug Substance would be reconstituted using aseptic technique immediately prior to use at the clinical trial site pharmacy using the histidine buffer described previously. This approach would negate the need for a lengthy and costly ICH stability study on A6-5188-LP in solution.

3.4 Aim 4: File IND

The stated milestone for this Aim was to file an IND to support first-in-human clinical trials by the end of Month 36.

3.4.1 Aim 4 Major activities and accomplishments

We are contracting with RTI for completion of this Aim. RTI is providing expert consultants to Navigen in the fields of Chemistry, Manufacturing and Controls (CMC), nonclinical toxicology and pharmacokinetics, and Regulatory Affairs to assist in completing this Aim. RTI will serve as Regulatory Contact with the FDA, will compile and assemble the IND in the required electronic common technical document (e-CTD) format, and file the IND on Navigen's behalf. RTI will also be responsible for any subsequent activities required for IND maintenance, e.g, filing of IND Annual Reports.

RTI consultants participate in our regular teleconferences with STA and WuXi. Also, we hold biweekly calls with RTI. We are currently building the framework for the IND, e.g, the layout and formatting of each IND module, and drafting/reviewing/finalizing CMC and nonclinical study reports. Our goal is to file the IND by the end of February 2022, i.e., by the end of Month 21.

3.5 Opportunities for Training and Professional Development

Nothing to report.

3.6 Dissemination of Results

Nothing to report.

3.7 Future Plans

Work will continue against our Specific Aims during Budget Period 2. The following activities will be undertaken:

1. GMP manufacture of A6-5188-LP Drug Substance (1.5 kg scale) and initiation of a 36-month ICH stability study. Finalization of study reports, batch records, etc.
2. GMP manufacture of lyophilized vials of A6-5188-LP Drug Substance for use as CTM and initiation of a 36-month ICH stability study. Finalization of study reports, batch records, etc.
3. Completion of IND-enabling nonclinical studies and finalization of all nonclinical study reports.
4. Preparation, compilation, and filing of an IND in e-CTD format around the end of February 2022 (Month 21), including communication with the FDA to ensure approval of the IND.

As mentioned briefly earlier, we plan to submit an R33 grant application to NHLBI by the October 2, 2021 deadline. We will propose to conduct a 3-arm clinical trial in healthy volunteers: (1) a single ascending dose (SAD) study to evaluate safety, tolerability, and PK of A6-5188, (2) a 7-day repeat dose multiple ascending dose study to evaluate safety, tolerability, and PK of repeated daily doses of A6-5188, and (3) a proof-of-mechanism study in which lipopolysaccharide (LPS) is administered to healthy subjects to induce a mild, self-limiting, and reversible acute lung injury as a model for ARDS to evaluate a pharmacodynamic (PD) response to A6-5188. The earliest that this grant could fund is July 2022. Having an IND filed is not required for this grant application, but initiation of the clinical trial within the first quarter after funding is required. Our planned IND filing date around the end of February 2022 fits nicely into this overall timeline.

3.7.1 Timeline

A high level timeline for completion of the Aims of this current grant as well as our proposed R33 NHLBI grant is provided in Figure 1.

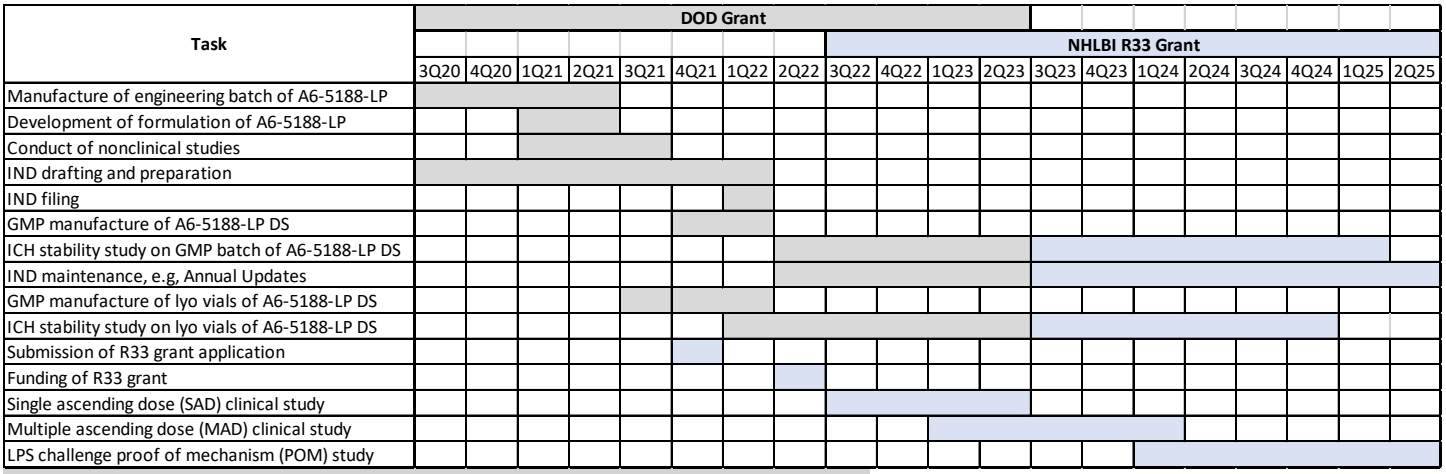

Figure 1. High level timeline for current DOD and future R33 grants.
Activities conducted under the current DOD grant and the future R33 grant are highlighted in gray and light blue, respectively. We will ensure that there is no overlap in activities between these two grants.

4 IMPACT

4.1 Overall impact

ARDS is a disease of high unmet medical need. Management of ARDS is challenging because care is limited to supportive measures, which are often inadequate. These measures consist of mechanical ventilation, fluid management, and, where possible, treatment of the underlying cause. There are no specific pharmacotherapies for prevention or treatment of ARDS. We are developing A6-5188, a first-in-class small-molecule ARF6 inhibitor

for the treatment and prevention of ARDS. Work conducted during this reporting period is critical to filing of an IND which will allow for the initiation of clinical trials on A6-5188.

4.2 Impact on other disciplines

Nothing to report.

4.3 Impact on technology transfer

Nothing to report.

4.4 Impact on society beyond science and technology

Nothing to report.

5 CHANGES/PROBLEMS

5.1 Major changes or changes in scope

No major changes or changes in scope have occurred. Minor changes have been discussed above and are summarized here.

1. We conducted salt and polymorph screening to identify A6-5188-LP as the most suitable Drug Substance.
2. A preformulation study led to the identification of an L-histidine buffer (pH 7.4) as the appropriate vehicle for preparation of A6-5188-LP in solution (Drug Product) for IV administration.
3. We manufactured an engineering batch of A6-5188-LP in sufficient quantity and purity to support conduct of IND-enabling nonclinical studies.
4. We elected to delay GMP manufacture of A6-5188-LP Drug Substance to delay the start of an ICH stability study and therefore prolong the length of time that this GMP batch would be available for human clinical trials.
5. We elected to manufacture lyophilized vials of A6-5188-LP Drug Substance as CTM for reconstitution at the clinical trial site immediately prior to use. This negates the need for long-term stability studies on A6-5188-LP in solution. This also simplifies shipment of CTM to the clinical trial sites.

5.2 Changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents

Nothing to report.

6 PRODUCTS

6.1 Publications, conference papers, and presentations

Nothing to report.

6.2 Websites of other internet sites

Nothing to report.

6.3 Technologies or techniques

Nothing to report.

6.4 Inventions, patent applications, and/or licenses

Nothing to report.

6.5 Other products

Nothing to report.

7 PARTICIPANTS

7.1 Individuals who have worked on the project

Table 3 lists the individuals who worked at least one person month per year on the project during the reporting period.

Table 3. Individuals contributing to the project

Name:	**Alan L. Mueller, PhD**
Project Role:	PI
Research Identifier:	Not applicable
Nearest person month worked:	6
Contribution to Project:	Dr. Mueller has overall responsibility for the project and manages the Contract Research Organizations (CROs) where work is conducted.
Funding Support:	Not applicable
Name:	**Jenny Lambson**
Project Role:	Project Manager/Grants Manager
Research Identifier:	Not applicable
Nearest person month worked:	9
Contribution to Project:	Ms Lambson is point of contact for the CROs for matters relating to the budget and invoicing. She also attends regularly scheduled teleconferences with the CROs, takes meeting minutes, and assigns action items.
Funding Support:	Not applicable

7.2 Changes in active other support of PI

Nothing to report.

7.3 Other organizations involved as partners

Nothing to report.

8 SPECIAL REPORTING REQUIREMENTS

8.1 Collaborative awards

Nothing to report.

8.2 Award chart

W81XWH2010239: Arf6 Inhibitor for Treatment of Acute Lung Injury/Acute Respiratory Distress Syndrome

PI: Alan Mueller, Navigen, Salt Lake City, Utah **Budget:** $3,266,291

Topic Area: Acute Lung Injury **Mechanism:** USAMRAA

Research Area(s): W81XWH **Award Status: Annual Report June 1, 2020 – May 31, 2021**

Study Goals: Advance nonclinical development of A6-5188 and file IND

Specific Aims: (1) GMP manufacture of A6-5188 Drug Substance, (2) Conduct GLP-compliant nonclinical toxicology and safety pharmacology studies to support IND filing, (3) GMP manufacture of A6-5188 Drug Product, (4) File IND.

Key Accomplishments and Outcomes: (1) Analytical methods were developed. Salt and polymorph screening were conducted to select A6-5188-LP (L-proline co-crystal) as desired Drug Substance (DS). A non-GMP batch of A6-5188 was manufactured (98.7% purity) to support the conduct of nonclinical trials. A preformulation study determined that a 20 mM histidine buffer was the appropriate vehicle. GMP manufacture of DS is forecast to begin late 4Q2021. (2) Several non-GLP nonclinical studies were conducted, e.g., a monkey pharmacokinetic (PK) study. IND-enabling GLP studies have begun or will begin soon, with draft audited study reports available late 3Q2021. (3) Based on results of preformulation and stability studies, A6-5188 Drug Product (DP) will be manufactured as a lyophilized powder in vials for reconstitution prior to use. (4) We plan to file the IND around the end of February 2022. Study reports are being drafted and finalized, and the outline of the IND has been generated for population as these reports become available.

Publications: none to date

Patents: none to date

Funding Obtained: none to date

Figure 2. Award Chart