PI: **Kaadige, Mohan Rao**	Title: Development of a potent and selective oral ENPP1 inhibitor for oncology	
Received: 04/05/2022	Opportunity: PA-21-259	Council: 08/2022
Competition ID: FORMS-G	FOA Title: PHS 2021-2 Omnibus Solicitation of the NIH, CDC and FDA for Small Business Innovation Research Grant Applications (Parent SBIR [R43/R44] Clinical Trial Not Allowed)	
1R44CA278144-01	Dual:	Accession Number: 4700831
IPF: 10050165	Organization: STINGRAY THERAPEUTICS, INC.	
Former Number:	Department:	
IRG/SRG: ZRG1 OTC1-T (10)B	AIDS: N	Expedited: N
Subtotal Direct Costs (excludes consortium F&A) Year 1: 977,757 Year 2: 682,961	Animals: Y Humans: N Clinical Trial: N Current HS Code: 10 HESC: N HFT: N	New Investigator: Early Stage Investigator:
Senior/Key Personnel:	*Organization:*	*Role Category:*
[redacted]	[redacted]	PD/PI
[redacted]	[redacted]	MPI

Always follow your funding opportunity's instructions for application format. Although this application demonstrates good grantsmanship, time has passed since the grantee applied. The sample may not reflect the latest format or rules. NCI SBIR posts new samples periodically: https://sbir.cancer.gov/small-business-funding/application-process

The text of the application is copyrighted. You may use it only for nonprofit educational purposes provided the document remains unchanged and the PI, the grantee organization, and NCI SBIR are credited.

Note on Section 508 conformance and accessibility: We have reformatted these samples to improve accessibility for people with disabilities and users of assistive technology. If you have trouble accessing the content, please contact the NCI SBIR Development Center at ncisbir@mail.nih.gov.

APPLICATION FOR FEDERAL ASSISTANCE
SF 424 (R&R)

3. DATE RECEIVED BY STATE	State Application Identifier

1. TYPE OF SUBMISSION*

❍ Pre-application ❍ Application ● Changed/Corrected Application

4.a. Federal Identifier

b. Agency Routing Number

2. DATE SUBMITTED 2022-04-05

Application Identifier

c. Previous Grants.gov Tracking Number GRANT13588688

5. APPLICANT INFORMATION **UEI*:** [redacted]

Legal Name*: Stingray Therapeutics, Inc.
Department:
Division:
Street1*: [redacted]
Street2:
City*: [redacted]
County:
State*: [redacted]
Province:
Country*: USA: UNITED STATES
ZIP / Postal Code*: [redacted]

Person to be contacted on matters involving this application
Prefix: r. First Name*: onathan Middle Name: Last Name*: Northrup Suffix:
Position/Title:
Street1*: [redacted]
Street2:
City*: [redacted]
County:
State*: [redacted]
Province:
Country*: USA: UNITED STATES
ZIP / Postal Code*: [redacted]
Phone Number*: [redacted] Fax Number: Email: [redacted]

6. EMPLOYER IDENTIFICATION NUMBER *(EIN) or (TIN)** [redacted]

7. TYPE OF APPLICANT* R: Small Business

Other (Specify):
Small Business Organization Type ❍ Women Owned ❍ Socially and Economically Disadvantaged

8. TYPE OF APPLICATION*

● New ❍ Resubmission
❍ Renewal ❍ Continuation ❍ Revision

If Revision, mark appropriate box(es).
❍ A. Increase Award ❍ B. Decrease Award ❍ C. Increase Duration
❍ D. Decrease Duration ❍ E. Other *(specify)* :

Is this application being submitted to other agencies?* ❍Yes ●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*
Development of a potent and selective oral ENPP1 inhibitor for oncology

12. PROPOSED PROJECT
Start Date* 09/01/2022
Ending Date* 08/31/2024

13. CONGRESSIONAL DISTRICTS OF APPLICANT
TX-013

SF 424 (R&R) APPLICATION FOR FEDERAL ASSISTANCE

14. PROJECT DIRECTOR/PRINCIPAL INVESTIGATOR CONTACT INFORMATION

Prefix: Dr. First Name*: [redacted] Middle Name: [redacted] Last Name*: [redacted] Suffix: [redacted]
Position/Title: [redacted]
Organization Name*: [redacted]
Department:
Division:
Street1*: [redacted]
Street2:
City*: [redacted]
County:
State*: [redacted]
Province:
Country*: USA: UNITED STATES
ZIP / Postal Code*: [redacted]
Phone Number*: [redacted] Fax Number: Email* [redacted]

15. ESTIMATED PROJECT FUNDING

a. Total Federal Funds Requested*	[redacted]
b. Total Non-Federal Funds*	$0.00
c. Total Federal & Non-Federal Funds*	[redacted]
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: r. First Name*: [redacted] Middle Name: Last Name*: [redacted] Suffix:
Position/Title*: Chief Executive Officer
Organization Name*: Stingray Therapeutics
Department:
Division:
Street1*: [redacted]
Street2:
City*: [redacted]
County:
State*: [redacted]
Province:
Country*: USA: UNITED STATES
ZIP / Postal Code*: [redacted]
Phone Number*: [redacted] Fax Number: Email* [redacted]

Signature of Authorized Representative*
Jonathan Northrup

Date Signed*
04/05/2022

20. PRE-APPLICATION File Name:

21. COVER LETTER ATTACHMENT File Name:

424 R&R and PHS-398 Specific
Table Of Contents

0MBNumber: 4040-0010
Expiration Date: 12/31/2022

Project/Performance Site Location(s)

Project/Performance Site Primary Location

O 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: Stingray Therapeutics Inc.
UEI: [redacted]
Street1•: [redacted]
Street2:
City•:
County: [redacted]
State•:
Province: USA: UNITED STATES
Country•: [redacted]
Zip / Postal Code•: [redacted]

Project/Performance Site Congressional Districr:

Project/Performance Site Location 1

O 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: Translational Genomics Research Institute
UEI: [redacted]
Street1•: [redacted]
Street2:
City•:
County: [redacted]
State•:
Province: USA: UNITED STATES
Country•: [redacted]
Zip / Postal Code•: [redacted]

Project/Performance Site Congressional Districr:

Project/Performance Site Location 2

O 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: Stingray Therapeutics, Inc.
UEI: [redacted]
Street1•: [redacted]
Street2: [redacted]
City•:
County: [redacted]
State•:
Province: USA: UNITED STATES
Country•: [redacted]
Zip/ Postal Code•: [redacted]
Project/Performance Site Congressional Districr:

Additional Location(s) File Name:

OMB Number: 4040-0010
Expiration Date: 12/31/2022

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 ❍ No

If YES, check appropriate exemption number: __ 1 __ 2 __ 3 __ 4 __ 5 __ 6 __ 7 __ 8

If 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 [redacted]

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 environmental assessment (EA) or environmental impact statement (EIS) been performed? ❍ Yes ❍ No

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*	SBIR_Abstract.pdf
8. Project Narrative*	SBIR_Project_Narrative.pdf
9. Bibliography & References Cited	SBIR_Bibliography_and_References_Cited.pdf
10.Facilities & Other Resources	SBIR_Facilities_and_Other_Resources.pdf
11.Equipment	SBIR_Equipment.pdf

Funding Opportunity Number: PA-21-259. Received Date: 2022-04-05T17:36:52.000-04:00

ABSTRACT

Compelling evidence suggests that careful and therapeutically relevant activation of the STING (STimulator of INterferon Genes) pathway is necessary to elicit potent anti-cancer innate immune responses. Ectonucleotide pyrophosphatase/phosphodiesterase 1 (ENPP1) is the STING pathway's only known direct negative regulator expressed in many tumor types,. and, when it is overexpressed, tumors show limited efficacy to front-line therapies. Such as, in triple-negative breast cancer (TNBC), high ENPP1 expression is associated with drug resistance and poor prognosis. If a safe and efficacious. ENPP1 inhibitor were available, it would have widespread utility for multiple cancer types and, if used in combination with other cancer therapies, may enhance their performance. Towards this end, we have developed an orall bioavailable otent small-molecule inhibitor of ENPP1 called SR-8541A. It inhibits hENPP1 activity with an nd demonstrates robust selectivity. We have established that it activates the STING pathway, promotes immune oell infiltration, and inhibits cancer spheroid growth. Furthermore, in syngeneic tumor mouse models, SR-8541A demonstrates a synergistic effect with radiation, and a preliminary study also shows synergy with checkpoint inhibitors. To date, we have completed preclinical development activities on SR-8541A that include API development and manufacturing, stability, pharmacokinetics, tolerability, and preliminary toxicology (mouse, rat, dog). The overall goal of thiis Direct to Phase II SBIR application is to complete non-GLP and GLP preclinical studies for our lead molecule SR-8541A with TNBC as our initial focus. In Aim 1, we will evaluate the efficacy of SR-8541A in combination with FDA-approved drug regimens (e.g., cisplatin, anti-mCTLA-4, anti-mPD-1, PARP inhibitor) in 4T-1 and EMT-6 breast cancer mouse models. In Aim 2, we will conduct IND enabling GLP toxicology study in dogs as the rat GLP study is complete. In Aim 3, we will develop and manufacture cGMP clinical-grade tablets necessary to conduct a Phase 1 clinical trial. Direct to Phase II SBIR success wm result in the completion of the requiired preclinical studies to see'k IND acceptance for a first-in-human Phase I clinical tri;al and to engage with private-sector investors in funding clinical trials in TNBC. If SR-8541A is approved for patient use, it would be the first-1in-class molecule to modulate the innate immune system, expanding the benefits of immunotherapy to more patients.

PROJECT NARRATIVE

Current immunotherapies activate only the adaptive immune system and prove ineffective when cancer silences the innate immune response, the other central arm of immunity. Stingray Therapeutics has developed a novel potent small-molecule inhibitor of ENPP1 (ectonucleotide pyrophosphatase/phosphodiesterase family member 1), a dual checkpoint regulator of innate and adaptive immune responses that alone or in combination with immune checkpoint inhibitors or standard of care treatments may produce superior outcomes in patients with high ENPP1-expressing tumors, such as triple-negative breast cancer. This Direct to Phase II SBIR application aims to complete non-GLP and GLP preclinical studies on our lead ENPP1 inhibitor SR-8541A necessary to seek IND acceptance for a first-in-human Phase I clinical trial.

Stingray Therapeutics believes in bringing the best people to the company wherever they might be located. As such, it has two office hubs, one in the Texas Medical Center Accelerator (TMCx) in Houston, Texas, and one in Dallas, Texas. The team is equipped with telecommunication software platforms such as Zoom and Teams, common shared cloud folders, and other software tools to allow collaboration, "whiteboards," and real-time editing and writing tools when traveling or at a distance. The team assembles virtually every week and periodically gets together in person to foster team building and provide personal connection among the group. With the lockdowns required during the last two years from the pandemic, it has been a strength for the company to be so accomplished at these types of communication.

In addition to offices, Stingray has also fostered a long-standing (since 2016) collaboration with Translational Genomics Research Institute (TGen), where it has been able to do sponsored research and work very closely with TGen and Dr. Sunil Sharma's lab. At TGen, we access the ability to do mouse efficacy models, immune-oncology *in vitro* testing, and where we have pioneered the human immune infiltration modeling, which has been so important to Stingray. This has been a great open collaboration where TGen has benefited from enhanced funding and several publications delivered at the American Academy of Cancer Research (AACR).

Early drug development requires many external connections with specialized providers. So many of the Investigational New Drug Application requirements must often be done by specialist firms in that ability who are set up to do the same kind of work over and over for many different biotechnology and pharmaceutical customers. Great examples are Charles River Laboratories, with deep expertise in cGLP toxicology who we are working with for cGLP toxicology in dog (Ashland, Ohio), and Catalent Oral Formulations group (San Diego), with similar deep expertise in all matters of oral formulations and the cGMP manufacture of clinical-grade tablets for Phase 1. It would be inefficient and expensive to set up this expertise for the occasion needs we would have to use it. Working like this, with our base team, critical specialized consultants to represent the company and review the work, and then great technical providers to do this work is the proven model for pulling together an IND for many biotech companies.

Brief Description of TGen

TGen, a formal affiliate of the City of Hope National Medical Center, is a non-profit biomedical research institution in Phoenix, Arizona. Its mission is to define 21st-century healthcare delivery with revolutionary advances in genomic-enabled medicine to prevent, diagnose, and treat diseases. TGen leverages best-in-class genomics, informatics, and high-throughput 'omics' expertise and technologies to effectively translate genomic discoveries into diagnostic tests and innovative therapies for cancer, neurological, and infectious diseases.

Environment

Faculty at TGen possess the high standards and rigor of a world-class academic research environment while afforded the flexibility of a small entrepreneurial company to translate discovery into clinical practice effectively. TGen's translational research enterprise provides an unparalleled environment for innovation, discovery, and creative collaboration with a global footprint of over 447 collaborations with academic, medical, and industry partners worldwide in 28 countries and U.S. territories. TGen has formal affiliations with three state universities, Arizona State University, Northern Arizona University, and the University of Arizona. Many TGen scientists hold university appointments at one of Arizona's three universities. Collaborations are bi-directional, with TGen currently providing appointments to 38 university faculty members.

The TGen facility houses approximately 310 scientists and staff with 20 independent investigators across 7 research divisions that occupy 100,000 sq ft of a centrally located research building, which is part of a 30-acre downtown Phoenix Biomedical Campus that incorporates buildings for translational and clinical research, health care education, and community critical care. TGen's research programs are supported by key core facilities and technology centers, which represent a concentration of resources and talent available to further the research of all TGen investigators and the wider scientific community. The TGen facility houses approximately 50,000 square

feet of wet lab space for DNA sequencing, the genetic basis of human disease, molecular diagnostics, target validation, neurogenomics, tissue microarray, and proteomics, including specialized infrastructure and facilities to support drug discovery and development. The laboratories at TGen are an open space design that fosters interaction and team-based science both within and across Divisions while also easily responsive to accommodate future needs.

Sharma Laboratory

[redacted] resides under the Division of Applied Cancer Research and Drug Discovery, of which [redacted] is the overall director. Division faculty deploy cutting-edge techniques in computer-aided drug design and systems biology to identify lead compounds along with proof-of-mechanism studies to rapidly advance compounds from discovery to clinical candidates. There are 14 scientists currently working within the division that are all well-versed in assay development, screening, biomarker identification, and *in vivo* models for preclinical efficacy and imaging studies. The lab occupies approximately 400 square feet of laboratory space that includes 4 full standard-sized bays with adjacent desk space. The lab is also outfitted with all the modern amenities for molecular, cellular, genomics, transcriptomics, and cell culture work. All research staff are fully trained to operate all equipment and perform all techniques proposed in this application.

Each lab member is equipped with personal computers and laptops, provided all applicable software tools, and full access to the high-performance computing facilities at TGen. All software licenses required to achieve the proposed research aims reside within the Sharma laboratory. [redacted] has personal office space that includes a desk, filing cabinets, a bookshelf, a small table for meetings, a dry/erase whiteboard, and full video-conferencing capabilities.

Other Shared Resources

IT Infrastructure

TGen's network infrastructure is comprised of Cisco and Dell Force10 10 Gigabit Ethernet core switches, with all servers being networked with either Gigabit Ethernet or 10 Gigabit Ethernet. There are redundant 10 Gigabit Ethernet connecting IDF closets in the main H.Q. building directly into the core network, providing high speed, low latency data transfers between scientific instruments and the data centers. All client drops are Gigabit Ethernet. TGen has implemented a Cisco wireless network utilizing 802.11a/b/g/n capable wireless access points, allowing for wireless transfer rates of up to 300Mbps. Wireless authentication is performed via Active Directory for internal users, and TGen makes a wireless network available for guest users at all TGen offices and laboratories.

File sharing is accomplished with a variety of servers providing access to data for collaborators and partners through ubiquitous protocols such as FTP, FTPS, HTTP, HTTPS, and SFTP. In addition, TGen has licensed an Aspera file transfer server utilizing the FASP technology to maximize throughput and utilize up to 800Mbps (megabits per second) to move large data sets as quickly as possible over the Internet.

TGen's Enterprise storage infrastructure includes several different technologies. There are two iSCSI-based Storage Area Networks (SAN), including 100 TB of Dell EqualLogic storage and 100 TB of Dell Powervault MD series. The other main SAN is based on an 8 Gbit fiber channel and includes 300 TB of Dell Compellent FluidData storage. This system is comprised of 200 GB SSDs, 300 GB 15,000 RPM drives, 600 GB 10,000 RPM drives, and 7,200 RPM 1TB drives. Data is automatically migrated between storage tiers as appropriate during processing.

Supporting TGen's Enterprise virtualization environment is a VMWare ESX cluster consisting of a production VMWare ESX environment running on 6 Dell M620 Blades. The M1000e Blade Center is directly connected to TGen's Core Networking environment utilizing multiple, redundant 10Gbe circuits to enable fast data transfer and high availability. This system has redundant fiber channel connectivity across multiple Brocade switches to the aforementioned Compellent SAN. This environment has been operated with as many as 200 virtual machinesat peak utilization.

For network perimeter and logical network security, TGen has deployed Palo Alto Networks Next-generation firewalls. In addition to providing stateful packet inspection, these firewalls are application-aware and capable of providing granular control of network traffic based on both the traditional Source / Destination pairs, as well as the application type and, in some cases, authenticated user. The Palo Alto Networks firewalls also provide URL filtering functionality and in-line evaluation of transferred files for malicious behavior. Features evaluated for deployment in Palo Alto include secure, VPN-free publication of web-based applications, third-party authentication capabilities, and risk-based demand for two-factor authentication.

TGen Datacenters are physically protected against power disruptions, fire, and unauthorized entry. Continuous power to TGen's data centers is ensured by using uninterruptible power supplies and backup generators. Fire suppression includes a combination of clean-agent fire suppression systems and dual-action, dry-pipe sprinkler systems. Card keys are required to access all TGen data center facilities, with some facilities also requiring biometric authentication and authorization. IR-enabled closed-circuit television cameras monitor individual areas with server equipment. The feeds from the CCTVs are recorded on DVR and reviewed periodically and as required.

CHARLES RIVER LABORATORIES

Charles River Laboratories is a well-known and long-standing company that performs many pharmaceutical and biotechnology services worldwide in preclinical and clinical laboratory services and gene and cell therapy services. In 1947 Henry Foster, a veterinarian, started in Boston, overlooking the Charles River; the company has become a leader in pharmacology and toxicology services.

Notably, Charles River launched the Humane Care Imperative in 2002, designed to raise awareness and train its employees on the importance of animal welfare. The same year, they were named "Company of the Year" by The Boston Globe. In 2008, Charles River signed a ten-year contract to partner with the National Cancer Institute and opened a facility in Frederick, Maryland. As Charles River Laboratories has grown, it has acquired several other toxicology vendors, such as WIL Research, Citoxlab, and ITR, and kept these sites as operating entities working together under the Charles River umbrella.

The Ashland, Ohio Charles River site has operated very well for many years and has a strong record of passing FDA inspections at the facility. We will be using this site for our "28-Day Oral Gavage Toxicity Study in Beagle Dogs followed by a 28-Day Recovery". The site is very capable of doing bioanalytical sample analysis, dose formulation preparation, analysis, and validation, appropriately storing test material, laboratory services, animal studies under current Good Laboratory Practices (cGLP), data protection, clinical pathology, toxicokinetic sample collection, and analysis, pharmacokinetics and pharmacodynamics, statistical analyses, terminal procedures required in a toxicology study such as macroscopic and microscopic examination, and histopathology. Also, appropriate animal housing, treatment, and examination meet all US FDA and accredited animal welfare guidelines for such studies.

Ashland is a large facility with all of the support labs and equipment needed for its purpose and all cGLP aspects appropriately monitored. This is one of the best toxicology facilities run by arguably the best toxicology company that could be available to Stingray Therapeutics.

CATALENT

Catalent San Diego is Catalent's West Coast Center of Excellence for Early Phase Development. Since 2007, it has been a 68,000 square foot facility focused on Phase 1 - 2 analytical and formulation development for small molecules. Services include pre-formulation testing, analytical and formulation development, and several oral dosage technologies.

The facility includes experts in specific equipment for oral manufacturing and development, dose form design, micronization, granulation, spray drying, small molecule analytical capabilities, overall development, and bioavailability. Clinical packaging, labeling, and worldwide distribution are also supported at the site.

The site makes numerous small molecule development efforts for pharmaceutical and biotechnology companies in the USA and Canada, has been successfully FDA inspected several times, and focuses on the pharmaceutics of early phase drug development.

Catalent is a global leader in enabling pharmaceutical and biotechnology companies to optimize product development from the early phase and onto the life cycle of pharmaceutical products. We have already worked with Catalent on some aspects of early pharmaceutical drug product development and found the organization responsive and first-class. We look forward to continuing our relationship with them.

EQUIPMENT

STINGRAY THERAPEUTICS

Dedicated Software
Computational and data analysis will be performed under the auspices of Stingray. Stingray has licensed software to perform these tasks including Mathematica; Macromedia Studio; Windows XP Professional Edition; Microsoft Office, Graphpad; Adobe Acrobat DC Writer, and SPSS statistics.

TRANSLATIONAL GENOMICS RESEARCH INSTITUTE (TGen)

General Molecular and Cellular Biology
The laboratory is equipped with modern molecular and cellular biology equipment and accompanying software that includes mini, medium-, large-, and ultra-centrifuges in addition to capillary electrophoresis, agarose gel running and documentation, PCR machines, a Viia7 RTPCR machine, a Perkin Elmer 96- and 384-well plate reader that reads luminescence, fluorescence, and absorbance, and an Eppendorf medium throughput liquid handler; histochem- and immuno-pathology that includes two epifluorescent microscopes and one confocal microscope; protein analysis that includes 6 protein gel apparatuses, an iBlot2 dry transfer system, a Li-Cor Odyssey CLx imaging system, and an MSD Multiplex Analysis system; and tissue culture facilities that includes 4 incubators, 4 biosafety cabinets, a Biotek Cytation5 and BioSpa8 live-cell imaging system, a Sony SH800 cell sorter, and a Macs Octo-Tissue Dissociator.

Synthetic Chemistry
All equipment and computer software for synthetic chemistry including mini, medium-, large-, and ultra-centrifuges, Combiflash chromatographic system, Biotage microwave reactor, rotary evaporator, 2 fume hoods, 2 weighing balances, 2 water bath sonicators, and a high vacuum pump with a sealed cryochamber for freeze drying. The team also has access to use the HPLC and LC-MS in the TGen Proteomics Center and the NMR facility at ASU.

Dedicated Software
(1) Open-source software: iterm2; Rstudio, Chrome

(2) Licensed software: IBM DB2 Universal Database v.8; IBM Java Virtual Machine; IBM Websphere Application, Graphpad Prism, Endnote, Microsoft Office Suite, Adobe Suite

CHARLES RIVER LABORATORIES

General: General equipment includes balances, pipettes, standard weights and animal weighing systems, High Performance Liquid Chromatography (HPLC) units, Tandem Mass Spectrometry (MS/MS), glassware, a Laboratory Information Management (LIMs) system, reagents, microscopes, surgical tools, refrigerators, and freezers, inspected and certified cGLP.

Software: Mathematica; Macromedia Studio; Windows XP Professional Edition; Microsoft Office; SAS; specialized cGLP recording and tracking systems.

CATALENT

Catalent San Diego has equipment to support the manufacture of solid oral dosage forms intended for the SR-8541A development program. The equipment anticipated to be used for manufacture, testing, and stability evaluation of SR8541A mini tablets includes the following:

Manufacturing Equipment
XPert Filtered Balance System, Mettler Top Loading Balance, Mettler Analytical Balance, Mettler Toledo Balance Printer, Nilfisk GM80 Vacuum, Portable; Portable Sentry Air; Flow meter, Disposable blow gun; Ezi-Flow plastic funnel; Patterson- Kelley V-blender with various shells; Gerteis Mini-Pactor; Quadro Comil 197S with round impeller and various screens; Stainless steel sieves of various sizes; Korsch XL100 Pro Tablet Press with gravity

feed frame, fill cam and various punches; Digital calipers; Hardness tester; Friability tester; Tablet and Capsule De-duster; Weight sorter; Metal detector; Arrow 1750 mixer or Lightnin' Labmaster mixer; Vector LDCS Hi-Coater with various perforated pan inserts, Induction sealer; Tablet counter

Analytical Equipment
Waters HPLC system with UV detector; Waters H-Class UPLC systems with UV detector; Orion Dual Star pH/ISE meter or Orion Versa Star Pro benchtop meter; Waters Empower™ Chromatography Data System software; Metrohm 851 Titrando Karl Fischer Coulometer; USP Dissolution Apparatus 2 (Paddles), X-ray powder diffractometer, Differential Scanning Calorimeter, Thermogravimetric Analyzer, Inductively Coupled Plasma Mass Spectrometer, or Inductively Coupled Plasma Atomic Emission Spectrometer.

Microbial Enumerations testing is outsourced by Catalent to SGS Lifesciences, Lincolnshire, IL and complies with the United State Pharmacopeia chapters <61> and <62>.

Stability Equipment
Validated and temperature-mapped stability chambers capable of storing products at 2-8°C, 25 ± 2°C/60 ± 5% relative humidity, 30 ± 2°C/65 ± 5% relative humidity, and 40 ± 2°C/75± 5% relative humidity. Stability testing will be performed with equipment listed in the Analytical Equipment section.

Software
Windows XP Professional Edition; Microsoft Office; SAS; specialized cGMP recording and tracking systems.

OMB Number: 4040-0001
Expiration Date: 12/31/2022

RESEARCH & RELATED Senior/Key Person Profile (Expanded)

PROFILE - Project Director/Principal Investigator

Prefix: Dr. First Name*: Mohan Middle Name Rao Last Name*: Kaadige Suffix: Ph.D

Position/Title*: [redacted]
Organization Name*: [redacted]
Department:
Division:
Street1*: [redacted]
Street2:
City*: [redacted]
County:
State*: [redacted]
Province:
Country*: USA: UNITED STATES
Zip / Postal Code*: [redacted]

Phone Number*: [redacted] Fax Number:

E-Mail*: [redacted]

Credential, e.g., agency login: [redacted]

Project Role*: PD/PI Other Project Role Category:

Degree Type: Ph.D. Degree Year: [redacted]

Attach Biographical Sketch*: File Name: [redacted]

Attach Current & Pending Support: File Name:

Funding Opportunity Number: PA-21-259 . Received Date: 2022-04-05T17:36:52.000-04:00

PROFILE - Senior/Key Person

Prefix: Dr. First Name*: Sunil Middle Name Last Name*: Sharma Suffix: M.D.

Position/Title*: [redacted]
Organization Name*:
Department:
Division:
Street1*:
Street2:
City*:
County:
State*:
Province:
Country*: USA: UNITED STATES
Zip / Postal Code*:

Phone Number* [redacted] Fax Number:

E-Mail*: [redacted]

Credential, e.g., agency login: [redacted]

Project Role*: PD/PI Other Project Role Category:

Degree Type: M.D. Degree Year: [redacted]

Attach Biographical Sketch*: File Name: Sharma_S_NIH_Biosketch_ENPP1.pdf

Attach Current & Pending Support: File Name:

Tracking Number: GRANT13588796 Funding Opportunity Number: PA-21-259 . Received Date: 2022-04-05T17:36:52.000-04:00

0MB No. 0925 0001 and 0925 0002 (Rev. 10/2021 Approved Through 09/30/2024)

BIOGRAPHICAL SKETCH

Prov de the fo ow ng nformat on for the Sen or/key personne and other s gn f cant contr butors.
Fo ow th s format for each person. **DO NOT EXCEED FIVE PAGES.**

NAME: Kaadige, Mohan

eRA COMMONS USER NAME (credential, e.g., agency login): mkaadige

POSITION TITLE: Associate Research Professor

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

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	Completion Date MM/YYYY	FIELD OF STUDY
Nizam's College, Osmania University, Hyderabad, India	B.S.	[redacted]	Microbiology, Botany, Chemistry
Madurai Kamaraj University, Madurai, India	M.S.	[redacted]	Biotechnology
Wayne State University, Detroit, MI, USA	Ph.D.	[redacted]	Gene regulation
Huntsman Cancer Institute, University of Utah, Salt Lake City, UT, USA	Postdoctoral	[redacted]	Cancer cell metabolism and signal transduction

A. Personal Statement

My qualifications to serve as the lead PI on this SBIR application stems from my decade long research career in the discovery and development of small molecule inhibitors for dysregulated proteins in oncology. I specialize in using computational drug design and synthetic medicinal chemistry to design better molecules with suitable physiochemical properties required for drug delivery and efficacy. I also have developed extensive skills in target selection and validation, development of screening assays for hit to lead discovery, lead optimization via structure activity relationship (SAR), *in vitro* ADME, pharmacokinetics and biomarker identification, and preclinical safety and efficacy studies. Between my appointments at Stingray Therapeutics and TGen, I have managed both small molecule and cell therapy development programs in oncology that target epigenetic, immune, and cell cycle pathways. Specific to this application, I am co-inventor of the proposed drug product SR- 8541A and serve as the Head of Biology at Stingray Therapeutics, where I oversee the ENPP1i drug development programs from hit to lead to preclinical development. I also have participated as co-investigator on federal-funded, multi-institutional grants, where I have attained successful administrative and management strategies to address collaborations that are both complex and diverse in nature. I will bring all of these experiences and resources to bear in support of this SBIR application.

Ongoing and recently completed projects that I would like to highlight include:

W81XWH1810617
Welm, Sharma
09/01/18 - 08/31/22
RON Kinase as a Multifaceted Therapeutic Target for Metastatic Breast Cancer

Citations:

1. **Kaadige MR**, Yang J, Wilde BR, Ayer DE (2015). MondoA:Mlx transcriptional activity is limited by mTOR-MondoA interaction. *Mol Cell Biol* 35(1):101-10 PMCID: PMC4295369.

2. Soldi R, Ghosh Halder T, Weston A, Thode T, Drenner K, Lewis R, **Kaadige MR**, Srivastava S, Daniel Ampanattu S, Rodriguez Del Villar R, Lang J, Vankayalapati H, Weissman B, Trent JM, Hendricks WPD, Sharma S (2020). The novel reversible LSD1 inhibitor SP-2577 promotes anti-tumor immunity in SWItch/Sucrose-NonFermentable (SWI/SNF) complex mutated ovarian cancer. *PLoS One* July 10;15(7):e0235705 PMCID: PMC7351179.
3. Weston A, Thode T, Rodriguez del Villar R, Dana S, Kasibhatla S, **Kaadige M**, Sharma S (2020). SR8541A is a potent inhibitor of ENPP1 and exhibits dendritic cell mediated antitumor activity. [Abstract]. Cancer Res Aug 2020, 80 (16 Supplement) LB-118; DOI: 10.1158/1538-7445.AM2020-LB-118.

Patent:

4. Vankayalapati H, Liu X, Ramamoorthy G, Sharma S, **Kaadige M**, Weston A, Thode T. (2020). Substituted-3H-imidazo [4,5-c] pyridine and 1H-pyrrolo[2,3-c]pyridine series of novel ectonucleotide pyrophosphatase/phosphodiesterase-1 (ENPP1) and stimulator for interferon genes (STING) modulators as cancer immunotherapeutics. WO EP US CN JP US10689376B2. Granted 06/23/2020.

B. Positions, Scientific Appointments, and Honors

Positions and Scientific Appointments

2020-	Head of Biology, Stingray Therapeutics, Inc., Dallas, TX
2017-	Associate Research Professor, Translational Genomics Research Institute, Phoenix, AZ
2016-2017	Research Associate, Clinical Trials Office and Center for Investigational Therapeutics, Huntsman Cancer Institute, University of Utah
2015-2016	Principal Scientist, Hughes Center for Research and Innovation, Nature's Sunshine Products, Salt Lake City, UT
2011-2015	Research Assistant Professor, Department of Oncological Sciences, Huntsman Cancer Institute, University of Utah

Honors

2009	Susan Cooper Jones Endowed Fellowship in Cancer Research, Huntsman Cancer Institute
2008	First Prize for Poster Presentation. FASEB Research Conference, Arizona
2002	Summer 2002 Dissertation Fellowship and a Travel Grant, Graduate School, Wayne State University
2001	Graduate Research Assistant Award, College of Science, Department of Biological Sciences, Wayne State University
1999	Thomas C. Rumble University Graduate Fellowship, Wayne State University
1997	Department of Biotechnology Scholarship, Ministry of Science and Technology, Government of India

C. Contributions to Science

I have built a comprehensive drug development program that integrates tools and technologies across the fields of computational drug design, synthetic medicinal chemistry, and biology in support of translating laboratory discoveries into patient care. My research lab works in collaboration with scientists and clinical investigators to facilitate the development of novel therapeutics to meet clinical unmet needs, with an emphasis in oncology. Currently, we have four active small-molecule drug development programs. First program is focused on targeting ENPP1, a protein involved in negative regulation of innate and adaptive immunity. IND-enabling studies are ongoing for the lead candidate SR-8541A. Second program is focused on CDK7, a kinase involved in both cell cycle and transcriptional regulation. We are initiating the IND-enabling studies for the lead candidate TGN-1062. Third program is focused on RON, a kinase involved in destruction of bone in breast cancer patients with bone metastasis. The lead candidate ZB-32 is currently undergoing *in vivo* testing in bone tumor models. The final program is focused on targeting KDM4A, an epigenetic regulator of histone modifications. This program is in the

hit to lead optimization stage. We also have ongoing collaborations to develop cell therapies and an organoid-based drug screen platform towards precision medicine. Much of my work has been published in peer-reviewed journals, presented at national scientific conferences, and to date, resulted in 12 patents. Below are relevant published works in this area.

a) Shi X, Hong T, Walter K L, Ewalt M, Michishita E, Hung T, Carney D, Pena P, Lan F, **Kaadige MR**, Lacoste N, Cayrou C, Davrazou F, Saha A, Cairns BR, Ayer D E, Kutateladze TG, Shi Y, Cote J, Chua KF, Gozani O (2006). ING2 PHD domain links histone H3 lysine 4 methylation to active gene repression. *Nature* 442**,** 96-99 PMCID: PMC3089773.

b) Parmenter TJ, Kleinschmidt M, Kinross KM, Bond ST, Li J, **Kaadige MR,** Rao A, Sheppard KE, Hugo W, Pupo GM, Pearson RB, McGee SL, Long GV, Scolyer RA, Rizos H, Lo RS, Cullinane C, Ayer DE, Ribas A, Johnstone RW, Hicks R, McArthur GA (2014). Response of BRAF-mutant melanoma to BRAF inhibition is mediated by a network of transcriptional regulators of glycolysis. *Cancer Discov* 4(4): 423-33 PMCID: PMC4110245.

c) Gupta S, Doyle K, Mosbruger T, Butterfield A, Weston A, Ast A, **Kaadige MR**, Verma A, Sharma S (2018). Reversible LSD1 inhibition with HCI-2509 induces the p53 gene expression signature in high-risk neuroblastoma cells. *Oncotarget* 9(11): 9907-9924 PMCID: PMC5839410.

d) Soldi R, Ghosh Halder T, Samson S, Vankayalapati H, Weston A, Thode T, Bhalla Md K, Ng S, Rodriguez Del Villar R, Drenner K, **Kaadige MR**, Horrigan SK, Batra S, Salgia R, Sharma S (2021). The small molecule BC-2059 inhibits Wnt-dependent gene transcription in cancer through disruption of the transducing beta-like 1 (TBL1)-β-catenin protein complex. *J Pharmacol Exp Ther*. Aug;378(2):77-86. PMID: 34006586.

Complete List of Published Work in MyBibliography:
https://www.ncbi.nlm.nih.gov/pubmed/?term=kaadige

OMB No. 0925-0001 and 0925-0002 (Rev. 12/2020 Approved Through 02/28/2023)

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: Sharma, Sunil

eRA COMMONS USER NAME (credential, e.g., agency login): SUNILSHARMA

POSITION TITLE: Physician-in-Chief

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 Delhi Hospital, New Delhi, India	AISSC	[redacted]	Biology
University of Delhi Hospital, New Delhi, India	Intern		Rotating Internship
University of Delhi, New Delhi, India	MD		Medicine
Post Graduate Institute of Medical Education and Research, Chandigarh, India	Junior Resident		Radiology
Michael Reese Hospital, Chicago, IL	Intern		Internal Medicine
Michael Reese Hospital, Chicago, IL	Resident		Internal Medicine
University of Texas Health Science Center, San Antonio, TX	Fellow		Medical Oncology
University of Massachusetts, Amherst, MA	MBA		Business Administration

A. Personal Statement

I am Physician-in-Chief, Professor, Deputy Director of Clinical Sciences, and Division Director of Applied Cancer Research and Drug Discovery at Translational Genomics (TGen). I am also the Chief of Translational Oncology Research and Drug Discovery and an Investigator at HonorHealth Research Institute. Additionally, I am Professor of Medicine at City of Hope Cancer Institute. I have extensive experience in drug development, including over 50 clinical trials. My research program focuses on the development of chemical inhibitors of identified targets, the preparation of investigation pharmaceuticals from promising chemical inhibitors, and then development of these drugs. The Sharma Lab recently developed potent activators of innate immunity (ENPP1 antagonists). I previously served as the Deputy Director of Huntsman Cancer Institute (HCI) to establish and implement strategic priorities. I was the Co-Leader of the Experimental Therapeutics Program to coordinate development of new therapeutics, including image-guided and targeted drug delivery systems. I was also the Director of the Center for Investigational Therapeutics, and an investigator at Huntsman Cancer Institute. For this grant, I will oversee the biomarker development in our discovery lab.

Ongoing and recently completed projects that I would like to highlight include:

W81XWH1810617
Sharma (Partnering PI)
[redacted]
RON Kinase as a Multifaceted Therapeutic Target for Metastatic Breast Cancer

Citations:

1. Shaw AT, Kim DW, Mehra R, Tan DS, Felip E, Chow LQ, Camidge DR, Vansteenkiste J, **Sharma S**, De Pas T, Riely GJ, Solomon BJ, Wolf J, Thomas M, Schuler M, Liu G, Santoro A, Lau YY, Goldwasser M, Boral AL, Engelman JA (2014). Ceritinib in ALK-rearranged non-small-cell lung cancer. N Engl J Med, 370(13), 1189-97 PMID: 24670165.
2. Weston A, Thode T, Rodriguez del Villar R, Dana S, Kasibhatla S, Kaadige M, **Sharma S** (2020). SR8541A is a potent inhibitor of ENPP1 and exhibits dendritic cell mediated antitumor activity. [Abstract]. Cancer Res Aug 2020, 80 (16 Supplement) LB-118; DOI: 10.1158/1538-7445.AM2020-LB-118.
3. Fiskus W, Mill C , Nabet B, Perera D, Birdwell C, Manshouri T, Lara B, Kadia T, DiNardo C, Takahashi K, Daver N, Bose P, Masarova L, Pemmaraju N, Kornblau S, Borthakur G, Montalban-bravo G, Garcia-Manero G, **Sharma S**, Stubbs M, Su X, Green M, Coarfa C, Verstovsek S, Khoury JD, Vakoc C. Superior efficacy of co-targeting GFI1/KDM1A and BRD4 against AML and post-MPN secondary AML cells. Blood Cancer J. 11, 98 (2021). https://doi.org/10.1038/s41408-021-00487-3.

Patent:

4. Vankayalapati H, Liu X, Ramamoorthy G, **Sharma S**, Kaadige M, Weston A, Thode T. (2020). Substituted-3H-imidazo [4,5-c] pyridine and 1H-pyrrolo[2,3-c]pyridine series of novel ectonucleotide pyrophosphatase/phosphodiesterase-1 (ENPP1) and stimulator for interferon genes (STING) modulators as cancer immunotherapeutics. WO EP US CN JP US10689376B2. Granted 06/23/2020.

B. Positions, Scientific Appointments, and Honors

Positions and Scientific Appointments

2020 - Present	Physician-In-Chief, Translational Genomics Research Institute, Phoenix, AZ
2018 - Present	California State License - Physician (MD)
2017 - Present	Arizona State License - Physician (MD)
2017 - Present	Adjunct Professor, Medicinal Chemistry, University of Utah, Salt Lake City, UT
2017 - Present	Deputy Director, Clinical Sciences, Translational Genomics, Phoenix, AZ
2017 - Present	Professor and Division Director, Applied Cancer Research and Drug Discovery, Translational Genomics, Phoenix, AZ
2017 - Present	Chief, Translational Oncology Research and Drug Discovery, HonorHealth Research Institute, Phoenix, AZ
2017 - Present	Professor, Department of Medical Oncology & Therapeutics Research, City of Hope National Medical Center, Duarte, CA
2017 - 2020	Founder, Proterus Therapeutics, Phoenix, AZ
2017 - 2017	Deputy Director, Huntsman Cancer Institute, Salt Lake City, UT
2016 - Present	Founder, Stingray Therapeutics, Dallas, TX
2015 - 2017	Leader, Experimental Therapeutics Cancer Center Program, Huntsman Cancer Institute, Salt Lake City, UT
2013 - 2017	Adjunct Professor, Department of Pharmaceutics and Pharmaceutical Chemistry, University of Utah, Salt Lake City, UT
2012 - 2017	Chief, Division of Medical Oncology, Huntsman Cancer Institute, Salt Lake City, UT
2011 - Present	Founder, Salarius Pharmaceuticals, Houston, TX
2009 - Present	Utah State License - Physician (MD)
2009 - 2017	Leader-Experimental Therapeutics Multidisciplinary Group, Huntsman Cancer Institute, Salt Lake City, UT
2009 - 2017	Jon and Karen Huntsman Presidential Professorship in Cancer Research Professor of Medicine, University of Utah, Salt Lake City, UT
2009 - 2017	Director, Center for Investigational Therapeutics, Division of Medical Oncology, Huntsman Cancer Institute, Salt Lake City, UT
2009 - 2017	Investigator, Huntsman Cancer Institute, University of Utah, Salt Lake City, UT

2009 - 2017	Senior Director, Clinical Research, Huntsman Cancer Institute, Salt Lake City, UT
2007 - Present	Fellow, American College of Physicians
2004 - Present	Nevada State License - Physician (MD)
2004 - 2008	Director, Clinical Trials Office, Nevada Cancer Institute, Las Vegas, NV
2004 - 2008	Associate Professor, University of Nevada School of Medicine, Las Vegas, NV
2004 - 2008	Director of Phase I programs, and Director of Gastrointestinal Oncology, Nevada Cancer Institute, Las Vegas, NV
1997 - Present	Member, American Association for Cancer Research
1995 - Present	Member, American Society of Clinical Oncology
1993 - 1995	Physician, Jackson County Rural Health Project, Scottsboro, AL

Honors

2019	Healthcare Leadership List 2019, Healthcare Researcher of the Year, AZ Business Magazine May-June 2019
2018	Health Care Power List 2018, Ranked #22, Phoenix Magazine April 2018
2007	Fellow of the American College of Physicians
2004	Annals of Oncology, Honorable Mention for Paper of the Year
1998	Pharmacia-Upjohn Award for exemplary research in oncology during fellowship
1988	Honors in Pathology, Pharmacology, Physiology and Gynecology, University of Delhi, India
1983	National Science Talent Scholarship

C. Contributions to Science

1. Early in my career I trained with Dr. Daniel Von Hoff who is a world renowned drug development expert in oncology and founder of ILEX Oncology and T-Gen. Subsequently, I also worked as a physician in the Division of Gastrointestinal Oncology at Memorial Sloan-Kettering Cancer Center, New York City.
   a. **Sharma S**, Raymond E, Soda H, Sun D, Hilsenbeck S.G., Sharma A, Izbicka E, Windle B, Von Hoff DD (1997). Preclinical and clinical strategies for development of telomerase and telomere inhibitors. Ann Oncol, 8, 1063-1067 PMID: 9426325.
   b. Soda H, Raymond E, **Sharma S**, Lawrence R, Cerna C, Gomez L, Schaub R, Von Hoff DD, Izbicka E (1999). Recombinant human interleukin-11 is unlikely to stimulate the growth of the most common solid tumors. Anticancer Drugs, 10, 97-101 PMID: 10194552.
   c. **Sharma S**, Kemeny N, Schwartz GK, Kelsen D, O'Reilly E, Ilson D, Coyle J, DeJager R, Ducharme M, Kleban S, Hollywood E, Saltz LB (2001). A phase I study of topoisomerase I inhibitor Exatecan Mesylate (DX-8951f) given as weekly 24-hour infusions every three of four weeks. Clin Cancer Res, 7(12), 3963-70 PMID: 11751488.
   d. Lobell RB, Liu D, Buser CA, Davide JP, DePuy E, Hamilton K, Koblan KS, Lee Y, Mosser S, Motzel SL, Abbruzzese JL, Fuchs CS, Rowinsky EK, Rubin EH, **Sharma S**, Deutsch PJ, Mazina KE, Morrison BW, Wildonger L, Yao SL, Kohl NE (2002). Preclinical and clinical pharmacodynamic assessment of L-778,123, a dual inhibitor of farnesyl:protein transferase and geranylgeranyl:protein transferase type-I. Mol Cancer Ther, 1(9), 747-58. PMID: 12479371.

2. Before joining HCI, I led a global oncology drug development program at the drug manufacturing company Novartis Pharmaceuticals. I then built a Phase I clinical trials program at the Nevada Cancer Institute, in Las Vegas, where I oversaw more than 25 clinical trials.
   a. Lee L, **Sharma S**, Morgan B, Allegrini P, Schnell C, Brueggen J, Cozens R, Horsfield M, Guenther C, Steward WP, Drevs J, Lebwohl D, Wood J, McSheehy PM (2006). Biomarkers for assessment of pharmacologic activity for a vascular endothelial growth factor (VEGF) receptor inhibitor, PTK787/ZK 222584 (PTK/ZK): translation of biological activity in a mouse melanoma metastasis model to phase I studies in patients with advanced colorectal cancer with liver metastases. Cancer Chemother Pharmacol, 57(6), 761-71 PMID: 16172907.
   b. **Sharma S**, Symanowski J, Wong B, Dino P, Manno P, Vogelzang N (2008). A Phase II Clinical Trial of Oral Valproic Acid in Patients with Castration-Resistant Prostate Cancers Using an Intensive Biomarker Sampling Strategy. Transl Onco 1(3), 141-7 PMCID: PMC2533142.

c. Symanowski J, Vogelzang N, Atadja P, Zawel L, Pass H, **Sharma S** (2009). A histone deacetylase inhibitor LBH589 downregulates X-linked inhibitor of apoptosis (XIAP) in mesothelioma cell lines which is likely responsible for increased apoptosis in combination with tumor necrosis factor alpha related apoptosis inducing ligand (TRAIL). J Thorac Oncol, 4(2), 149-60 PMID: 19179889.

3. I started a translational research laboratory effort at HCI, focused on drug discovery and clinical trials support. In this arena, I initially recruited scientists from biotechnology companies to help synthesize and focus on computational drug discovery efforts. The concept is to develop compounds against breakthrough targets that can move the cancer research field in collaboration with basic and physician scientists at the University of Utah and HCI.
   a. Sorna V, Theisen ER, Stephens B, Warner SL, Bearss DJ, Vankayalapati H, **Sharma S** (2013). High-throughput virtual screening identifies novel N'-(1-phenylethylidene)-benzohydrazides as potent, specific, and reversible LSD1 inhibitors. J Med Chem, 56(23), 9496-508. PMID: 24237195.
   b. Soldi R, Horrigan SK, Cholody MW, Padia J, Sorna V, Bearss J, Gilcrease G, Bhalla K, Verma A, Vankayalapati H, **Sharma S** (2015). Design, Synthesis, and Biological Evaluation of a Series of Anthracene-9,10-dione Dioxime beta-Catenin Pathway Inhibitors. J Med Chem, 58(15), 5854-62. PMID: 26182238.
   c. Fiskus W, **Sharma S**, Saha S, Shah B, Devaraj SG, Sun B, Horrigan S, Leveque C, Zu Y, Iyer S, Bhalla KN (2015). Pre-clinical efficacy of combined therapy with novel beta-catenin antagonist BC2059 and histone deacetylase inhibitor against AML cells. Leukemia, 29(6), 1267-78 PMCID: PMC4456205.
   d. Velinder M, Singer J, Bareyan D, Meznarich J, Tracy CM, Fulcher JM, McClellan D, Lucente H, Franklin S, **Sharma S**, Engel ME (2016). GFI1 functions in transcriptional control and cell fate determination require SNAG domain methylation to recruit LSD1. Biochem J. PMID: 27480105.

4. At Translational Genomics Research Institute (TGen), in the Applied Cancer Research and Drug Discovery Division, we are identifying clinical candidate compounds as possible cancer-fighting agents to be considered for Phase I (first-in-man) clinical trials. Our preclinical program features computational and medicinal chemistry; including computer-aided drug design; cancer biology; including assay development, screening, biomarker identification and in vivo models for preclinical efficacy and imaging studies. Our laboratory program has also developed immune organoid programs that can be used to develop novel vaccine therapies for cancer.
   a. Saenz D, Fiskus W, Mill CP, Perera D, Manshouri T, Lara BH, Karkhanis V, **Sharma S**, Horrigan SK, Bose P, Kadia TM, Masarova L, DiNardo CD, Borthakur G, Khoury J, Takahashi K, Bhaskara S, Lin CY, Green MR, Coarfa C, Crews CM, Verstovsek S, Bhalla KN. Mechanistic basis and efficacy of targeting β-catenin-TCF7L2-JMJD6-MYC axis to overcome resistance to BET inhibitors. Blood. 2020 Apr 9;135(15):1255-1269. doi: 10.1182/blood.2019002922.. PMID: 32068780.
   b. Griffiths JI, Wallet P, Pflieger LT, Stenehjem D, Liu X, Cosgrove PA, Leggett NA, McQuerry JA, Shrestha G, Rossetti M, Sunga G, Moos PJ, Adler FR, Chang JT, **Sharma S**, Bild AH. Circulating immune cell phenotype dynamics reflect the strength of tumor-immune cell interactions in patients during immunotherapy. Proc Natl Acad Sci U S A. Jul 2020, 117 (27) 16072-16082; DOI:10.1073/pnas.1918937117. PMID: 32571915.
   c. Soldi R, Ghosh Halder T, Weston A, Thode T, Drenner K, Lewis R,. Kaadige MR, Srivastava S, Daniel Ampanattu S, Rodriguez del Villar R, Lang J, Vankayalapati H, Weissman B, Trent JM, Hendricks WP, **Sharma S.** The novel reversible LSD1 inhibitor SP-2577 promotes anti-tumor immunity in SWItch/Sucrose-NonFermentable (SWI/SNF) complex mutated ovarian cancer. 2020 July PLoS ONE 15(7): e0235705. https://doi.org/10.1371/journal.pone.0235705. PMID:32842875.
   d. Lapidot M, Case AE, Weisberg EL, Meng C, Walker SR, Garg S, Ni W, Podar K, Hung YP, Carrasco RD, Knott A, Gokhale PC, **Sharma S**, Pozhitkov A, Kulkarni P, Frank DA, Salgia R, Griffin JD, Saladi SV, Bueno R, Sattler M. Essential role of the histone lysine demethylase KDM4A in the biology of malignant pleural mesothelioma (MPM). Br J Cancer. 2021 Aug;125(4):582-592. doi: 10.1038/s41416-021-01441-7. Epub 2021 Jun 4. PMID: 34088988; PMCID: PMC8368004.

Complete List of Published Work in MyBibliography:
https://www.ncbi.nlm.nih.gov/myncbi/1zUufao4qst5-/bibliography/public/

OMB Number: 4040-0001
Expiration Date: 12/31/2022

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

UEI*: [redacted]

Budget Type*: ● Project ○ Subaward/Consortium

Enter name of Organization: Stingray Therapeutics, Inc.

Start Date*: 09-01-2022 **End Date*:** 08-31-2023 **Budget Period: 1**

A. Senior/Key Person

Prefix	First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base Salary ($)	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits ($)*	Funds Requested ($)*
1 . Dr.	Mohan		Kaadige	Ph.D	PD/PI	[redacted]	[redacted]			[redacted]	[redacted]	[redacted]

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

Additional Senior Key Persons: File Name:

Total Senior/Key Person [redacted]

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						
1	Senior Manager of Biology	[redacted]			[redacted]	[redacted]	[redacted]
1	**Total Number Other Personnel**					**Total Other Personnel**	[redacted]
						Total Salary, Wages and Fringe Benefits (A+B)	[redacted]

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

Funding Opportunity Number: PA-21-259 . Received Date: 2022-04-05T17:36:52.000-04:00

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

UEI*: [redacted]

Budget Type*: ● Project ❍ Subaward/Consortium

Organization: Stingray Therapeutics, Inc.

Start Date*: 09-01-2022 **End Date*:** 08-31-2023 **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*: [redacted]

Budget Type*: ● Project ❍ Subaward/Consortium

Organization: Stingray Therapeutics, Inc.

Start Date*: 09-01-2022 **End Date*:** 08-31-2023 **Budget Period: 1**

F. Other Direct Costs	Funds Requested ($)*
1. Materials and Supplies	
2. Publication Costs	
3. Consultant Services	[redacted]
4. ADP/Computer Services	
5. Subawards/Consortium/Contractual Costs	[redacted]
6. Equipment or Facility Rental/User Fees	
7. Alterations and Renovations	
8. Dosage Toxicology Studies	[redacted]
9. Drug Product Effort	
Total Other Direct Costs	[redacted]

G. Direct Costs	Funds Requested ($)*
Total Direct Costs (A thru F)	[redacted]

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)*
1 . Modified Total Direct Cost	[redacted]	[redacted]	[redacted]
Total Indirect Costs			[redacted]

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)	[redacted]

J. Fee	Funds Requested ($)*
	0.00

K. Total Costs and Fee	Funds Requested ($)*
	[redacted]

L. Budget Justification*	File Name:
	Stingray_Therapeutics_Budget_Justification.pdf

RESEARCH & RELATED Budget {F-K} (Funds Requested)

OMB Number: 4040-0001
Expiration Date: 12/31/2022

RESEARCH & RELATED BUDGET - SECTION A & B, Budget Period 2

UEI* [redacted]

Budget Type*: ● Project ○ Subaward/Consortium

Enter name of Organization: Stingray Therapeutics, Inc.

Start Date*: 09-01-2023 **End Date*:** 08-31-2024 **Budget Period: 2**

A. Senior/Key Person

	Prefix	First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base Salary ($)	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits ($)*	Funds Requested ($)*
1.	[redacted]	[redacted]		[redacted]	[redacted]	[redacted]	[redacted]	[redacted]			[redacted]	[redacted]	[redacted]

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

Additional Senior Key Persons: File Name: **Total Senior/Key Person** [redacted]

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						
1	Senior Manager of Biology	[redacted]			[redacted]	[redacted]	[redacted]
1	**Total Number Other Personnel**					**Total Other Personnel**	[redacted]
						Total Salary, Wages and Fringe Benefits (A+B)	[redacted]

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

Funding Opportunity Number: PA-21-259 . Received Date: 2022-04-05T17:36:52.000-04:00

RESEARCH & RELATED BUDGET - SECTION C, D, & E, Budget Period 2

UEI*: [redacted]

Budget Type*: ● Project ❍ Subaward/Consortium

Organization: Stingray Therapeutics, Inc.

Start Date*: 09-01-2023 **End Date*:** 08-31-2024 **Budget Period: 2**

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 2

UEI*: [redacted]

Budget Type*: ● Project ❍ Subaward/Consortium

Organization: Stingray Therapeutics, Inc.

Start Date*: 09-01-2023 **End Date*:** 08-31-2024 **Budget Period: 2**

F. Other Direct Costs	Funds Requested ($)*
1. Materials and Supplies	
2. Publication Costs	
3. Consultant Services	[redacted]
4. ADP/Computer Services	
5. Subawards/Consortium/Contractual Costs	[redacted]
6. Equipment or Facility Rental/User Fees	
7. Alterations and Renovations	
8. Dosage Toxicology Studies	0.00
9. Drug Product Effort	[redacted]
Total Other Direct Costs	[redacted]

G. Direct Costs	Funds Requested ($)*
Total Direct Costs (A thru F)	[redacted]

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)*
1 . Modified Total Direct Cost	[redacted]	[redacted]	[redacted]
		Total Indirect Costs	[redacted]

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)	[redacted]

J. Fee	Funds Requested ($)*
	0.00

K. Total Costs and Fee	Funds Requested ($)*
	[redacted]

L. Budget Justification*	File Name:
	Stingray_Therapeutics_Budget_Justification.pdf

RESEARCH & RELATED Budget {F-K} (Funds Requested)

BUDGET JUSTIFICATION
STINGRAY THERAPEUTICS, INC.

PERSONNEL ( )

Mohan Kaadige, PhD., Principal Investigator (51% effort | 6.12 calendar months): primary appointment for the duration of this project will be as Head of Biology at Stingray Therapeutics, Inc. for over half of his annualized effort. He will dedicate 6.12 calendar months to the project in years one and two. is interested in developing targeted cancer therapies. His current research is focused on pre-clinical drug development. Prior to this, he had extensively worked in areas of gene regulation, cancer cell metabolism, and signal transduction pathways. In collaboration with will be responsible for design, execution, and overarching task-driven responsibilities of the project. He will lead and participate in recurring team meetings as well as dissemination of findings and computational analysis. Funding for salary and benefits commensurate with proposed effort devoted is requested.

Senior Manager of Biology (50% effort / 6.0 calendar months): will have a half-time appointment with Stingray Therapeutics for the duration of this project as Senior Manager of Biology She has seven years of experience in drug development and medicinal chemistry. Specifically, for this project, will be primarily responsible for analyzing data received from the studies performed at TGen. She may also assist with analysis of findings from the drug toxicology studies. Funding for salary and benefits commensurate with proposed effort devoted is requested.

FRINGE BENEFITS ( )

Fringe benefits are calculated at 27% for Dr. Kaadige and at 13.5% for . Stingray Therapeutics does not have a federally negotiated fringe benefit rate but the rates proposed are reasonable rates per the collaborating research institution.

TOTAL SALARY AND FRINGE requested for the proposed research project is **( ).**

Other Direct Costs ( )

Consultants ( )

Funds are requested to support consultants for: regulatory compliance; Chemistry, Manufacturing & Controls (i.e., CMC); toxicology; quality assistance & quality control; and pk/pd development. In keeping in tune with the NIH executive level II salary cap, funds are requested to support consultants for the first for each hour of support provided to Stingray Therapeutics for their assistance with the proposed studies. Other funds will be sourced by Stingray Therapeutics to finance hourly consultant rates over the an hour. In the first year of the project 790 in labor hours of consultant support is anticipated and in year two 1,320 labor hours of consultant support is anticipated. Calculations are as follows for the breakdown of the anticipated number of labor hours of support provided for the duration of the project:

Name, Classification of Support	Est. Hourly Rate	Est. Labor Hours	Total
		920	
		60	
		35	
		380	
		360	
		135	
		220	
Total			

Subaward Costs ( )

Translational Genomics Research Institute (TGen) subaward for total cost of direct costs and indirect costs). TGen will complete the immune marker analysis of tumor and blood specimens for aim one.

Drug Toxicology Studies ( )

Funds are requested for support of canine drug toxicology studies through a Contract Research Organization (i.e., CRO) who will perform the canine toxicology studies on a fee for service basis under the direction of Stingray Therapeutics staff in accordance with the S9 Nonclinical Evaluation for Anticancer Pharmaceuticals – Guidance for Industry (http://www.fda.gov/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/default.htm). With the recent shortage of approved canines the CRO, Charles River Laboratories Ashland LLC, is equipped to handle the proposed aim two dog toxicology studies which we anticipate will buttress the existing rat toxicology studies in the planned Investigational New Drug (IND) application for SR-8541A. Dosage level guidance will be provided by Stingray Therapeutics staff and findings from the proposed studies will be passed onto Stingray Therapeutics staff for evaluation and analysis. in funds are requested in year one in support of the proposed drug toxicology studies.

Drug Product Effort ( )

Funds are requested for partial support of cGMP drug product effort through the CRO Catalent Pharma Solutions who will assist with the oral formulation of SR-8541A for aim three on a fee for service basis under the direction of Stingray Therapeutics. Because cGMP develop and manufacture require very specialized personnel, facilities and equipment, it is typical industry practice for smaller companies to outsource these efforts. Other funds will be sourced by Stingray Therapeutics to cover additional costs which exceed the cost estimations within the proposed budget. in funds are requested in year one and in funds are requested in year two in support of the proposed drug product effort studies.

TOTAL DIRECT COSTS REQUESTED:

Stingray Therapeutics Fee ( )

No for-profit fee is requested for the work being done by Stingray Therapeutics. The reason for this is to allow for more operational support of the proposed work.

INDIRECT COSTS ( )

Facilities and Administrative (F&A) costs are calculated at 8% as a *de minimus* rate for operational expenses of Stingray Therapeutics Inc. Up to 40% can be requested based on NIH SBIR policy guidance, however a *de minimus rate* of 8% is requested to allot for more operational support of the proposed study.

RESEARCH & RELATED BUDGET - Cumulative Budget

	Totals ($)	
Section A, Senior/Key Person		[redacted]
Section B, Other Personnel		[redacted]
Total Number Other Personnel	2	
Total Salary, Wages and Fringe Benefits (A+B)		[redacted]
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		[redacted]
1. Materials and Supplies	0.00	
2. Publication Costs	0.00	
3. Consultant Services	[redacted]	
4. ADP/Computer Services	0.00	
5. Subawards/Consortium/Contractual Costs	[redacted]	
6. Equipment or Facility Rental/User Fees	0.00	
7. Alterations and Renovations	0.00	
8. Other 1	[redacted]	
9. Other 2	[redacted]	
10. Other 3	0.00	
11. Other 4	0.00	
12. Other 5	0.00	
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)		[redacted]
Section H, Indirect Costs		[redacted]

Section I, Total Direct and Indirect Costs (G + H)	[redacted]
Section J, Fee	0.00
Section K, Total Costs and Fee (I + J)	[redacted]

OMB Number: 4040-0001
Expiration Date: 12/31/2022

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

UEI*: [redacted]

Budget Type*: ❍ Project ● Subaward/Consortium

Enter name of Organization: Translational Genomics Research Institute (TGen)

Start Date*: 09-01-2022 **End Date*:** 08-31-2023 **Budget Period: 1**

A. Senior/Key Person

	Prefix	First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base Salary ($)	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits ($)*	Funds Requested ($)*
1 .	Dr.	Sunil		Sharma	MD	PD/PI	[redacted]	[redacted]			[redacted]	[redacted]	[redacted]

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

Additional Senior Key Persons: File Name:

Total Senior/Key Person [redacted]

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						
1	Research Scientist III	[redacted]			[redacted]	[redacted]	[redacted]
1	**Total Number Other Personnel**					**Total Other Personnel**	[redacted]
						Total Salary, Wages and Fringe Benefits (A+B)	[redacted]

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

Funding Opportunity Number: PA-21-259 . Received Date: 2022-04-05T17:36:52.000-04:00

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

UEI*: [redacted]

Budget Type*: ❍ Project ● Subaward/Consortium

Organization: Translational Genomics Research Institute (TGen)

Start Date*: 09-01-2022 **End Date*:** 08-31-2023 **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*: [redacted]

Budget Type*: ❍ Project ● Subaward/Consortium

Organization: Translational Genomics Research Institute (TGen)

Start Date*: 09-01-2022 **End Date*:** 08-31-2023 **Budget Period: 1**

F. Other Direct Costs	Funds Requested ($)*
1. Materials and Supplies	[redacted]
2. Publication Costs	
3. Consultant Services	
4. ADP/Computer Services	
5. Subawards/Consortium/Contractual Costs	
6. Equipment or Facility Rental/User Fees	
7. Alterations and Renovations	
Total Other Direct Costs	[redacted]

G. Direct Costs	Funds Requested ($)*
Total Direct Costs (A thru F)	[redacted]

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)*
1 . Modified Total Direct Cost	[redacted]	[redacted]	[redacted]
Total Indirect Costs			[redacted]

Cognizant Federal Agency [redacted]
(Agency Name, POC Name, and POC Phone Number)

I. Total Direct and Indirect Costs	Funds Requested ($)*
Total Direct and Indirect Institutional Costs (G + H)	[redacted]

J. Fee	Funds Requested ($)*

K. Total Costs and Fee	Funds Requested ($)*
	[redacted]

L. Budget Justification* File Name: TGen_Budget_Justification.pdf

RESEARCH & RELATED Budget {F-K} (Funds Requested)

Tracking Number: GRANT13588796 Funding Opportunity Number: PA-21-259 . Received Date: 2022-04-05T17:36:52.000-04:00

OMB Number: 4040-0001
Expiration Date: 12/31/2022

RESEARCH & RELATED BUDGET - SECTION A & B, Budget Period 2

UEI*: [redacted]

Budget Type*: ❍ Project ● Subaward/Consortium

Enter name of Organization: Translational Genomics Research Institute (TGen)

Start Date*: 09-01-2023 **End Date*:** 08-31-2024 **Budget Period: 2**

A. Senior/Key Person

Prefix	First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base Salary ($)	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits ($)*	Funds Requested ($)*
1 . Dr.	Sunil		Sharma	MD	PD/PI	[redacted]	[redacted]			[redacted]	[redacted]	[redacted]

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

Additional Senior Key Persons: File Name:

Total Senior/Key Person [redacted]

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						
1	Research Scientist III	[redacted]			[redacted]	[redacted]	[redacted]
1	**Total Number Other Personnel**					**Total Other Personnel**	[redacted]
						Total Salary, Wages and Fringe Benefits (A+B)	[redacted]

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

Funding Opportunity Number: PA-21-259 . Received Date: 2022-04-05T17:36:52.000-04:00

RESEARCH & RELATED BUDGET - SECTION C, D, & E, Budget Period 2

UEI*: [redacted]

Budget Type*: ❍ Project ● Subaward/Consortium

Organization: Translational Genomics Research Institute (TGen)

Start Date*: 09-01-2023 **End Date*:** 08-31-2024 **Budget Period: 2**

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 2

UEI*: [redacted]

Budget Type*: ❍ Project ● Subaward/Consortium

Organization: Translational Genomics Research Institute (TGen)

Start Date*: 09-01-2023 **End Date*:** 08-31-2024 **Budget Period: 2**

F. Other Direct Costs	Funds Requested ($)*
1. Materials and Supplies	[redacted]
2. Publication Costs	
3. Consultant Services	
4. ADP/Computer Services	
5. Subawards/Consortium/Contractual Costs	
6. Equipment or Facility Rental/User Fees	
7. Alterations and Renovations	
Total Other Direct Costs	[redacted]

G. Direct Costs	Funds Requested ($)*
Total Direct Costs (A thru F)	[redacted]

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)*
1 . Modified Total Direct Cost	[redacted]	[redacted]	[redacted]
		Total Indirect Costs	[redacted]

Cognizant Federal Agency [redacted]
(Agency Name, POC Name, and POC Phone Number)

I. Total Direct and Indirect Costs	Funds Requested ($)*
Total Direct and Indirect Institutional Costs (G + H)	[redacted]

J. Fee	Funds Requested ($)*

K. Total Costs and Fee	Funds Requested ($)*
	[redacted]

L. Budget Justification* File Name: TGen_Budget_Justification.pdf

RESEARCH & RELATED Budget {F-K} (Funds Requested)

Tracking Number: GRANT13588796 Funding Opportunity Number: PA-21-259 . Received Date: 2022-04-05T17:36:52.000-04:00

BUDGET JUSTIFICATION
TRANSLATIONAL GENOMICS RESEARCH INSTITUTE (TGen)

PERSONNEL ([redacted])

Sunil Sharma, M.D., Principal Investigator (5% effort | 0.60 calendar months): Dr. Sharma is the Deputy Director of Clinical Sciences as well as Professor and Division Director of Applied Cancer Research and Drug Discovery at TGen. His focus has been on the development of chemical inhibitors for identified targets and then advance these drugs for Phase I clinical trials. He has extensive experience in drug development, including over 20 clinical trials. Dr. Sharma will be responsible for design, execution, and overarching task-driven responsibilities of the project. He will lead and participate in recurring team meetings as well as dissemination of findings. Although salary support for Dr. Sharma has been requested for 3% calendar months Dr. Sharma is committing 5% effort to this project. Funding for salary and benefits commensurate with proposed effort devoted is requested.

TBN, Research Scientist (30% effort / 3.6 calendar months): A research scientist will work with Dr. Sharma for the *in vivo* efficacy testing. S/He will participate in recurring team meetings and will also assist in facilitating information to Stingray Therapeutics staff for data analysis. Funding for salary and benefits commensurate with proposed effort devoted is requested.

FRINGE BENEFITS ([redacted])
Fringe benefits are calculated at 18.6% in accordance with TGen's negotiated fringe benefit burden rate.

TOTAL SALARY AND FRINGE requested for the proposed research project is **([redacted]).**

SUPPLIES ([redacted])

Funds are requested to support supply costs for testing of the *in vivo* efficacy models and *ex vivo* analysis described in Aim 1. Calculations are as follows for the project:

Description	Cost	Number Needed	Total
SR-8541A + Checkpoint Abs *in vivo* efficacy model (4T-1)	[redacted]	1	[redacted]
SR-8541A + Chemo *in vivo* efficacy model (4T-1)	[redacted]	1	[redacted]
SR-8541A + Checkpoint Abs *in vivo* efficacy model (EMT-6)	[redacted]	1	[redacted]
SR-8541A + PARP Inhib *in vivo* efficacy model (EMT-6, BRCA1 KO)	[redacted]	1	[redacted]
Antibodies	[redacted]	10	[redacted]
Imaging Consumables	[redacted]	2	[redacted]
Adenosine Assays Kit	[redacted]	2	[redacted]
Adenosine Assays Consumables	[redacted]	2	[redacted]
MSD Plates	[redacted]	1	[redacted]
MSD Consumables	[redacted]	1	[redacted]
Total			

TOTAL DIRECT COSTS REQUESTED: [redacted]

INDIRECT COSTS ([redacted])
Facilities and Administrative (F&A) costs are calculated as per TGen's on site Federal negotiated rate of 92% on Modified Total Direct Costs.

RESEARCH & RELATED BUDGET - Cumulative Budget

	Totals ($)	
Section A, Senior/Key Person		[redacted]
Section B, Other Personnel		[redacted]
Total Nu8 mer Other Personnel	2	
Total Salary, b ages and Wringe BeneFits (Af B)		[redacted]
Section C, E+ui98 ent		. 0. .
Section D, Traqel		. 0. .
1. Domestic	. 0. .	
20Woreign	. 0. .	
Section E, Partici9ant/Trainee Su99ort Costs		. 0. .
10Tuition/Wees/Health Insurance	0.00	
20Sti9ends	. 0. .	
50Traqel	. 0. .	
40Sumsistence	. 0. .	
p0Other	. 0. .	
30Nu8 mer oFPartici9ants/Trainees	.	
Section W, Other Direct Costs		[redacted]
10v aterials and Su99lies	[redacted]	
20Pumlication Costs	. 0. .	
50Consultant Serqices	. 0. .	
40ADP/Co8 9uter Serqices	. 0. .	
p0Suma6 ards/Consortiu8 /Contractual Costs	. 0. .	
30E+ui98 ent or Wacility Rental/User Wees	. 0. .	
w0Alterations and Renoqations	. 0. .	
70Other 1	. 0. .	
M0Other 2	. 0. .	
1. 0Other 5	. 0. .	
110Other 4	. 0. .	
120Other p	. 0. .	
150Other 3	. 0. .	
140Other w	. 0. .	
1p0Other 7	. 0. .	
130Other M	. 0. .	
1w0Other 1.	. 0. .	
Section G, Direct Costs (A thru W)		[redacted]
		[redacted]

Section H, Indirect Costs

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Section I, Total Direct and Indirect Costs (G f H)	[redacted]
Section J, Wee	. 0. .
Section K, Total Costs and Wee (I f J)	[redacted]

Total Direct Costs less Consortium F&A

NIH policy (NOT-OD-05-004) allows applicants to exclude consortium/contractual F&A costs when determining if an application falls at or beneath any applicable direct cost limit. When a direct cost limit is specified in an FOA, the following table can be used to determine if your application falls within that limit.

Categories	Budget Period 1	Budget Period 2	Budget Period 3	Budget Period 4	Budget Period 5	TOTALS
Total Direct Costs less Consortium F&A	[redacted]	[redacted]	0	0	0	[redacted]

OMB Number: 4040-0001
Expiration date: 12/31/2022

SBIR/STTR Information

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

❍ DOE ● HHS ❍ USDA ❍ Other:

SBC Control ID:* 002195643

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: Therapeuti

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 ❍ No

1b. Anticipated Number of personnel to be employed at your organization at the time of award.* 7

1c. Is your small business majority owned by venture capital operating companies, hedge funds, or private equity firms?* ❍ Yes ● No

1d. Is your small business a Faculty or Student-Owned entity?* ❍ Yes ● No

2. 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 ● No

4. 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 ● No

7. 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_Commercialization_Plan.pdf

OMB Number: 4040-0001
Expiration date: 12/31/2022

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.*

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COMMERCIALIZATION PLAN

A. Value of the SBIR / STTR Project, Expected Outcomes, and Impact

Overview. Stingray Therapeutics, Inc. (ST) is a preclinical stage biotechnology company founded by [redacted] and Dr. Sunil Sharma in mid-2016. ST has pioneered inhibitors of a novel immune-oncology target in innate immunity, ectonucleotide pyrophosphatase/phosphodiesterase family member 1 (ENPP1).

Technology and Product Description. ST has developed three clinical candidate series of inhibitors against ENPP1, all chemically distinct, with [redacted] potency against the target and highly selective for human and mouse ENPP1. Multiple selectivity studies, cancer cell line panels, normal cells, 7-day tolerability in mouse, rat, and dog, 28-day toxicology in rat, and 28-day tolerability in dog, show no direct cytotoxic activity or harmful effect from ENPP1 inhibition across these time frames. [redacted]

As an immune-oncology agent, it must not have direct cytotoxic activity but work through the immune system to generate cancer-killing activity. Our ENPP1 inhibitor (ENPP1i) is highly potent, extremely selective for ENPP1, well-tolerated, and has suitable properties for an oral small molecule for patients. We think we have achieved these properties in compound SR-8541A. While we anticipate SR-8541A to perform as described, we recognize it is prudent to have other viable compounds as backup for high-risk early drug development. Our backup lead compounds include SR-8626 and the different chemical scaffold candidates SR-8542-3 and SR-8649, which we will take toward IND should anything adverse occur with SR-8541A.

Value of the SBIR Project. The last decade has shown a revolution in immune-oncology agents that have enhanced cancer treatments. Chimeric antigen T cell (CAR-T) therapies in hematologic cancers and immune checkpoint inhibitors (ICIs) in solid tumors (especially melanoma and lung cancer) have provided initial solid responses and extended patient lives for many years. However, response rates decline to less than 50% over several years with CAR-T therapies. With ICIs, resistance builds, and only 20% of patients are alive at the 5-10-year mark in melanoma, the cancer indication that shows the best response to date with these agents. We need to do more to help patients. CAR-Ts and ICIs activate only the adaptive immune system and are ineffective when cancer silences innate immunity.

Our clinical hypothesis is that activating the innate immune response, the other central arm of immunity, may strongly increase the breadth of response and durability with adaptive immune modulators in roughly half of all cancers where the cancer has compromised the innate immune system response. These two critical arms of immunity are highly synergistic, and by not modulating innate immunity, we often lose the benefit of this part of the immune system to the cancer's suppressive actions. Unfortunately, there is no suitable approved innate immune modulator to provide this needed functionality.

It has been shown that ENPP1 is the critical immune-suppressive molecule cancers use to suppress innate immunity and interferon production, rechanneling the pathway to produce adenosine, a very important broadly acting immunosuppressive and pro-metastatic molecule (1). Therefore, ENPP1i may be among the most exciting targets in innate immunity today. We expect an ENPP1i with an ICI regimen will achieve superior synergy by engaging both adaptive and innate immunity responses.

We believe adding an ENPP1i to ICI will extend the utility of ICI into many more cancers where they currently have low efficacy and overcome the ICI resistance that develops over time. There is a strong correlation between tumors with high ENPP1 expression and high resistance and low effectiveness for ICI. One such tumor that we plan as our first-in-man study is triple-negative breast cancer [TNBC - estrogen, progesterone, and HER2 (human epidermal growth factor receptor 2) negative].

Lead Product. Our lead product is SR-8541A. This small molecule exhibits [redacted] potency in enzymatic assays, is highly selective for human and mouse ENPP1, and is orally bioavailable. In addition, it has shown activity in *in vivo* as a single-agent and in combination mouse tumor models while also adverse event-free in tolerability and toxicology studies [redacted]

Current Stage of Development. We are completing *in vivo* mouse efficacy studies and need to do a final 28-day dog toxicology, a cardiovascular (C/V) safety pharmacology study in telemetry-implanted dogs, and manufacture cGMP drug product for Phase 1-2 clinical trials. We expect to file our IND in Y2 Q3 given the current delays in starting dog toxicology studies at the USA's major Contract Research Organizations (CROs). Therefore, we are applying for a Direct-to-Phase II SBIR award to fund several exploratory preclinical efficacy studies, perform remaining IND-enabling toxicology study per FDA requirements, assemble our IND application, and do the prework and contracting with the clinical sites for our Phase 1-2 clinical trials.

Expected Outcomes and Impact. We plan for SR-8541A to be given orally [redacted] We believe it will likely be valuable in the settings of high expression ENPP1 solid tumors and in combination initially with checkpoint inhibitors - for the purpose to extend the utility of checkpoint inhibitor therapy to tumor types that are currently unresponsive or to those tumors that develop drug resistance. **Figure 1** displays cancer types with high ENPP1 expression and a functioning innate immune system include **BRCA - breast cancer**, TGCT - tenosynovial giant cell tumor, SARC - sarcoma, PAAD - pancreatic adenocarcinoma, MESO - mesothelioma, READ - renal cell adenocarcinoma, STAD - stomach adenocarcinoma, COAD - colon adenocarcinoma, KIRC - kidney renal clear cell carcinoma, ESCA - esophageal carcinoma, OV - ovarian cancer, LUAD - lung adenocarcinoma cancer (1).

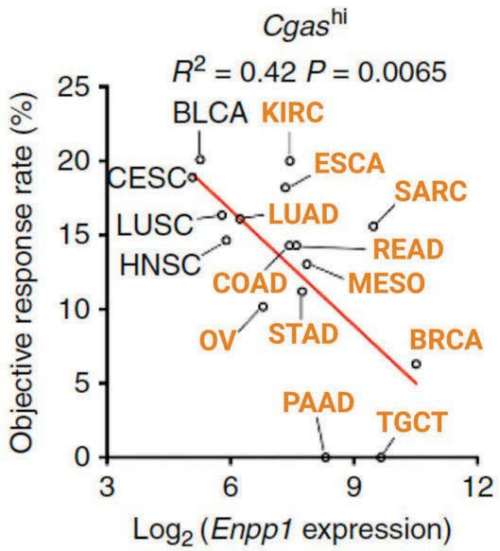

Figure 1. ORR to checkpo nt nh b tor n response to ENPP1 express on by cancer type for tumors w th h gh cGAS express on. Data reported n (1).

Table 1 shows important cancers in the USA. Breast Cancer, and within it, Triple Negative Breast Cancer (TNBC) are among the most important needs for new therapies. We plan to test our drug preclinically and initially clinically with the standard of care (SOC) and in combination with ICI for TNBC. As is typical in oncology, we would expect to start in the relapse/refractory setting and, as we show response and value, earn the right to move forward to the next step in earlier disease with the next set of trials. Should this study be successful, we believe we can then do a registration-directed study in TNBC. With additional Phase 2's and 3's, we would broaden the label to include other ENPP1 high cancers with the potential for an eventual label encompassing all ENPP1 high tumors.

Table 1. Important Cancers in the USA.

USA (000)	Breast	Lung	Prostate	Colon	Pancreas	Melanoma
A Cases	**3,676**	583	3 245	1 365	84	1 295
New Cases	**282**	236	249	150	60	106
Cancer Deaths		132	29	53	48	
5 year Sur9 9a	**0**	22	98	65	11	93

SOC for TNBC and Clinical Development. TNBC is not sensitive to endocrine or molecular-targeted therapies. SOC for TNBC consists of chemotherapy. Regimens include the PARP (poly-ADP ribose polymerase) inhibitors olaparib (Lynparza) and talazoparib (Talzenna) for germline breast cancer 1/2 gene (BRCA1/2) mutation-associated breast cancer; and ICI and nab-paclitaxel for PD-L1 (programmed cell death ligand 1) advanced TNBC. In PD-L1-positive patients, ICI may also be used in combination with chemotherapy (2). Once patients progress, sacituzumab (Trodelvy, Trop-2-directed antibody conjugated to topoisomerase inhibitor) has been approved and used in patients with relapsed/refractory TNBC. We believe ENPP1i plus sacituzumab with ICI may be a beneficial treatment that will outperform SOC alone.

The initial study would be a Phase 1-2 dose-escalation POC (Proof of Concept) study to evaluate safety and establish dose and evaluate efficacy in the expansion part of the study. Patients that have progressed on

chemotherapy with or without ICI would be enrolled and treated with SR-8541A, sacituzumab, and ICI. Phase 1 would enroll approximately 9-18 patients, and the Phase 2 expansion would enroll approximately 20 patients at the RP2D (Recommended Phase 2 Dose) of SR-8541A. In Phase 2, only patients overexpressing ENPP1 would be eligible for the study.

If sufficient activity is established in Phase 2, then the Phase 3 study would be a randomized, pivotal registration study vs. SOC. We would have an EOP2 (End of Phase 2) meeting with the FDA (Food and Drug Administration) to discuss our data and plans for Phase 3.

Potential Societal, Educational, and Scientific Benefits. Cancer is clearly of enormous societal impact, both in early death and expensive morbidity. And advanced TNBC is one of the most difficult to treat and with poor outcomes (**Table 2**). It is a particularly aggressive form of breast cancer, exhibiting drug resistance, progression, and a poor prognosis. From a health equity perspective, black women have the highest rate of new cases of TNBC, and progress with this disease would be beneficial to this minority community.

Table 2. Subtypes of Breast Cancer

Subtype	HR+/HER2- "Luminal A"	HR-/HER2- "TNBC"	HR+/HER2+ "Luminal B"	HR-/HER2+ "HER2 Enriched"	Unknown
Age-adjusted rate of new cancers per 100 000	88.1	**13.1**	13.4	5.5	8.8
Percent of cases	68%	**10%**	10%	4%	7%
5-year re at ve surv va	94.3%	**76.9%**	90.5%	84.0%	76.1%

HR, hormone receptor; HER2, human ep derma growth factor receptor 2
Data from NCI SEER, https://seer.cancer.gov

While immune oncology is only one of our mainstays to fight cancer, almost half of all cancers do not respond well to current immune agents. As a result, we are left with only chemotherapy and targeted therapies to fight the disease in these tumors systemically. Enabling immune oncology to work in far more tumors, starting with the very deadly disease of TNBC, would start us on the path of providing broad and dramatic benefit to the human situation in the fight against cancer.

SBIR project integration with the overall business plan of the company. ST has spent [redacted] and six years to develop potent and selective oral ENPP1i's. We wish to complete preclinical testing and regulatory requirements for SR-8541A, file and have accepted our Investigational New Drug (IND) application, and contract with our Phase 1-2 clinical trial sites. This is both the primary focus of the company and the subject of this Direct-to-Phase II SBIR application. If successful in this phase of our journey, we will go on to do the Phase 1-2 clinical trial and see the objective proof of our hypothesis.

B. Company

Company objectives and team competencies, experience, and history. ST is a preclinical immune-oncology cancer biotech working on ENPP1 inhibition since 2016. We were the first company to choose this target and have been steadily advancing our technology during this time, doing our own chemistry and biology. We are interested in all technologies and approaches toward ENPP1i, including novel small molecules and monoclonal antibody approaches. Our lead program is SR-8541A, which will be first into the clinic among our staple of molecules. As a small biotech, we have focused our efforts on everything ENPP1, especially related to immune-oncology treatment.

We have no revenue and raise funds to support our programs. We have raised a [redacted] Seed Series and a [redacted] M Convertible Note into our Series A, which we are raising now as a [redacted] Preferred Security at a [redacted] pre-money. This is our first SBIR or any NIH application for funding, and we do not have any other grant funding history. We have raised private equity to support our efforts to move as fast as possible during the previous years

in the ever more recognized field of ENPP1i as a key new immune-oncology technology. We have received investment from angel investors and high net worth individuals as small as [redacted] and from regional venture capital and venture philanthropy in amounts as great as [redacted] We are confident that we can raise our [redacted] Series A that just started early this year.

We have a strong team in the company and are supported by excellent specialty consultants. We specialize in preclinical to Phase 2 clinical studies and then plan to partner our programs with a larger biotech or pharma capable of assisting in the management of Phase 3 through the marketing of the products and building out the therapeutics for additional cancer indications. We believe this specialization fits our team and fits larger pharmaceutical companies built for many clinical trials at once on a promising therapeutic and the ensuing worldwide marketing effort. In addition, because of the breadth of ENPP1i utility, this model especially fits this type of therapy that could literally support hundreds of clinical trials in parallel should it be successful.

Team competencies, experience, and history:

[redacted]**, Cofounder and CEO.** [redacted] is the CEO and Chair of Stingray therapeutics and will coordinate CMC and toxicology activities with the help of consultant drug product and toxicology specialists. With Iterion therapeutics (previously Beta Cat Pharmaceuticals), [redacted] was Cofounder and CEO and oversaw all of Iterion's in vivo animal studies, pharmacology and toxicology work, drug substance and drug product formulation efforts, IND assembly, and Iterion's accepted IND, start of clinical studies, and raised [redacted] in financing and [redacted] in award funding. [redacted] continued as a Board Member through 2020. In Salarius Pharmaceuticals, [redacted] was also co-founder and CEO until 2015, overseeing in vivo studies, API manufacture, formulation efforts, and raising many millions for this company. [redacted] continued as Executive Chair of Salarius Pharmaceuticals until 2020. In 2020, [redacted] left both positions to concentrate on Stingray. [redacted] was COO of Jubilant Innovation, the venture arm of Jubilant Life Sciences, a large Indian contract research operation, where he oversaw the development of several nonclinical and clinical programs. [redacted] was 28 years at Eli Lilly and company, ascending through marketing and sales management positions and retiring as SVP of Corporate Business Development. At Lilly, [redacted] did 40 deals for Lilly during his BD career and, in sales and marketing launched five new Lilly products into the marketplace in the USA. [redacted] is a published author, contributing the foundational section to "The Business of Healthcare Innovation," 2012 and 2005, Cambridge University Press.

[redacted]**, M.D., FACP, Chief Medical Officer, Founder, SAB Chair.** [redacted] is Co-founder, Board Member and Chief Medical and Scientific Officer, Stingray Therapeutics. He is also Founder and SAB Chair for Iterion Therapeutics; Founder, former Board Member, and SAB Chair, Salarius Pharmaceuticals; Deputy Director and Chief Physician, Translational Genomics Research Institute (TGen), Honor Health, City of Hope; previous Deputy Director of Huntsman Cancer Institute, University of Utah; previous head, G/I cancer program, Nevada Cancer Institute; previous VP, early development, Novartis and has a strong interest in cancer drug discovery where he has discovered and developed multiple drugs with several in clinical studies today. [redacted] has been the principal investigator in over 150 oncology Phase 1-2 clinical trials. [redacted] has received 12 grants from NCI and various companies and foundations.

[redacted]**, Ph.D., Head of Biology.** [redacted] has been working with Stingray Therapeutics under an independent contractor agreement since 2020. As the Head of Biology, he has played a key role from hit identification to the development of SR-8541A. He is a co-inventor on all 7 patents filed by Stingray Therapeutics. [redacted] also works as an Associate Research Professor at the Translational Genomics Research Institute and has been a Research Assistant Professor at the Huntsman Cancer Institute and received his Ph.D. at the Wayne State University. [redacted] is an expert in cancer biology and cancer biology related to therapeutics.

[redacted]**, Chief Business Officer.** [redacted] is currently CBO of Stingray Therapeutics. He has been CFO of Iterion Therapeutics, the CFO and head of Investor relations of Salarius Pharmaceuticals. As CFO of Salarius, he is the primary architect of Salarius' current reverse merger with Flex Pharma to go to the NASDAQ [redacted] is knowledgeable about many aspects of the biotech business, was an investment banker with Healthios Capital, and has a proven ability to raise money in the angel and high net worth area and with venture and on the public markets. [redacted] also worked at Neopharm and Abbott.

[redacted], **VP Development.** [redacted], PharmD, MBA, is the Chief Development Officer for Stingray. Before Stingray, [redacted] was the CDO for WindMIL Therapeutics and had over 20 years of pharmaceutical and biotechnology industry experience in oncology drug development. Prior to WindMIL, [redacted] was the Chief Operating Officer of IRX Therapeutics /Brooklyn ImmunoTherapeutics. Prior to that, he was the Medical Affairs Lead for Immuno-Oncology at Bristol Myers Squibb. In addition, [redacted] was Head of Clinical Operations and Development at Ventrus Biosciences prior to its merger with Assembly Biosciences. Prior to Ventrus, [redacted] led the solid tumor development programs and led the clinical portfolio and strategic planning function at Celgene Corporation. In addition, he led the Oncology Development and Operations activities at Fibrogen and Novacea. [redacted] began his career at Novartis in the Oncology Early Development Group leading clinical trials prior to joining the Medical Sciences Group at Amgen. [redacted] received his BS and PharmD degrees from Rutgers University in New Jersey and his MBA from the Florida Institute of Technology.

[redacted], **CPA, Chief Financial Officer.** [redacted] has been CFO and President for Magnolia Tejas, a venture-backed biotech developing treatments for neuropathies; she has been Controller for Iterion therapeutics and Controller and VP of Salarius Pharmaceuticals. She has deep experience with domestic and international accounting and has been invited and spoken on appropriate accounting procedures at several meetings.

[redacted], **MA.** [redacted] has been Senior Manager of Biology at Stingray Therapeutics, Lab Manager and Research Associate at Translational Genomics Research Institute, and Lab Manager at the Huntsman Cancer Institute.

Consultants (see Letters of Support):

[redacted], Senior pharmaceutical R&D and regulatory executive with over 25 years of industry experience across major global markets. Highly experienced in determining and executing efficient and cost-effective global development programs, including successful leadership in the preparation and conduct of Agency meetings (PIND and equivalent, EOP1, EOP2, and PNDA/MAA and regional equivalents), obtaining agreement on data requirements for introducing pharmaceutical products to the clinic, progressing through development, and preparation/submission of market applications. Regulatory strategy development includes obtaining orphan drug status, Fast Track status (and global equivalents) and accelerated approval for orphan indications and/or breakthrough therapies. Successful leadership in executing development programs through preclinical development to market approvals and supporting commercial products (i.e., post-approval studies to support label changes, promotional materials, and CMC changes). Has Led technical due diligence in both in-licensing and out-licensing business development, including M&A. Served as the primary lead in technical due diligence on behalf of financial institutions (private equity, venture capital, investment banking).

[redacted] is co-founder and CEO of a contract research laboratory and consulting group specializing in customized cardiovascular preclinical drug discovery and safety studies. He has provided these services for over 500 international biopharmaceutical clients and is an expert in cardiovascular models and regulatory issues. Because the family of phosphodiesterases (but not ENPP1) can have cardiovascular effects and because the oncology FDA group is more and more asking for cardiovascular safety studies beyond traditional hERG testing, we have engaged Mike as part of our team.

[redacted] is also a co-founder of COR Solutions and a physician, and his specialty is the regulatory and clinical aspects of cardiovascular effects and adverse effects, whereas [redacted] is a preclinical and regulatory expert.

[redacted] is a Chemistry, Manufacturing, and Controls expert specializing in developing small molecule new chemical entities and CMC due diligence review. [redacted] is an expert in analytical method development/validation and transfers, stability programs and data assessment, chemical process development, API manufacturing, formulation development, drug product process development and validation, clinical supplies manufacturing, and preparation of CMC-related regulatory documentation (IND/CTD). He has held senior positions of responsibility in his field at Pfizer and MGI Pharma and has consulted for 15 years.

[redacted] has worked full-time as a consultant for twenty-one years. Before that, she had 19 years in the pharmaceutical and food ingredient industries. [redacted] specializes in preclinical toxicology, product safety assessment, and GLP consulting and training. Clients range from other consultants and start-up companies to universities and Fortune 500 corporations. Clients encompass pharmaceutical, biologic, biotechnology, gene therapy, and other industries. She is experienced with small and large molecules, nanoparticles, nanomaterials, and botanicals and has performed safety/risk assessments for main products, excipients, contaminants, degradants, and metabolites. The majority of my efforts support clients in getting FDA regulatory approval for their products (IND, BLA, NDA, FAP, GRAS, FCN, etc.).

[redacted] Quality Assurance professional with 12+ years of experience in the FDA and DEA regulated pharmaceutical field. Keen interest in supporting Early Phase pharmaceuticals throughout the drug development process resulting in FIH clinical trials. Enjoy working with CMC and CMO teams in ensuring phase-appropriate, quality operations that span from starting materials to the drug product and successful IND filings. Proven experience in taking drugs from development through validation, PAI, and commercialization. My expertise encompasses cGMP auditing, domestic and international, of API & DP manufacturers, contract packagers, and contract laboratories in the small molecule, solid dosage space.

[redacted] Broad drug developer and PK/PD expert of large and small molecules, immuno-oncology molecules, and ADC molecules with a focus on Clinical Pharmacology, preclinical pharmacology study design, preclinical to clinical translation, exploratory exposure-response in Phase I / II studies to evaluate dosing regimens (improving safety profiles while maintaining efficacy) and first in human dose selection and beyond. Expertise in pharmacology, pharmacotherapy, pharmacokinetics, and pharmacodynamics focused on developing new therapeutics in Oncology.

C. Market, Customer, and Competition

Market size. This early in development, it is more challenging to access a market size and potential sales that do not have an enormous error bar. However, these markets include the very largest in oncology. Success in one or two markets would open multi-billion potential to the drug, and success in several could allow the drug to cross above the $[redacted] mark eventually. In addition, the potential to expand the utility of checkpoint inhibitors, radiation, PARP inhibitors, and targeted therapy suggests that many large pharmaceutical companies may be interested in augmenting their current therapies. We already see this interest in the preclinical setting, and with human proof of concept, the interest would only expand.

For example, checkpoint inhibitors are forecast to be a $[redacted] worldwide sales market this decade (3). At a similar price point, about $[redacted] per year, and assuming we could be used in combination beneficially half the time, that would be a $[redacted] worldwide market for our inhibitor.

Further, success with our drug is based on the combination settings where it would add value; it is not based on just a small orphan indication and a very high price. As a result, we have a better opportunity to withstand possible future adverse pricing events in the pharmaceutical industry both in the USA and worldwide.

Pharmaceuticals typically start in one smaller market for their initial indication and expand through additional clinical trials and marketing authorizations. In our case, this would be TNBC as the initial indication, although we would hope the eventual indication might be all high-expressing ENPP1 tumors. In TNBC, we estimate this is 10% of breast cancer cases. That would be 28,155 new US cases/year and 367,000 total US cases/year. If our therapy is successful in TNBC in displacing SOC and we were used in just 15% of all new cases (4,223 patients) and 5% of all patients (18,350) facing recurring disease at a price point of $[redacted] per case, that would be an eventual sales level of 22,573 US patients/year or around $[redacted] just in TNBC.

Therefore, licensing and acquisition deals by large pharmaceutical companies in innate immune modulators have been quite lucrative. **Table 3** shows the straight arithmetic average of published deal terms by companies who have been acquired or, more typically, done a licensing deal with a big pharmaceutical company.

Table 3: Recent Innate Immune Modulator Deals

Sellers / Licensees	Buyers / Licensors	
V raTherapeut cs	Boehr nger Inge he m	**Average Upfront** $[redacted] on
BeneV r	Johnson & Johnson	
V ra yt cs	Johnson & Johnson	**Average Milestones** $[redacted] on
R gontec	Merck	
IFM Therapeut cs	BMS	
Aduro B otech	Novart s	
MavuPharma	Abbv e	
Vo astra	BMS	

Competition.

Table 4: Competitive Landscape

Company	Approach	Compound	Phase
Abbv e (MavuPharma)	Ora ENPP1 nh b tor	MV 626/MV 104	Stopped
R bosc ence	Ora [redacted] ENPP1 nh b tor	RBS 2418	Phase 1a started
Stingray	**Oral ENPP1 inhibitor**	**SR-8541a**	**Late Preclinical**
Zensh ne (Ch na)	Ora ENPP1 nh b tor	ZXP 8202	Prec n ca
Angarus	IV Phosphonate based ENPP1 Inh b tor	ANG 1623	Prec n ca
Vo astra	Suspect an ENPP1 Inh b tor program	No Cand date	Ear y Prec n ca
Avammune (Ind a)	ENPP1 Inh b tor program	AVA NP n test ng	Ear y Prec n ca
SparcB o (US/Ind a)	ENPP1 nh b tor program	IBS715 n test ng	Ear y Prec n ca
KU Leuven/CD3 (Be g um)	ENPP1 nh b tor program	No Cand date	Ear y Prec n ca
Acu eus (AUS)	ENPP1 Inh b tor program	No Cand date	Ear y Prec n ca

Mavupharma, backed by Frazier Ventures with $[redacted] was acquired by Abbvie in July 2019. This exit showed that big pharma is interested in ENPP1i. However, since the Abbvie purchase, the program has been discontinued. We believe Abbvie used the inhibitor at too high a dose, which compromised efficacy and may have inhibited other phosphodiesterases causing toxicity.

Riboscience out of Stanford (mostly an infectious disease company) began Phase 1a (March 2022) with their oral [redacted]nhibitor. Such inhibitors do not enter cells and work only extracellularly. This works for ENPP1i and enhances safety to some degree. Still, these compounds are poorly absorbed [redacted] making careful dosing difficult, [redacted]

Zenshine out of China reported on an ENPP1i at last year's AACR. This year they are reporting again but with a new compound.

Angarus Therapeutics out of Stanford has developed a phosphonate-based ENPP1i program that will have to be dosed IV – ANG-1623[redacted] Due to the nature of phosphonate compounds, a pro-drug would have to be generated to have an oral therapy. Phosphonates have some potential liabilities as small molecule drugs. They need a pro-drug to be oral, they are excreted by the kidney and, in high doses, can cause some kidney injury. Their levels in the blood can be strongly affected by renal impairment in patients, a significant issue in elderly cancer patients. Angarus has had difficulty financing the program, and no recent progress has been reported.

Volastra Therapeutics was founded out of Memorial Sloan Kettering by Bakhoum, who published the Cancer Discovery article in 2021 and is VC backed by Vida and Polaris. They are publicly focused on chromosomally unstable tumors and have not announced that they are working on an ENPP1i, but we believe they are based on our sources. We also think their ENPP1i may be part of their recent deal with BMS.

Avammune Therapeutics out of India and Aculeus/ Cancer Therapeutics CRC out of Australia have indicated that they have an ENPP1i program in development but have not released any data or statements in the last year. Therefore, we do not believe they have achieved a clinical candidate to start the march to IND.

SparcBio is a small US-based LLC with the owner on the Board of Integral Biosciences, an Indian CRO. Together they have announced a program in ENPP1 inhibition.

KU Leven, a Belgian university, and CD3, Belgium's national university accelerator, have announced a program, as have Aculeus announced that they had licensed a program from CRC in Australia.

We believe our major advantage in the field is our chemistry, as we have three separate, chemically distinct ENPP1i scaffolds, all with highly potent compounds. We are also ahead of most players and close to catching up to Riboscience.

It is early to understand competitive positioning, advantages, and disadvantages within the class except for the likely weaknesses of the Angarus program and the Riboscience program, already discussed. Small molecule programs can often be substituted for each other therapeutically. The smaller differences will not show themselves until later in development or may be created through smart clinical development. However, being among the first or second to market is a powerful advantage, not lost on pharma companies. We believe we are in an attractive position in the next few years to do a partnering deal with a pharma interested in the field who wants to pick up the jump start of collaborating with us to quickly get to the clinic with the program.

Marketing Strategy. The most likely development program for a biotech like Stingray is partnering with a large pharmaceutical house well before marketing. However, it is still important to know the value created at each stage of development and the ultimate sales return to the end marketer to do an effective deal with the pharma company – and be prepared if the opportunity to go the full distance avails itself.

Traditional pharmaceutical pricing depends on several factors, including the pricing of alternative treatments, the value from the level of performance of the drug, and the overall impact of paying for the drug on insurance providers. We can look to several benchmarks for a reasonable estimate for the traditional pricing of this therapeutic. Novel new therapies were recently launched, and the now older checkpoint inhibitors and PARP inhibitors provide launches in roughly similar therapeutic space and for which we would hope to achieve similar cancer relevance to patients.

Table 5. Recent Pricing for New Oncolytics

Company Brand/Generic Name	Therapeutic Type	Est. Wholesale Cost/ year
Q n ock (r pret n b) Dec phere	TKI for 4th ne GIST	$ [redacted]
Tazver k (tazemetostat) Ep zyme	Demethy transferase nh b tor	$ [redacted]
Sarc sa (satux mab rfc) Sanof	CD38 nh b tor	$ [redacted]
Tukysa (tucat n b) Seatt e Genet cs	HER2 TKI	$ [redacted]
Trode vy (sac tuzumab gov tecan hz y) Immunomed cs	Ant body drug conjugate	$ [redacted]
Pemazyre (pem gat n b) Incyte	Ora f brob ast growth factor receptor 2	$ [redacted]
Tabrecta (capmat n b) Novart s	Ora TKI MET nh b tor	$ [redacted]
Retevmo (se percat n b) Loxo Onco ogy/ L y	Ora RET nh b tor	$ [redacted]
BMS Opd vo (n vo umab)	Checkpo nt nh b tor	$ [redacted]
Merck Keytruda	Checkpo nt nh b tor	$ [redacted]
Pf zer Bavenc o (ave umab)	Checkpo nt nh b tor	$ [redacted]
AstraZeneca Imf nz (durva umab)	Checkpo nt nh b tor	$ [redacted]
Roche Tecentr q (atezo zumab)	Checkpo nt nh b tor	$ [redacted]
Sanof /Regeneron L btayo (cem p mab rw c)	Checkpo nt nh b tor	$ [redacted]
AstraZeneca Lynparza (o apar b)	PARP nh b tor	$ [redacted]
Zeju a (n rapar b)	PARP nh b tor	$ [redacted]
Rubraca (rucapar b)	PARP nh b tor	$ [redacted]

PARP inhibitor prices rom nstitute or Clinical and Economic Review (CER) checkpoint inhibitor prices rom multiple sources

The average price of these agents across the **Table 5** is $[redacted] per year. Based on these prices, an assumed price of $ [redacted] would appear reasonable

compared to the table average of $ [redacted]. And this is without referencing the much higher CAR-T adoptive therapy prices that exist, well over $ [redacted]. Several similar most recent launches have been over $ [redacted] per year. Therefore, we have used $[redacted].

Traditional pricing is easy to understand. Much more difficult is how the US pricing environment for pharmaceuticals might evolve and what will be required to show value for therapy in this market. There are many competing approaches, and it is far from clear which method might win and what would eventually become the standard. Approaches include using quality-adjusted days of life given and comparing the average cost of each day from the therapy to just comparing a basket of prices for the same therapy in other countries. The UK actively evaluates costs and decides which therapies may be reimbursed and when. Often, European pricing negotiations include the nationality of the requesting company, that company's jobs in the country, and generic and other brand name prices for similar drugs.

Our approach will be to focus on proving clinical safety and efficacy. Following a good efficacy report-out in Phase 1-2, we will immediately start considering what clinical trials might be beneficial to show value to providers and payors, given the current state of play in how reimbursement may be shaped and how economic value is being studied for cancer pharmaceuticals.

D. Intellectual Property (IP) Protection

Our intellectual property portfolio (**Table 6**) comprises the seven patent families listed below, six of which are wholly owned by ST and one which is co-owned with the University of Utah (U of U). We have a license agreement with U of U that provides ST exclusive worldwide commercialization rights for the co-owned subject matter. Our patents and patent applications are directed to novel compositions of matter and their use as inhibitors of ENPP1 to treat various ENPP1-associated diseases and conditions. Our expected patent term runs through 2038-2041, and we will apply for all regulatory patent term extensions available, extending these patent terms by up to five years. Stingray's lead candidate, SR-8541A, and several backup compounds are specifically disclosed and claimed in pending applications throughout our portfolio. We are aggressively pursuing patent protection for SR-8541A and prosecuting claims directed to backup and related compounds to keep competitors out of our immediate space.

Table 6. Stingray's Intellectual Property Portfolio

Title	Filing Date	App. Nos.	Status	Owner
Nove ENPP1 and STING Modu ators as Cancer Immunotherapeut cs	27Ju 2018	[redacted]	Granted: US Pend ng: CN, EP & JP	St ngray Therapeut cs/ U of U
Qu no ne and Qu nazo ne Compounds and Methods of Use	17Mar2020	[redacted]	Granted: US Pend ng: PCT	St ngray Therapeut cs
Nove ENPP1 and STING Modu ators as Cancer Immunotherapeut cs	01Aug201	[redacted]	Pend ng: US To be f ed n: CN, EP, and JP	St ngray Therapeut cs
Inh b tors of ENPP1 and Methods of Use	04 eb2020	[redacted]	Pend ng: US	St ngray Therapeut cs
Im dazo e Compounds as Inh b tors of ENPP1	02Dec2020	[redacted]	Pend ng: US	St ngray Therapeut cs
Phosphonates as Inh b tors of ENPP1 and CDNP	0 Dec2020	[redacted]	Pend ng: US	St ngray Therapeut cs
Phosphonates as Inh b tors of ENPP1 and CDNP	0 Dec2020	[redacted]	Pend ng: US	St ngray Therapeut cs

We have a clear path to obtaining patent protection on SR-8541A. ST commissioned Seed Intellectual Property Law Group LLP ("Seed") to perform a novelty and patentability analysis for SR-8541A. Seed performed structure-based searching and determined that SR-8451A is a novel compound. This analysis also identified several compounds, including those published by Mavupharma (Abbvie). [redacted]

[redacted]

We have not identified any third-party positions that would prevent the commercialization of an ENPP1 therapeutic comprising SR-8541A. In addition to the patentability analysis, Seed also performed a freedom-to-operate analysis at our request. Seed performed both structure-based searching and keyword searching in an attempt to identify third-party blocking positions. The structure search identified 17 patent families for review, none of which were determined to present a blocking position. Seed's keyword searching identified 228 hits, including our patent publications and those of competitors such as [redacted] Each of these hits was analyzed given ST's commercialization plans, and no blocking positions were identified. Accordingly, we are not aware of any intellectual property impediments to our current commercialization plans for SR-8541A.

Beyond intellectual property for composition of matter and method of use, there is limited protection available for most small molecules. Our product processes are straightforward, and other blocking approaches are limited. We can work to study our molecule better in clinical studies, more tightly define the relevant patients who will benefit, enhance our clinical profile compared to competitors, and study with the key opinion leaders in immune oncology so they know our drug well. This would be the best way to enhance our competitive profile for several years.

E. Finance Plan

The Founders of ST (Dr. Sunil Sharma, Jon Northrup) have a substantial track record of raising capital from non-dilutive (CPRIT, NIH, other) and retail / institutional capital sources resulting in two oncology development programs (Seclidemstat – Salarius; Tegavivint – Iterion) successfully advancing into Phase 1 / 2 clinical studies.

ST targets raising money at earlier stages of development with angels and high net worth (HNW) individuals and applying for grant funding wherever possible. We then transition to institutional capital at the Series A or B point when millions of dollars rather than hundreds of thousands are required.

ST was started with money contributed by company founders and one successful biotech investor in 2016-2017. In 2018/2019, ST raised a $ S[redacted]d Series Preferred financing round with participation from accelerator/ incubator platforms (Dallas Health Wildcatters), venture capitalists (Green Parks and Golf – GPG (Dallas), Springhood Ventures (Boston), Early Investment Partners (New Jersey), foundations (Carson Leslie Foundation) and hospital venture (HonorHealth, Phoenix). In 2020/2021, ST raised a $ convertib[redacted]te, which supported the completed Pre-IND and IND-directed studies. GPG has provided Stingray a letter of support and is currently considering a second investment in Stingray.

In 2022, ST has started a $[redacted] Series A Preferred Security at a $[redacted] pre-money valuation. ST has initiated outreach to institutional investors in the 1Q 2022 to help finance the remaining preclinical studies and complete Phase 1 clinical studies with the lead asset, SR-8541A. Stingray is interfacing with leading institutional investors, family offices, and angel groups via direct outreach and online digital conferences (e.g., JPMorgan Week - BIO, Biotech Showcase, RESi).

[redacted] We expect additional investors to advance under confidentiality, given the number of productive meetings held to date.

The company is also actively evaluating transactions leading to rewarding shareholders, including a sale to a large biopharmaceutical company (M&A), public listing through an initial public offering (IPO), or SPAC (Special Purpose Acquisition Company). Historical transactional data from similar immune-oncology companies suggests the most likely and earliest development stage when ST would execute a liquidity event (sale, IPO) would be after Phase 1 / 2 studies. In addition, the Founders of ST may leverage their extensive experience and contacts (buy/sell-side bankers and analysts) in transitioning companies from the private-to-public markets (e.g., Salarius Pharmaceuticals listed on NASDAQ in 2019) to facilitate a public offering for ST increasing the company's access to capital and providing liquidity to shareholders.

F. Production and Marketing Plan

We require specialized manufacturing facilities from contractors who are FDA certified for cGMP active pharmaceutical ingredient (API) and cGMP formulation and tableting drug product (DP) at this time. We have already produced 15 kilograms (KG) of cGMP API, which has been on stability for a year with no issues. Therefore, we do not expect any additional need for API until Phase 3 studies. If and when marketing authorization is achieved, we could consider our API manufacture, but given the potency of the product and the likely small tablet size [redacted], it is more likely we will continue with contract API manufacture.

We are using Catalent, a leading US contractor working in San Diego, for formulation and tableting. The plan is for them to manufacture our clinical trial needs for Phase 1 and Phase 2. We plan a tablet run for each phase, likely 600 bottles of 60 tablets of [redacted] for Phase 1 and 1200 bottles of 60 tablets of [redacted] for Phase 2. We plan to expand into Phase 3 production and initial marketing and sales runs with Catalent and will reconsider our tableting strategy once marketing authorization is achieved.

For marketing and sales, once FDA authorization is achieved, we plan to partner with a major US pharmaceutical company and potentially participate in these activities. Outside the US, we would partner to access sales. The strategy we take will depend on the ultimate product profile of our drug and how broadly it might be used. If it looks like we might achieve an eventual label across many cancers and many combination therapies as we think today, we will likely pick a number of specialist oncologists who treat a set of diseases to focus on and work with our partner to reach the additional oncologists of importance. We would also coordinate marketing message and communications. If the marketing use is more confined, we might lean toward specializing as a research and development house and have our partner do the sales and marketing. On our team, Jon and Scott have run organizations that marketed and sold pharmaceutical products to physicians and would be capable of building such an organization if needed by ST. This decision would be made close to the Phase 3 readout of our pivotal trial.

The internet is an essential source of information sharing and communication in biotech. Still, sales require a physician to write a prescription or an order and a pharmacist to sell the drug to the patient.

G. Revenue Stream

Our business model is to develop our products through a Phase 2 clinical study and partner with a large pharmaceutical company. The partnering deal then provides validation and funding for the company, allowing the company to consider an initial public offering and become a public company or continue as a private company and bring forward additional products for partnering.

Our CEO worked 28 years in a large pharmaceutical company – Eli Lilly – and has launched six new pharmaceuticals into the US market, run significant aspects of the sales force and the marketing department, and has done 40 deals on the buy-side for Lilly with companies much like ST. Our Chief Medical and Scientific

Officer, Dr. Sharma, has done over 150 Phase1-2 clinical oncology trials as principal investigator and is a capable and experienced oncology trialist.

Upon a successful large pharmaceutical deal to power the company to the next level, we would grow the team in research and development to handle additional targets to bring forward and enhance the team in clinical development so that we could work alongside the big pharma in additional Phase 2's and eventually Phase 3's. This would allow us to take the next successful project further and capture a larger percentage of the ultimate economics. With several successes, we might be able to consider taking a project all the way to market and beginning to exploit the "idea to medicine cabinet" business model of a typical pharmaceutical company. All of these possibilities depend on a successful first ENPP1i delivering a good readout in its Phase 1-2 clinical experience and completing the IND enabling studies so that the IND may be accepted, and the clinical experience can start.

PHS 398 Cover Page Supplement

0MB Number: 0925-0001

Expiration Date: 09/30/2024

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). 0therwise, leave this section blank.

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

Tracking Number: GRANT13588796

Funding Opportunity Number: PA-21-259 . Received Date: 2022-04-05T17:36:52.000-04:00

PHS 398 Cover Page Supplement

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. 0r, 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. 0ne 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

OMB Number: 0925-0001
Expiration Date: 09/30/2024

Introduction	
1. Introduction to Application (for Resubmission and Revision applications)	
Research Plan Section	
2. Specific Aims	SBIR_Specific_Aims.pdf
3. Research Strategy*	SBIR_Research_Strategy.pdf
4. Progress Report Publication List	
Other Research Plan Section	
5. Vertebrate Animals	SBIR_Vertebrate_Animals.pdf
6. Select Agent Research	
7. Multiple PD/PI Leadership Plan	SBIR_Multiple_PI_Leadership_Plan.pdf
8. Consortium/Contractual Arrangements	TGen_LOI.pdf
9. Letters of Support	LOS_Packet.pdf
10. Resource Sharing Plan(s)	Resource_Sharing_Plan.pdf
11. Authentication of Key Biological and/or Chemical Resources	SBIR_Authentication_of_Key_Resources.pdf
Appendix	
12. Appendix	

Funding Opportunity Number: PA-21-259. Received Date: 2022-04-05T17:36:52.000-04:00

SPECIFIC AIMS

Ectonucleotide pyrophosphatase/phosphodiesterase 1 (ENPP1) is a highly accessible protein located on the surface of cancer cells that acts as a control switch for immune suppression and metastasis. It allows cancer cells to thrive in an unfavorable inflammatory environment by intercepting the warning signals of tumor formation before they can reach nearby immune cells. Essentially, when chromosomally unstable tumors release DNA fragments into the cytosol, they are bound by the enzyme cGAS, that in turn, catalyzes the formation of 2'3'-cyclic GMP-AMP (2'3'-cGAMP), which triggers an immune response through activation of a downstream pathway called STING (**ST**imulator of **IN**terferon **G**enes). ENPP1 functions as a negative regulator of the STING pathway by cleaving extracellular 2'3'-cGAMP, preventing it from activating STING in neighboring immune cells in the tumor microenvironment (TME). The cleavage of 2'3'-cGAMP also releases the molecule adenosine, known to promote immune suppression and cancer cell migration. Analysis of ENPP1 gene expression in tumors from The Cancer Genome Atlas found that it is expressed in many tumor types, and those with high ENNP1 expression are associated with immune suppression, cancer metastasis, and poor patient outcomes. Moreover, cancers such as breast, and in particular triple-negative breast cancer, show limited efficacy to front-line treatments, including immunotherapies, when tumors express high ENPP1 levels. Altogether, ENPP1 is an attractive target to pursue for several reasons: (1) it is readily accessible, (2) its expression is relatively specific to cancer cells, (3) it is highly expressed in a variety of cancers, and (4) it can be leveraged to sensitize "cold" tumors to immunotherapies. Moreover, while STING is also an interesting target, it, unlike ENPP1, is broadly expressed; hence STING agonists indiscriminately activate STING in multiple cells and tissues, resulting in "off- target" side effects. The effects of an ENPP1 inhibitor would be localized to the TME not only because of its limited expression but also due to the high levels and short half-life of 2'3'-cGAMP.

If a safe and efficacious ENPP1 inhibitor were available, it would have widespread utility for multiple cancer types and, if used in combination with other cancer therapies, may enhance their performance. Towards this end, we have developed an orally bioavailable potent small-molecule inhibitor of ENPP1 called SR-8541A. It inhibits hENPP1 activity with an IC_{50} of 3.6 nM (K_i=1.9 nM) and demonstrates robust selectivity. We have established that it activates the STING pathway, promotes immune cell infiltration, and inhibits cancer spheroid growth. Furthermore, in syngeneic tumor mouse models, SR-8541A demonstrates a synergistic effect with radiation, and a preliminary study also shows synergy with checkpoint inhibitors. To date, we have completed preclinical development activities on SR-8541A that include API development and manufacturing, stability, pharmacokinetics, tolerability, and preliminary toxicology (mouse, rat, dog). Ongoing efforts to be completed before the proposed studies commence include *in vitro* safety pharmacology and PK/PD modeling.

The current objective for our ENPP1 program is to complete non-GLP and GLP preclinical studies necessary to seek IND acceptance for a first-in-human phase I clinical trial. Our initial indication of focus will be in TNBC. To position us to meet these goals, we propose in this Direct to Phase II SBIR application the following aims for our lead molecule SR-8541A:

- **AIM 1: Preclinical evaluation of SR-8541A in combination with FDA-approved drug regimens.** We will work with Charles River Laboratories to evaluate the efficacy of SR-8541A in combination with the chemotherapy drug cisplatin, the checkpoint inhibitors CTLA-4 and PD-1, and the PARP inhibitor olaparib, using syngeneic tumor mouse models of breast cancer.
- **AIM 2: Perform IND enabling dog GLP toxicology study on SR-8541A.** We will work with Charles River Laboratories to conduct a GLP toxicology study in dogs as the rat GLP toxicology is complete.
- **AIM3: cGMP tablet development, manufacture, and initial stability.** We will work with Catalent to determine the human dosage based on the PK/PD modeling data and manufacture clinical-grade tablets necessary to conduct a Phase I clinical trial.

At the conclusion of this work, we will have completed the necessary preclinical research and development of SR-8541A, developed a clinical strategy to test as a single agent or in combination, and submitted the IND application. Next, we will assemble a clinical team to identify the sites where SR-8541A can be clinically tested.

RESEARCH STRATEGY

A. Significance

Mounting preclinical evidence suggests that careful and therapeutically relevant activation of the STING (**ST**imulator of **IN**terferon **G**enes) pathway is necessary to elicit potent anti-cancer innate immune responses. A key limitation with the development of STING agonists is the widespread expression of STING in normal tissues, whereby the hyperactivation of STING can lead to a systemic cytokine storm (1). To avoid such adverse events, direct intratumoral STING agonists have been developed, showing robust anti-tumor activity, including complete cures, in several preclinical models (2). Yet, their performance in clinical trials has been dissatisfactory. Thus, there is a need to identify alternative approaches to activate STING in a controlled manner. **ENPP1 is the only known direct negative regulator of the STING pathway, and targeting ENPP1 is superior and safer for several reasons:**

- ENPP1 inhibition would achieve the dual purpose of reducing adenosine levels, an immune suppressor, while simultaneously increasing the levels of the immunostimulatory STING ligand 2'3'-cGAMP.
- ENPP1 is selectively upregulated in metastatic and chromosomally unstable tumor cells, in stromal cells within the tumor microenvironment (TME), and in tumor cells that have become resistant to standard of care.
- As tumor cells produce the highest levels of 2'3'-cGAMP, the effects of inhibition of ENPP1 will be limited to the TME.
- The half-life of 2'3'-cGAMP is short; therefore, the increased levels of 2'3'-cGAMP due to inhibition of ENPP1 will primarily stimulate STING in the TME and stromal compartment.
- Finally, expression of ENPP1 is low in most normal tissues except for liver and bone. In addition, the majority of immune cells also have low ENPP1 expression, except for neutrophils and plasma cells.

We reason that the restricted expression of ENPP1 and the short half-life of 2'3'-cGAMP will minimize any potential side effects that have been observed with the systemic administration of direct STING agonists. ENPP1 is uniquely positioned to function as a dual checkpoint regulator of innate and adaptive immune responses, and thus, developing a safe and efficacious ENPP1 inhibitor would be immensely valuable for cancer patients.

1. Role of the STING signaling pathway in innate immunity response to cancer. STING is a pattern-recognition receptor anchored in the endoplasmic reticulum with a pivotal surveillance role that includes the response to cytosolic DNA introduced by either pathogens or leaked from the nucleus as a result of genomic instability. The STING protein is expressed in various epithelial, endothelial, and hematopoietic cell types in addition to cancer cells. As depicted in **Figure 1**, the activation of the STING signaling pathway is triggered when the cGAS enzyme senses and binds to cytosolic DNA, which in turn catalyzes the formation of the cyclic dinucleotide (CDN), 2'3'-cGAMP. The binding of 2'3'-cGAMP with STING induces conformational changes that lead to perinuclear migration of STING, followed by recruitment of interferon (IFN) regulatory factor 3 (IRF-3) and TANK-binding kinase 1 (TBK1). IRF-3 is phosphorylated by TBK1 and subsequently translocates into the nucleus to drive transcription of the innate immune genes, such as IFNβ and IFN-stimulatory genes (ISGs). 2'3'-cGAMP is also readily exported to the extracellular space, where it promotes an anti-tumor immune response by activating STING in host cells present in the TME. Hence, STING activation leads to the abundant secretion of type I IFNs and concomitant activation of autocrine and paracrine pathways that support the cross-priming and migration of immune cells (e.g., dendritic cells, T cells, and natural killer cells) to the TME (3-8).

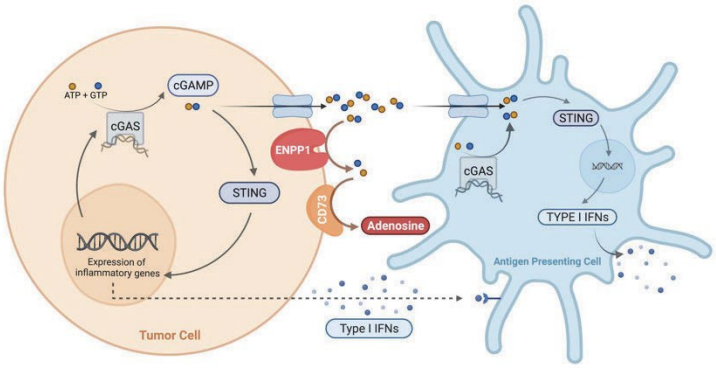

Figure 1. Scheme of cGAS-STING s gna ng pathway and ts nh b t on by ENPP1 through hydro ys s of 2'3'-cGAMP.

2. Targeting STING with synthetic CDNs fails to produce a clinical response with off-target effects. As 2'3'-cGAMP is a potent immune-stimulatory molecule, STING has become an attractive therapeutic target for cancer immune therapy, leading to the development of hydrolysis-resistant synthetic CDNs as a new class of cancer therapeutics (9-11). These direct STING agonists have been shown to possess potent preclinical efficacy where complete cures were demonstrated in multiple preclinical cancer models (6). Two of these resistant CDNs (ADU-S100 and MK-1454) have entered Phase I clinical trials but failed to produce any responses independently and showed only a modest response when combined with a PD-1 inhibitor. A few other CDNs are currently in Phase I clinical trials (12). While activating STING with synthetic CDNs is quite attractive, it has several limitations. First, these molecules are bulky and suffer from poor permeability, resulting in low drug accumulation in the tumor. Second, STING is commonly expressed in cells and tissues, and systemic administration of synthetic CDNs can lead to indiscriminate activation of STING in both cancer cells and normal tissues. T cells express high amounts of STING, and prolonged or hyperactivation of STING leads to apoptosis of T cells (13, 14). Hyperactivation of STING is also associated with severe autoimmune diseases. Third, intratumoral administration of synthetic CDNs is technically challenging, especially in patients with metastatic disease, and no abscopal effects have been reported from the terminated or ongoing clinical trials (10-13, 15). Given the widespread expression of STING, the magnitude of STING activation needs to be tightly controlled to minimize toxicity to normal tissues and concurrently have robust anti-tumor activity in the TME. These challenges dampen the enthusiasm for developing systemic or intratumoral STING agonists.

3. ENPP1, a direct negative regulator of the STING pathway. Cancer cells have evolved multiple mechanisms to evade cGAS-STING-dependent immune surveillance. One mechanism involves silencing downstream type I IFN signaling and promoting NF-KB-dependent migratory programs. Another mechanism involves upregulation of the enzyme ectonucleotide pyrophosphatase/phosphodiesterase I (ENPP1) in chromosomally unstable tumor cells, whereby it selectively degrades 2'3'-cGAMP and thus, inhibits STING activation (**Figure 1**). Furthermore, the cleavage of 2'3'-cGAMP releases the immuno-suppressant metabolite, adenosine, which is associated with reduced immune cell infiltration, increased metastasis, and resistance to immune checkpoint blockade therapy (16, 17). ***We hypothesize that inhibition of ENPP1 activity with an orally available small molecule would be a novel approach to activate the STING pathway compared with CDNs, which require an intratumoral route of administration and may lead to cytokine release syndrome.***

ENPP1 is a member of a family of nucleotide pyrophosphatases /phosphodiesterases (NPPs), consisting of seven structurally related ectoenzymes that hydrolyze various substrates, including nucleotides, lysophospholipids, and choline phosphate esters (18, 19). It is a membrane-bound enzyme with many functions (**Figure 2**) and is expressed in tissues such as cartilage, heart, kidney, parathyroid, and skeletal muscle. It is highly expressed in osteoblasts and chondrocytes, where it regulates extracellular levels of inorganic pyrophosphate (PPi) by hydrolyzing ATP, thus playing an essential role in bone mineralization. It also has a vital role in preventing soft tissue mineralization, as demonstrated in ENPP1 deficient mice, which present with an abnormal gait and progressive calcification in ectopic sites. Many inherited mineralization disorders have been linked to inactivating mutations of ENPP1, such as generalized arterial calcification of infancy (GACI). Yet, synthetic analogs of PPi known as bisphosphonates have been used successfully to reduce calcification in GACI patients, affording them the ability to live fairly normal lives.

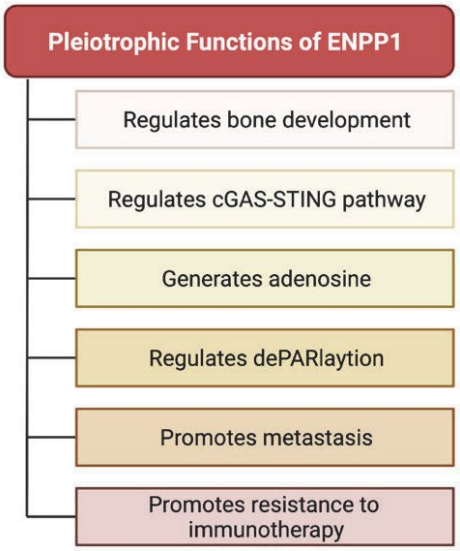

Figure 2. B o og ca and pathophys o og ca processes of ENPP1.

In addition to ATP, ENPP1 hydrolyzes the following substrates: UTP, cAMP, AP_4A (diadenosine polyphosphates), and 2'3'-cGAMP (20, 21). Except for UTP, hydrolysis of these substrates by ENPP1 results in the production of AMP, which is subsequently hydrolyzed by CD73, an ecto-5'-nucleotidase (NT5E), to generate adenosine (**Figures 1-3**). Adenosine plays a major role in establishing an immunosuppressive TME by suppressing the activities of immune cells: T cells - proliferation, cytokine production, and cytotoxicity; NK cells

– cytotoxicity; macrophage/dendritic cells - antigen presentation, cytokine production; and neutrophils - oxidative burst. Moreover, adenosine produced by cancer cells can influence myeloid cell differentiation and the accumulation of M2-type tumor-associated macrophages and myeloid-derived suppressor cells (MDSC) into the TME. These cell types express high levels of both CD73 and CD39 - another ectonucleotidase that generates AMP by hydrolysis of ATP, thereby contributing to the vicious cycle of adenosine production within the TME (17, 22, 23). ENPP1 is differentially expressed in immune cells with low levels in NK cells, DC, and macrophages and high levels in neutrophils. ENPP1 is also expressed in a small subset of B-cells, and studies suggest that these cells may be involved in the modulation of T-cell activity. Interestingly, elevated ENPP1 expression was reported in the M2 subtype of macrophages that are known to play a role in tumor promotion (24-26). Lastly, the expression of ENPP1 was also shown to be elevated in endothelial cells in response to hypoxia or response to the constitutively active form of HIF1a (27). ***Thus, targeting ENPP1 provides a unique opportunity to stimulate innate and adaptive immune responses through the modulation of 2'3'-cGAMP and adenosine levels in the tumor microenvironment.***

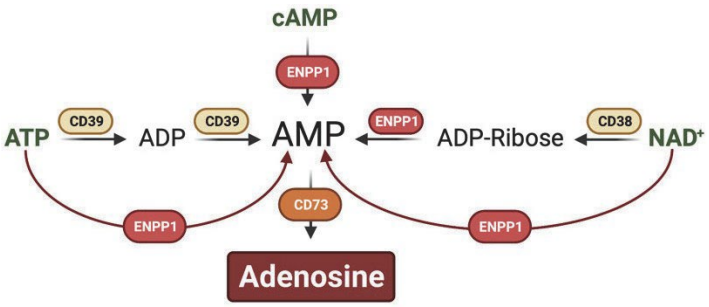

Figure 3. Scheme of adenosine generation by sequential hydrolysis of ATP or other substrates via ENPP1 and CD73.

4. Clinical relevance of ENPP1 in cancer. Analysis of *ENPP1* gene expression in tumors from The Cancer Genome Atlas found that it is expressed in many tumor types such as brain, breast, lung, and melanomas, and those with high *ENPP1* expression are associated with immune suppression, cancer metastasis, and poor patient outcomes (**Figure 4**) (17, 28-30). Moreover, breast and ovarian cancers show limited efficacy to front-line treatments, including immunotherapies, when tumors express high *ENPP1* levels (17).

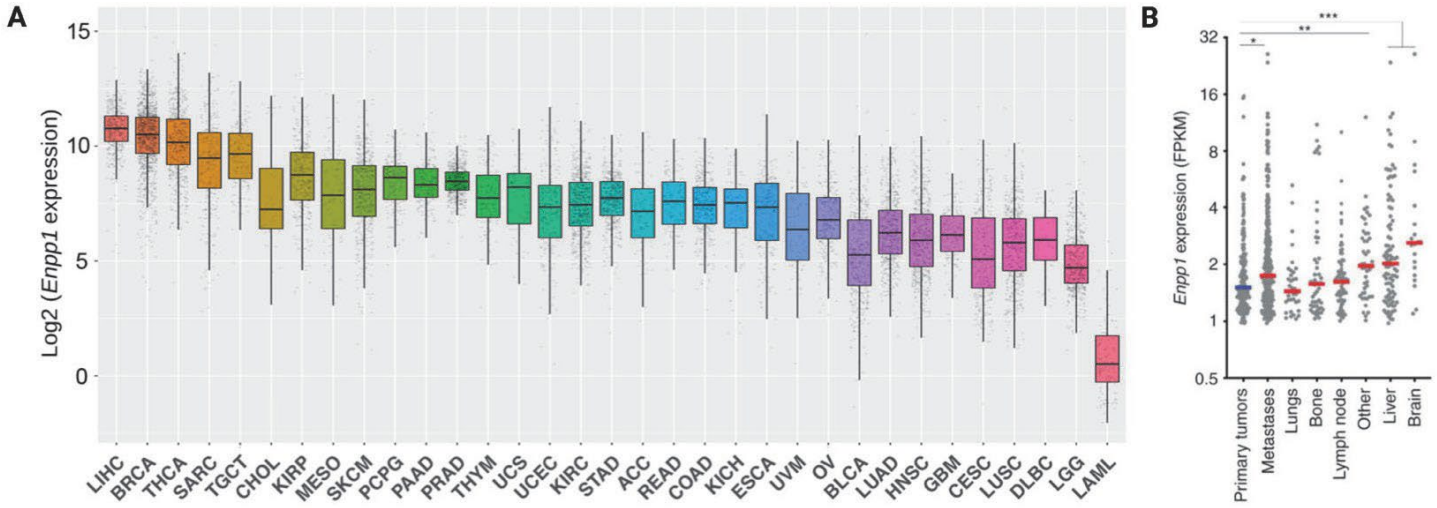

Figure 4. *ENPP1* expression in human cancer. (A) *ENPP1* mRNA expression across 33 human cancer types found in the TCGA database. **(B)** *ENPP1* expression is higher in metastatic tumors compared to primary tumors. Data reported in Ref (17).

Role of ENPP1 in breast cancer. As breast cancer is a heterogeneous disease, treatment strategies differ according to molecular subtype. In general, breast cancer treatment usually involves a combination of surgery, radiation, chemotherapy, hormone, and targeted therapies. Although mortality has been substantially reduced in early breast cancer and important milestones, have been achieved in minimizing toxicity and improving quality of life, more and better treatments are needed. *ENPP1* is highly expressed in breast cancer and is associated with poor prognosis, irrespective of ER status (**Figure SA-B**) (17). In addition, it is known to facilitate metastasis, cancer stem cell generation, and promote drug resistance (28, 31-34). Specific to the latter, elevated *ENPP1* expression has been observed in breast cancer patients that developed tamoxifen resistance (32, 33). Similarly, high ENPP1 expression was reported to increase resistance to docetaxel in breast cancer cells (34). Although

the mechanism for ENPP1-mediated drug resistance remains unclear, it has been suggested it's through an association with ABC transporters. For example, ENPP1 was shown to directly associate with and promote surface localization of the ABCG2 drug transporter (34).

With respect to immune suppression, it has been shown that high expression of ENPP1 in breast cancer cells and its surrounding stroma correlated with poor immune cell infiltration in the TME (**Figure SC**). Interestingly, growth rates of ENPP1-KO tumors were shown to be significantly reduced compared to wild-type tumors when both were treated with checkpoint inhibitors (**Figure SD**) (17). Similarly, ENPP1 has been shown to impair the anti-tumor immune response post- radiation. Mechanistically, ENPP1-generated adenosinergic metabolites enhance the expression of haptoglobin, a pro-inflammatory mediator that elicits invasion of myeloid-derived suppressor cells and promotes neutrophil extracellular trap formation in breast tumors (35).

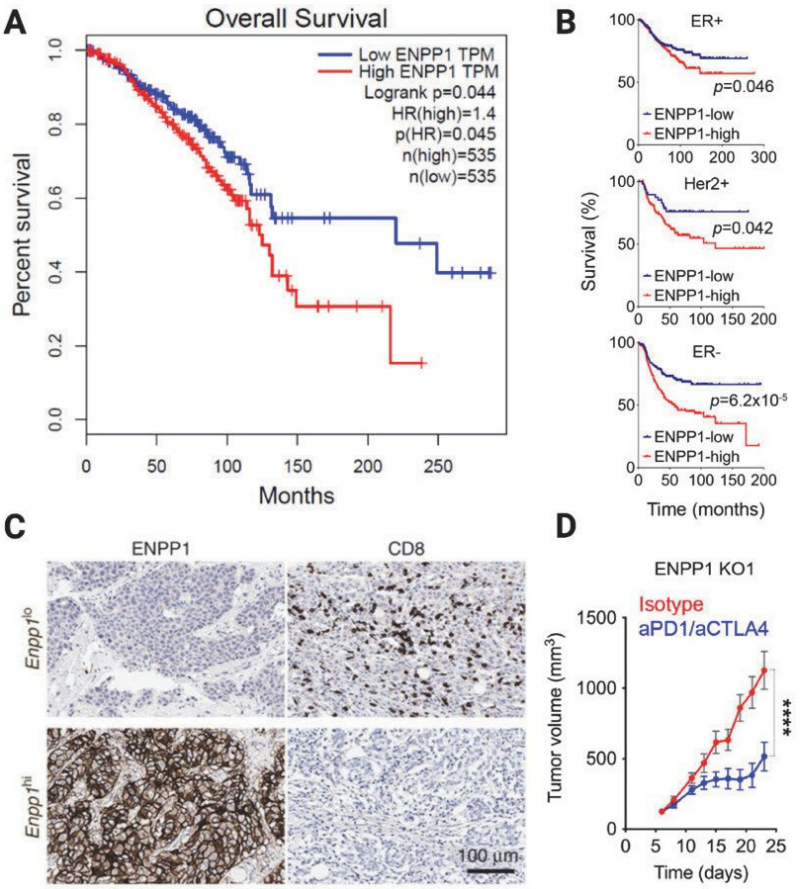

Figure 5. (A) Overa surv va of breast cancer pat ents (GEPIA). **(B)** Surv va of breast cancer pat ents strat f ed by tumor receptor status and *ENPP1* express on eve s. **(C)** CDS+ T-ce dens ty s reduced n the presence of h gh ENPP1 express on n breast cancer. **(D)** Growth curves of orthotop ca y transp anted ENPP1 KO 4T-1 tumors upon treatment w th checkpo nt nh b tors. Data adopted from Ref (17).

ENPP1 is also proposed to function as a PARG (poly-ADP-ribose glycohydrolase), an enzyme that can catalyze and reverse protein ADP-ribosylation, suggesting that ENPP1 may have a role in the recycling of PARP proteins. As PARP inhibitors are approved for BRCA-mutated, HER2-negative breast cancers and inhibition of PARP was shown to stimulate STING-dependent anti-tumor immunity; there is a possibility of potential synergy between PARP and ENPP1 inhibitors in breast cancers (36-39).

Altogether, these studies suggest that ENPP1 inhibitors, when combined with chemotherapeutic agents or targeted therapeutics, including immunotherapies, may improve the overall treatment of breast cancer patients.

B. Innovation

Targeted therapeutics alone or in combination with conventional treatments have improved the outcome for breast cancer patients. Still, modest response rates and drug resistance remain the major impediments to treatment success. In recent years much emphasis has been placed on improving adaptive immune responses to suppress tumor evasion. **We propose ENPP1 as a novel dual immunosuppressive checkpoint target that can directly modulate innate and adaptive immune responses within the tumor microenvironment.** By destroying a crucial second messenger, 2'3'-cGAMP, and simultaneously increasing adenosine levels and upregulation of its expression as tumors advance and become metastatic, ENPP1 can handicap the therapeutic efficacy of conventional and targeted treatments in breast cancer. Our innovation lies in developing a novel potent small-molecule inhibitor of ENPP1 that can be delivered orally and help overcome resistance in breast cancer treatment. We believe that inhibition of ENPP1 alone or in combination with other immune checkpoint inhibitors or standard of care treatments can produce superior outcomes for breast cancer patients.

C. Preliminary Data

1. Discovery of SR-8541A: Medicinal chemistry strategy. Pfizer scientists reported that many of the ENPP1 inhibitors from the quinazolin-4-piperidin sulfamide series suffer from high-affinity binding to hERG potassium channels, which can cause drug-induced QT prolongation (40). We started optimizing SR-8345 to address this liability and introduce additional novelty. The initial goal was to improve the hERG inhibitory activity of SR-8345. Using the structure-based hERG homology model, the authors identified two important interactions to the hERG binding pocket: stacking the quinazoline with the Phe656 residues and extensive hydrogen bonding of the sulfamide to Ser624. These two quinazoline and sulfamide pharmacophore moieties are also essential for ENPP1 activity.

SR-8541A was nominated as the development candidate. A provisional application was filed in Feb 2020.

Figure 7. Med c na chemistry strategy and development of the ENPP1 nh b tor SR-S541A.

2. SR-8541A is a potent inhibitor of ENPP1

2a. ENPP1 enzymatic assay. Biochemical enzymatic assays were performed to determine the potency of ENPP1 inhibitors with recombinant human ENPP1 (hENPP1) and TMP as a substrate, and the reaction was quantified by measuring absorbance at 405 nm. As shown in **Figure 8A**, SR-8541A inhibited hENPP1 activity

2b. Thermal shift assay. To demonstrate the direct binding of SR-8541A, we employed a fluorescent-based protein unfolding thermal shift assay. The principle behind this assay is the drug's ability to bind and directly increase the target protein's stability. As reported in the literature, a thermal shift of greater than 2 degrees is considered a potential binder (41, 42). Protein melt reactions were conducted with recombinant hENPP1 in the presence or absence of SR-8541A.
As a positive control, 2'3'-cGAMP was used with a change of 2 degrees.

2c. Selectivity studies. Among the seven members of the NPP family, hENPP1 is closely related to hENPP2 and hENPP3, with 40-50% identity at the protein level. To demonstrate the selectivity of the ENPP1 inhibitors, we employed a biochemical hENPP2 enzymatic assay along with a cell-based enzymatic assay for hENPP1, hENPP3, and mouse ENPP1 (mENPP1). As shown in **Figure 8C-E**, SR-8541A did not affect the enzymatic activity of hENPP2 and showed 300-fold selectivity against hENPP3. SR-8541A was 15-fold less potent on mENPP1 when compared with hENPP1. Importantly, SR-8541A was tested against a full kinome (468 members, DiscoverX), GPCR (168 members, Eurofins), bromodomain (40 members, Eurofins), PDE (13 members, Eurofins), and p450 enzyme (6 members, ThermoFisher) panels and observed no activity. Lastly, SR-8541A showed an activity of >10 μM against hERG (ThermoFisher).

- 0/6 hits in **p450 Enyme Panel** at 10 μM
- >10 μM against **hERG**
- 0/468 hits at 1 μM in **Kinome Panel**
- 0/13 hits in **PDE Panel**
- 0/40 hits in **Bromodomain Panel**
- 0/168 hits in **GPCR Panel** at 10 μM

Figure 8. SR-S541A nh b ts ENPP1 act v ty. **A)** hENPP1 b ochem ca assay w th SR-S541A. **B)** Therma sh ft prote n unfo d ng assay w th hENPP1 prote n n the presence or absence of SR-S541A and 2'3'-cGAMP. **C)** hENPP2 b ochem ca enzymat c assay w th a pos t ve contro PF-S3S0, and SR-S541A. **D)** ENPP enzymat c assay us ng ce ysates from HEK293T ce transfected w th var ous constructs and treated w th SR-S541A. **E)** Se ect v ty prof ng of SR-S541A.

2d. SR-8S41A binds the catalytic pocket of hENPP1. With the help of computational modeling, specific amino acids in the catalytic pocket that interact with SR-8541A were identified, and mutant hENPP1 constructs were generated using site-directed mutagenesis. As shown in **Figure 9**, SR-8541A showed a 2.5- and 100-fold weaker activity against F257L and Y371L mutant hENPP1 proteins, respectively, compared with wild-type hENPP1 protein. Importantly, combining both mutations resulted in a 1000-fold loss of activity for SR-8541A. Recently, the crystal structure of human ENPP1 bound with QPS2 (a quinazolin-4-piperidin sulfamide compound) was reported (21) and our docking results suggested a similar binding pattern for SR-8541A (data not shown). Together, these results suggest that SR-8541A binds the catalytic pocket of hENPP1 and inhibits its activity.

Figure 9. SR-S541A b nds the cata yt c pocket of hENPP1. **A)** Image shows the cr t ca res dues n the cata yt c pocket of ENPP1. **B-C)** Mutat on of key res dues n the cata yt c pocket of ENPP1 d srupts the b nd ng of SR-S541A. **B)** Western b ot show ng the express on of mutated hENPP1 prote ns n transfected HEK293T ce s. **C)** ENPP1 enzymat c assay us ng ce ysates prepared from the transfected ce s.

3. SR-8541A stimulates an immune response in vitro

3a. Activation of the STING pathway by SR-8S41A. We hypothesized that inhibition of ENPP1 will not impact tumor cells in the absence of an immune system. Therefore, we developed an *in vitro* 3D-spheroid assay to evaluate the effect of ENPP1 inhibitors on the activation of the STING pathway and migration and infiltration of immune cells into tumor spheroids.

[redacted]

Briefly, single-cell suspension of cancer cells was seeded in a 96-well ultra-low affinity plate and incubated for 72 hours to form spheroids. Then, the culture medium was replaced with a fresh medium containing DMSO or SR-8541A. A 5 µm transwell was placed into the well and labeled PBMCs were added. Lymphocyte infiltration was assessed by fluorescence imaging after 48 hours of co-culture (**Figure 10A**). Basal lymphocyte infiltration was increased in triple-negative breast cancer (MDA-MB-231) ENPP1 KO cells compared with parental cells (**Figure 10B**). Notably, a dose-dependent increase in lymphocyte infiltration was observed only in the parental cells treated with SR-8541A, indicating that SR-8541A was ineffective in the absence of ENPP1 (**Figure 10C**). SR-8541A treated parental spheroids and culture medium were collected and analyzed for gene expression changes to assess STING pathway activation. There was a dose-dependent increase in the expression of IFN, CXCL10, and ISG15 genes (**Figure 10D**), along with an increase in the amount of secreted IFNand CXCL10 proteins (**Figure 10E**).

[redacted]

3b. Combination of SR-8S41A with immune checkpoint inhibitors potentiates the infiltration of immune cells in a breast cancer spheroid model. We treated human breast cancer spheroids (MDA-MB-231) with SR-8541A in the presence or absence of anti-CTLA-4/anti-PD-1. As shown in **Figure 11**, both the single-agent SR-8541A and the anti-CTLA-4/anti-PD-1 control showed a significant increase in immune infiltration, but the strongest effect was observed in the SR-8541A + anti-CTLA-4/anti-PD-1 combination group.

4. SR-8541A inhibits the growth of tumors in syngeneic mouse models and enhances the efficacy of checkpoint inhibitors

4a. Pharmacokinetics studies of SR-8S41A. Mice, rats, and dogs were administered a single intravenous (IV) or oral dose of SR8541A. Oral bioavailability was higher in rats and dogs compared to mice. Pharmacokinetic parameters are presented in **Table1**.

Table 1. Pharmacokinetic parameters of SR-8541A in mice, rats, and dogs following intravenous or oral administration.

Species	Mouse[c]		Rat[c]		Dog[b]	
&*t	V	'	V	'	V	'
&s (mg/kg)						
					a	a
C_{max}/C_o (ng/m)						
t_{max} (h)						
AUC_{ast} (ng/m *h)						
AUC_{nf} (ng/m *h)						
$t_{1/2}$ (h)						
CL (mL/m n/kg)						
Vss (L/kg)						
% F						

AUC_{ast} = area under plasma concentration time curve rom time O to last plasma collection time point AUC_{inf} = area under the plasma concentration time curve rom time O to in inity C_{max} = pea plasma concentration CL = Clearance N = number o animals NA = not applicable t_{max} = time o maximum plasma concentration $t_{/2}$ = elimination hal -li e Vss = volume o distribution at steady state [a] cross over design [b] values calculated rom the mean o male and emale dogs [c] mean ± SD

4b. SR-8S41A inhibits the growth of 4T-1 breast cancer and CT-26 colorectal cancer cells in vivo. SR-8541A has no direct cytotoxic effects on cancer cells, and it requires an intact immune system to impact tumor cells. Therefore, we have conducted efficacy studies in immunocompetent mouse models of breast and colorectal cancers. 4T-1 or CT-26 tumor cells were implanted orthotopically or subcutaneously in mice, respectively, and when tumors reached about 100-150 mm^3, SR-8541A was administered [redacted] In both models, SR-8541A showed a 34-36% tumor growth inhibition compared to the vehicle-treated tumors (**Figure 12A-B**). Importantly, there was an increase in the infiltration of both CD3 and CD8 positive T cells in CT-26 tumors treated with SR-8541A (**Figure 12C**). [redacted] The mechanism behind this observation is currently unclear, but similar results were reported for an approved PARP inhibitor, Olaparib (43).

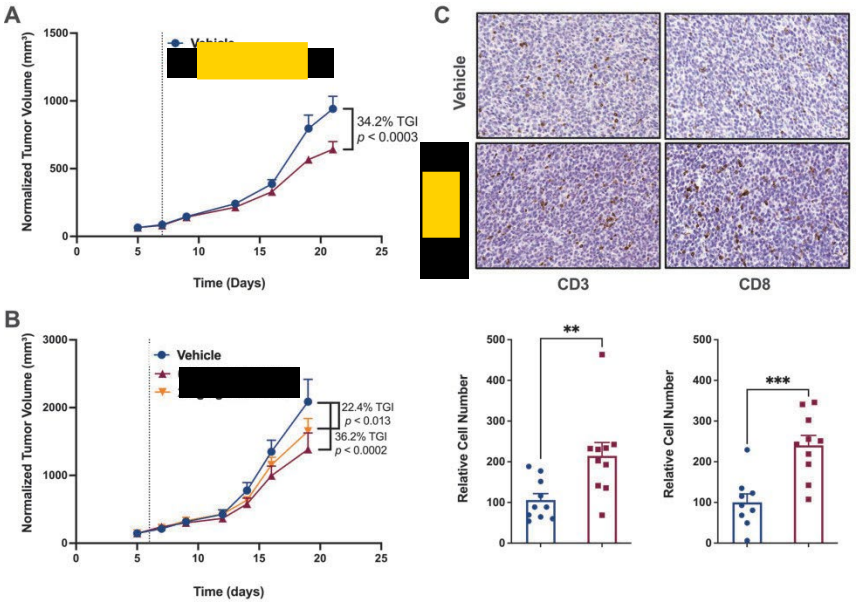

Figure 12. A) 4T-1 breast cancer ce s were mp anted orthotop ca y nto BALB/c m ce and treated w th SR-S541A ora y tw [redacted] ce da y at 0.2 mg/kg. **B)** CT- 26 co orecta cancer ce s were mp anted subcutaneous y nto the r ght f anks of BALB/c m ce and treated w th SR-S541A [redacted] Tumor vo ume and body we ght measurements were taken every three days for the durat on of the study and 10 m ce were used per arm. Data po nts represent mean ± SEM; t-test. **C)** Representat ve IHC mage (top) and quant f cat on (be ow) of ant -CD3 and ant -CDS mmune ce s n the CT-26 tumors treated w th veh c e or SR-S541A. Data po nts represent mean ± SEM; n = ≥ 9 tumors/group; ** $p < 0.01$, *** $p < 0.001$; t-test.

4c. SR-8S41A augments the effects of anti-PD-1 and anti-CTLA4 antibodies in a CT-26 colorectal cancer tumor model. We next tested if SR-8541A can work in combination with checkpoint inhibitors. We chose to test this first using the CT-26 tumor model as it is known to be sensitive to anti-mPD-1 and anti-mCTLA-4 treatment. CT-26 tumor cells were implanted subcutaneously in mice, and when tumors reached about 100-150 mm^3, SR-8541A was administered [redacted] daily at 0.2 mg/kg. In addition, Anti-mPD-1 and anti-mCTLA-4 antibodies were administered intraperitoneally weekly at 10 mg/kg. As shown in **Figure 13**, about 30% (4/14 mice) of SR-8541A treated mice, about 60% (9/15 mice) of the checkpoint only treated mice, and about 85% (11/13) of the combo treated mice had tumors less than the smallest tumor in the vehicle-treated group. These results suggest that SR-8541A enhances the effects of checkpoint inhibitors. We have similar studies planned for 4T-1 (resistant to anti-mPD-1 inhibitor), EMT-6 (moderately resistant to checkpoint inhibitors), and E0771 (sensitive to anti-mPD1) syngeneic breast tumor models.

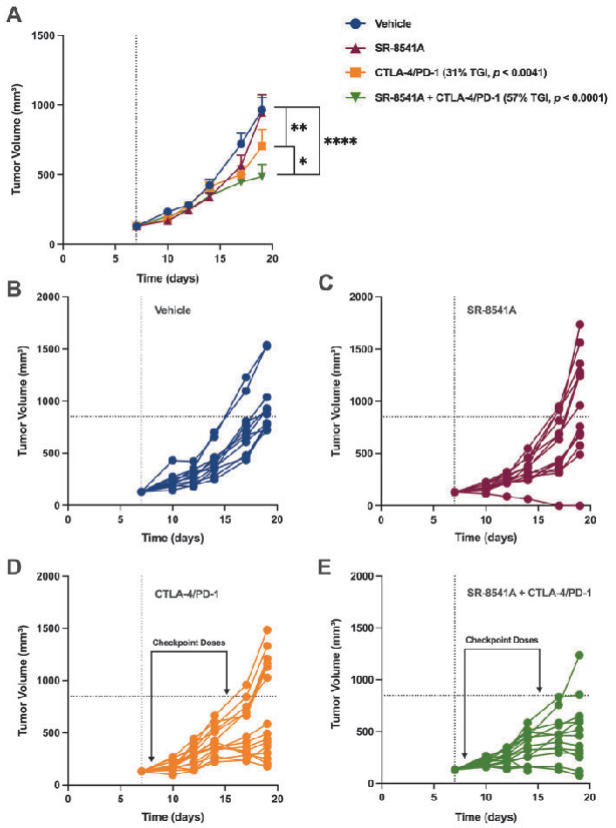

Figure 13. **A)** CT-26 co orecta cancer ce s were mp anted subcutaneous y nto the r ght f anks of BALB/c m ce and treated w th SR-S541A [redacted] M ce were a so treated w th ant - mPD-1 and ant -mCTLA-4 ant bod es once week y at 10 mg/kg. Tumor vo ume and body we ght measurements were taken every three days for the durat on of the study. Data po nts represent mean ± SEM; n = ≥10 m ce; * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$; t-test. **B-E)** Data for nd v dua m ce for each group p otted separate y.

5. Toxicology and tolerability studies of SR-8541A. The nonclinical safety/toxicology program was tailored to advance the clinical development of SR-8541A for oncology indications and support administration to patients with advanced cancer. SR-8541A was evaluated per International Conference on Harmonization (IHC) guidelines.

Sa. Genetic Toxicology: Bacterial cytotoxicity and AMES fluctuation test are complete for SR-8541A with no major findings.

Sb. Formulation analysis, plasma method validation, and stability: Formulation, method development, and validation studies have been completed for SR-8541A to support GLP studies. Extended stability studies (12 months) of SR-8541A under various environmental factors such as temperature and humidity are completed without any findings.

Sc. ADME: Stability of SR-8541A in mouse, rat, dog, and human liver microsomes and S9 fractions, along with the detection of the expected metabolites, are completed. Stability in human blood and human hepatocytes are completed. Binding to mice, rats, dogs, and human plasma proteins is completed. Preliminary findings from CYP induction and inhibition suggest that SR-8541A is not an inducer or an inhibitor of CYP enzymes tested.

Sd. Toxicology studies (Charles River Laboratories): We completed 7-day tolerability and 28-day toxicology studies in rats with no findings. SR-8541A was administered orally at 0, 100, 300, or 600 mg/kg in rats, and the no observed adverse effect level (NOAEL) was reported as 600 mg/kg. We have completed 7-day and 28-day tolerability studies in dogs where SR-8541A was administered orally at 0, 50, 100, or 200 mg/kg. We saw no [redacted] toxicity from ENPP1 inhibition,

D. Approach

AIM 1: Preclinical evaluation of SR-8S41A in combination with FDA-approved drug regimens

***Rationale*:** The standard of care for cancer patients is rapidly evolving, and numerous challenges remain, especially *de novo* or acquired resistance to therapies. Monotherapies have been less efficient primarily because of tumor cells' ability to activate alternate pathways and promote growth. Combination therapies have come to the forefront to combat this resistance and improve overall therapeutic efficacy. With a rational combination of therapeutic agents, physicians can extract maximum benefit for patients with minimal undesirable effects. We have shown that inhibition of ENPP1 with SR-8541A results in suppression of growth in breast and colorectal cancer cells, and importantly, SR-8541A improved the effects of checkpoint inhibitors on the growth of colorectal cancer cells *in vivo*. To further investigate SR-8541A as a combinatorial agent, we will conduct combination studies with chemotherapeutic and targeted therapeutics in breast cancer mouse models.

***Design and Methodology*:** We will partner with a CRO (either Charles River Laboratories or Covance) to conduct drug combination studies using a syngeneic mouse model platform. Drug combinations and breast cancer models for these studies are detailed in **Table 2**. We have selected cisplatin, an effective primary chemotherapeutic agent for breast cancer, checkpoint inhibitors anti-PD-1 and anti-CTLA-4, and the PARP inhibitor olaparib, approved for specific cancers carrying *BRCA* germline mutations. Briefly, treatment will be initiated 7-10 days post-implantation (orthotopic) or when the tumors have reached 150 mm^3 (subcutaneous). Each study will include a vehicle group, a single-agent group, and a combination group with fifteen mice per group. The study will consist of appropriate isotype IgG controls for checkpoint inhibitors. Mice will be monitored and euthanized if they lose 20% of their initial weight, develop ascites, cachexia, or display extreme weakness or inactivity. Body weights and tumor volume measurements will be collected every three days. The study will be terminated when the vehicle control group reaches 2000 mm^3. Tumors and blood will be collected at study termination, and samples shipped to TGen for immune marker analyses by the Sharma laboratory. Immunohistochemistry for CD45, CD3, CD4, CD8, CD206, and CD86 will be performed on tumor specimens. Plasma will be analyzed for cytokines and chemokines using the MSD multiplex platform. Markers will include IFNβ, IFNy, TNFa, IL6, IL10, IL15, IL1β, MCP1, and CXCL10. In addition, adenosine levels in the plasma will be measured using a fluorometric-based assay (Abcam). We expect to finish the in-life portion of the proposed studies in the first year and *ex vivo* analysis in the second year of the granting period.

Table 2. Drug regimens and cancer models to be used for combination studies.

Study #	Drug Combination	Breast Cancer Model
1	SRS541A + C sp at n	4T-1 (orthotop c)
2	SRS541A + Checkpo nt nh b tors	4T-1 (orthotop c)
3	SRS541A + Checkpo nt nh b tors	EMT-6 (orthotop c)
4	SRS541A + PARP nh b tor	EMT-6 (*BRCA1* KO, orthotop c)

[redacted]
Chemotherapy cisplatin 70 mg/kg via intraperitoneal injection once weekly
Checkpoint inhibitors anti-mC LA-4 and anti-mPD-1 10 mg/kg via intraperitoneal injections biweekly
PARP inhibitor Olaparib 100 mg/kg via oral gavage once daily

***Statistical approaches*:** An unpaired two-sided Student's t-test will be used to determine statistical significance between the control and treatment groups. All data will be screened for parametric statistical test assumptions. All statistical tests' *a priori* alpha level will be set at $p < 0.05$.

***Expected outcomes*:** These are routine studies readily performed by the identified CROs, so we do not anticipate any issues. Based on our preliminary data, we expect ENPP1 inhibition by SR-8541A, especially in combination, will significantly reduce the overall tumor burden in mice. In addition, our extensive *ex-vivo* analyses of both tumor and plasma should reveal the immune cell types that infiltrate and contribute to the growth inhibition of tumors. We also expect no toxicity with the proposed drugs and the dosage regimen.

***Limitations and alternative approaches*:** Mouse syngeneic tumor models possess unique tumor-immune and mutation profiles with differential responses to therapeutics, especially immunotherapies (44-46). Our data shows that SR-8541A has reduced activity on mENPP1 compared to hENPP1, but still, yet, it demonstrates anti-tumor activity in mouse models. However, SP-8541A may show partial or no effects in the models and combination studies proposed herein. Since appropriate tumor mouse models are limited, an alternative approach is to test the effects of SR-8541A alone or in combination using fresh patient-derived tumoroids for

breast cancer. Tumoroids are the best methodology for drug testing because they recapitulate the genomic, transcriptomic, proteomic, and immunogenic profiles of the tumor of origin (47, 48). Nilogen Oncosystems has pioneered this tumoroid technology for multiple cancer indications with various therapeutics. We will pursue additional funding to conduct these studies with Nilogen Oncosystems if it proves warranted.

AIM 2: Perform IND enabling dog GLP toxicology study on SR-8S41A

***Rationale*:** Stingray has completed a 28-day GLP rat toxicology study on SR-8541A with no related adverse clinical signs, changes in body weight, changes in food consumption, ophthalmic changes, or related clinical pathology changes.

***Design and Methodology*:** The objective of this study is to determine the potential toxicity of SR-8541A for the treatment of multiple cancer types when given orally for 28 days to dogs and to evaluate the potential reversibility of any findings. In addition, the toxicokinetic characteristics of SR-8541A will be determined. The following parameters and endpoints will be evaluated in this study: mortality, clinical observations, body weights, bodyweight gains, food consumption, ophthalmology, electrocardiography, clinical pathology parameters (hematology, coagulation, clinical chemistry, and urinalysis), toxicokinetic parameters, organ weights, and macroscopic and microscopic examinations. The design of this study follows the study design guidelines of the Committee for Human Medicinal Products for Human Use (CHMP), OECD Guideline 417, and ICH Harmonized Tripartite Guidelines M3 (R2), S3A, and S7B. The test article, SR-8541A, will be identified, analyzed, recorded and reserve samples kept. Doses will be formulated per instructions from Stingray, given per protocol, and reserve samples kept for analysis. Animals will be housed, handled, and attended to as specified in the USDA Animal Welfare Act (9 CFR, Parts 1, 2, and 3) and described in the Guide for the Care and Use of Laboratory Animals (49). An animal enrichment toy, certified dog treats, appropriate food, available water, regulated environmental conditions, and veterinary care will be provided to all study animals. The animals will be evaluated at cage side, post-dose, for daily food consumption, for detailed clinical observations, body weights weekly, and ophthalmic and electrocardiology. Standard clinical pathology tests will include hematology, coagulation, clinical chemistry, urinalysis, and representative tissue samples taken and analyzed from organs. Histology and histopathology will be performed. We will partner with Charles River Laboratories (CRL) to conduct this dog GLP toxicology study (see *Letter of Support*). We expect to initiate the study in Y1 Q1, which means we secure our birth in the CRL queue, and CRL begins study preparations and ordering for the study. Study initiation is planned for the end of Y1 Q1, in-life completion by Y1 Q3, and the draft report in Y2 Q1.

***Statistical approaches*:** Means, standard deviations (or % coefficient of variation or standard error, when deemed appropriate), ratio, percentages, numbers, and/or incidences will be reported as appropriate by dataset. All statistical tests' *a priori* alpha level will be set at $p < 0.05$. In addition, all pairwise comparisons will be conducted using 2-sided t-tests and reported at $p < 0.01$ and $p < 0.05$.

***Expected outcomes*:** We expect to complete a 28-day dog GLP toxicology, establishing a no observable adverse effect level (NOAEL) at [redacted].

***Limitations and alternative approaches*:** This study is an FDA requirement, and the only alternative would be to use non-human primates, which would be costly, and, we believe, unjustified.

AIM 3: cGMP tablet development, manufacture, and initial stability

***Rationale*:** SR-8541A is a small molecule with a 5.09-hour half-life and 73.95% oral bioavailability in dogs. It is soluble, permeable, and stable in storage. [redacted] the exact dosage size, and mini-tablet development will be sufficiently robust to accommodate variations to the final dosage requirements.

***Design and Methodology*:** Catalent Pharma Solutions supports many pharmaceutical and biotechnology companies in oral dosage design and has the specialized expertise and facilities to optimize these formulations for early clinical use within all regulatory guidelines. Catalent will be providing Stingray their cGMP API storage, drug product development, tableting, packaging, labeling, and analytical services to the SR-8541A program to complete the drug product requirements for IND acceptance and Phase 1 clinical needs (see *Letter of Support*). Stingray has provided Catalent with the analytical methods for drug substances and has shipped them 5 kg of API. Another 5 kg remains at the API manufacturer's site. Catalent will develop analytical methods for assay and related substances, content and blend uniformity, dissolution, microbial quality, and cleaning verification. These methods will be validated as appropriate for Phase 1 clinical use. Catalent will develop a formulation, including excipient compatibility, compressibility, and roller compaction feasibility, to identify suitable granulation blends with appropriate density and powder flow for tableting. Based on this work, tableting development will commence by establishing compression profiles for each granulation blend. Tablets will be evaluated for hardness, weight uniformity, disintegration, dissolution, friability, assay and related substances, uniformity of content, and water content. Once suitable tablets have been developed, Catalent will develop a coating process and pick two mini-tablet formulations for a 3-month stability study under two storage conditions [25°C/60% relative humidity (RH) and 40°C/75% RH]. Stability testing will include visual appearance, assay, related substances, dissolution, water content, content uniformity, and hardness. Based on these results, Catalent will manufacture one engineering batch at a scale consistent with the equipment used for the GMP batch, which will support release testing and ICH stability testing. Stability will again be at 25°C/60% RH and 40°C/75% RH for 12 months with the same tests done as previously mentioned. Catalent will then manufacture one GMP drug product batch at a scale of 200,000 tablets. Testing will include blend uniformity, hardness, weight uniformity, and friability. The mini-tablets will be film-coated and packaged in 60 count HDPE bottles for stability and clinical use, with some remaining in bulk for bulk stability purposes and later packaging. Clinical supply will go to a clinical pharmacy after appropriate cGMP release testing and procedure for eventual shipment to the clinical sites. We expect to begin the development in Y1Q1 and have tablets manufactured for clinical development in Y2Q1.

***Expected outcomes*:** We expect to emerge with an oral formulation appropriate for human clinical use and delivery to patients.

***Limitations and alternative approaches*:** We have bid the project with additional oral tablet suppliers and believe Catalent is the best combination of price, expertise, and quality. Capsule manufacture or other oral formulations could be considered. However, we think that mini tablets are the most versatile and best option for this program.

At the conclusion of this work, we will have completed the necessary preclinical research and development of SR-8541A, developed a clinical strategy to test as a single agent or in combination, and submitted the IND application.

PHS Human Subjects and Clinical Trials Information

0MB Number: 0925-0001

Expiration Date: 09/30/2024

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

VERTEBRATE ANIMALS

All portions of the studies requiring the manipulation of live animals with be conducted by a reputable CRO. Specimen samples collected in Aim 1 will be mailed via dry ice to TGen for further analysis.

Specific Aim 1: Preclinical evaluation of SR-8541A in combination with FDA-approved drug regimens.

1. Description of procedures. The purpose of this study is to evaluate SR-841A as a combinatorial agent with chemotherapeutic and targeted therapies in breast cancer using a syngeneic mouse model platform. We will partner with a CRO, either Charles River Laboratories or Covance, to conduct these studies. We will use 6- to 8-week-old female Balb/c mice and the murine orthotopic breast cancer models (4T-1, EMT-6, and EMT-6 BRCA KO) to evaluate the anti-tumor activity and biological effects of the combination therapies as detailed in **Table 1**. In this study, we will utilize 15 animals/group x 4 treatment groups (vehicle, single agent 1, single agent 2, and combination) x 4 study arms x 25% failure rate = **300 animals**. Treatment will be initiated 7-10 days post-implantation (orthotopic) or when the tumors have reached 150 mm^3 (subcutaneous). Body weights and tumor volume measurements will be collected every three days. Tumors and blood will be collected at study termination and samples shipped to TGen for immune marker analyses by the Sharma laboratory.

Table 1. Drug regimens and cancer models to be used for combination studies.

Study Arm	Drug Combination	Breast Cancer Model
1	SR8541A + C sp at n	4T-1 (orthotop c)
2	SR8541A + ant -mCTLA-4/ ant -mPD-1	4T-1 (orthotop c)
3	SR8541A + ant -mCTLA-4/ ant -mPD-1	EMT-6 (orthotop c)
4	SR8541A + PARP nh b tor	EMT-6 (*BRCA1* KO, orthotop c)

[redacted]
Chemotherapy cisplatin 70 mg/kg via intraperitoneal injection once weekly
Checkpoint inhibitors anti-mC LA-4 and anti-mPD-1 10 mg/kg via intraperitoneal injections biweekly
PARP inhibitor Olaparib 100 mg/kg via oral gavage once daily

2. Justifications. Orthotopic syngeneic tumor models are a preferred preclinical model for evaluating the efficacy of novel anti-cancer agents as it permits the tumor to grow in the organ of origin in their natural tumor environment, while also providing an effective approach for studying tumor immunity and immunotherapy response in the presence of a fully functionally immune system. The Balb/c mouse is the background strain for the selected orthotopic murine breast cancer models to be tested.

3. Minimization of pain and distress. All efforts will be made to limit distress of the animals; however, there are some studies which may cause unavoidable stress, including the described tumor studies. Mice will be monitored daily for signs of distress. IACUC approved methodology will be used to rank each individual mouse on a daily basis in terms of overall health, hygiene, and distress levels. Mice will be euthanized if they lose 20% of their initial body weight, develop ascites, cachexia, or display extreme weakness or inactivity.

Specific Aim 2: Perform IND enabling dog GLP toxicology study on SR-8541A.

1. Description of procedures. Charles River Laboratories will conduct this dog GLP toxicology study. The objective of this study is to determine the potential toxicity of SR-8541A for the treatment of multiple cancer types when given orally for 28 days to dogs and to evaluate the potential reversibility of any findings. Therefore, there will be a 28-day recovery period following dosing. The study design is detailed in **Table 2** and will use 5- to 7-month-old male and female Beagle dogs (Marshall BioResources, North Rose, NY). In addition, the toxicokinetic characteristics of SR-8541A will be determined. The following parameters and endpoints will be evaluated in this

Table 2. A 28-day dog GLP toxicology study on SR-8541A.

Group	Test Material	Dose Level (mg/kg/day)	Dose Volume (mL/kg)	Number of Animals			
				Main Study		Recovery Study	
				Males	Females	Males	Females
1	Veh c e	0	10	4	4	2	2
2	SR-8541A	TBD	10	4	4	0	0
3	SR-8541A	TBD	10	4	4	2	2
4	SR-8541A	TBD	10	4	4	2	2

study: mortality, clinical observations, body weights, bodyweight gains, food consumption, ophthalmology, electrocardiography, clinical pathology parameters (hematology, coagulation, clinical chemistry, and urinalysis), toxicokinetic parameters, organ weights, and macroscopic and microscopic examinations.

2. Justifications. The design of this study follows the study design guidelines of the Committee for Human Medicinal Products for Human Use (CHMP), OECD Guideline 417, and ICH Harmonized Tripartite Guidelines M3 (R2), S3A, and S7B. This study is a requirement for Investigational New Drug Application and Acceptance from the Oncology Division, FDA.

3. Minimization of pain and distress. Animals will be housed, handled, and attended to as specified in the USDA Animal Welfare Act (9 CFR, Parts 1, 2, and 3) and described in the Guide for the Care and Use of Laboratory Animals. An animal enrichment toy, certified dog treats, appropriate food, available water, regulated environmental conditions, and veterinary care will be provided to all study animals. The animals will be evaluated at cage side, post-dose, for daily food consumption, for detailed clinical observations, body weights weekly, and ophthalmic and electrocardiology.

MULTIPLE PI LEADERSHIP PLAN

Rationale and Justification for Choosing the Multiple PI Approach. Drs. Kaadige and Sharma are members of the multiple PI team on the application. Dr. Kaadige has extensive experience in discovering and developing small-molecule inhibitors for dysregulated proteins in oncology. Dr. Sharma is a distinguished physician-scientist with extensive expertise in translational drug discovery, including over 50 clinical trials. The multiple PI approach is justified because each PI brings expertise to the project that is non-overlapping with their counterpart. By jointly leading from their respective strengths, the design of the research plan is strengthened, the proximity of the in vivo evaluations is kept close, and the pace of feedback for improved hypothesis testing is accelerated beyond what the leaders could deliver independently or in a hierarchical approach.

Governance and Organizational Structure. Dr. Kaadige is the contact PI for the proposal and will be the lead PI for the grant due to his experience as Principal Scientist at Stingray Therapeutics on the proposed drug product. Drs. Kaadige and Sharma will select three external advisory board (EAB) members from the scientific and/or life science business community. The multi-PI team considers these II external II because they will have no vested interest in the proposed work. Members of the EAB will be senior scientists with the rank of Professor or higher and/or CEOs, and they will not be members of Stingray Therapeutics or TGen. The EAB will be utilized for conflict resolution when Drs. Kaadige and Sharma are not in agreement or cannot reach an agreement.

Procedures for Resolving Conflicts. Drs. Kaadige and Sharma do not expect any issues regarding conflicts or resolution of conflicts that may arise. However, they recognize the potential need for a plan to resolve conflicts. They will employ the EAB as an arbitration panel for conflicts that cannot be resolved through communication between the two PIs. The decision of the EAB will be final.

Process for Making Decisions on Scientific Direction and Allocating Resources and Funds. As lead PI Dr. Kaadige will make decisions regarding the scientific direction and the allocations of resources and funds. He will do this in collaboration with Dr. Sharma; however, if an agreement cannot be reached, the EAB will step in to resolve the conflict.

Communication. The two PIs hold regular standing laboratory meetings within their respective groups. In addition to these more general laboratory meetings, the PIs will hold joint monthly laboratory meetings entirely focused on the experiments pertinent to this grant. At these laboratory meetings, both teams will also share raw and processed data with each other. Both sites will therefore have access to the data in a timely fashion, and the storage of the raw data at two sites will act as an additional safeguard against data corruption (above and beyond the multiple protections already employed within each laboratory).

Data Sharing Within the Research Team. All data will be shared freely and openly in both raw and processed form each month during the combined laboratory meeting. This meeting will serve as a notification of completed experiments and the deposition of the data related to those experiments in a freely accessible shared folder.

Collaborative Publication Policies. Publications and Conference Abstracts will be led by the team responsible for the creation of the first draft. The team responsible for the first draft will be based on the decision of Dr. Kaadige as lead PI. We anticipate submitting two conference abstracts during the grant period.

Intellectual Property. Rights in any pre-existing intellectual property will remain the property of the party that created and/or controls it. Stingray Therapeutics and TGen have executed agreements previously related to IP and expect to do so without issue related to the IP associated with this grant proposal.

Change in PI Location. In the event that one of the PIs moves to a new institution or business, attempts will be made to transfer the relevant portion of the grant to the new business/institution. In the event that a PI cannot carry out his/her duties, a new PI will be recruited as a replacement, subject to the approval of the business/ institution involved and the NIH funding institute staff.

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COOPERATING INSTITUTION CONSORTIUM STATEMENT

The Translational Genomics Research Institute (TGen) is proposing to participate in this application as described below:

PRIME GRANTEE/CONTRACTOR ORGANIZATION: Stingray Therapeutics, Inc.

Principal Investigator:	Dr. Mohan Kaadige
Sponsoring Agency:	NIH-NCI
Sponsor Number (if known):	PA-21-259
Project Title:	Development of a potent and selective oral ENPP1 inhibitor for oncology
Next Budget Period:	09/01/2022-08/31/2023
Total Project Period:	09/01/2022-08/31/2024

SUB-GRANTEE/CONTRACTOR: Translational Genomics Research Institute (TGen)

Project Director:	Dr. Sunil Sharma
Phone:	[redacted]
E-Mail:	[redacted]
Project/Subproject Title:	Development of a potent and selective oral ENPP1 inhibitor for oncology

Human Subjects: ☐ Yes ☒ No IRB Approval Date:
Animal Subjects: ☒ Yes ☐ No IACUC Approval Date: pending
IACUC Location:

First Year Budget Period Costs:	Direct:	[redacted]
	F&A:	[redacted]
Total Project Period Costs:	Direct:	[redacted]
	F&A:	[redacted]

F & A Cost Rate: ☒ MTDC ☐ TDC ☐ S/W ☐ Other (Explain):

The appropriate programmatic and administrative personnel of Translational Genomics Research Institute (TGen) involved in this grant application are aware of the PHS consortium grant policy and will establish the necessary inter-institutional agreement(s) consistent with that policy. TGen makes all applicable assurances/certifications, and has implemented a written policy for Investigator Financial Disclosure and Conflict of Interest consistent with PHS requirements. **Additionally, TGen supports Dr. Mohan Kaadige transitioning his primary employment to Stingray Therapeutics, Inc. throughout the proposed project.**

[redacted]
Jatan Clark, M.B.A.
Sr. Director, Office of Sponsored Research

04/05/2022
Date

RESOURCE SHARING PLAN

As Stingray Therapeutics is a for-profit venture, the securing of intellectual property will precede any public disclosures. Stingray Therapeutic and TGen's resource sharing plan includes:

A. Data Sharing Plan

Awardees will retain custody of and primary rights to their data and intellectual property developed under the award subject to current government policies regarding rights of access. Pursuant to NIH policies, data will be released immediately following the exercise of intellectual property rights, if applicable, and the receipt of notification of acceptance to publish, if applicable. NIH recommended time period will be adhered to whenever practicable. Our dissemination plan also includes: (i) presentations at scientific conferences; (ii) presentations to individual investors and investor groups; and (iii) presentations to prospective pharmaceutical partners. In addition, we will continue our practice of making available to the research community reagents and resources presented in publications using standard Material Transfer Agreements.

B. Sharing Model Organisms

Awardees adhere to the NIH Grant Policy on Sharing of Unique Research Resources and the Policy on Sharing Model Organisms for Biomedical Research (NOT-OD- 42-042, NOT-OD-04-066). Any unique model organism resources generated under this award will be distributed freely or deposited in a repository available to the broader research community, either before or immediately after publication. In addition, these resources will be made available for use at academic or not-for-profit institutions at no cost except for standard transportation expenses, and if applicable, the cost of producing the materials/models.

C. Genomic Data Sharing

Not applicable for this proposal.

AUTHENTIFICATION OF KEY BIOLOGICAL AND/OR CHEMICAL RESOURCES

The proposed research will utilize the following key biological resources:

Experimental studies outlined in this proposal will be conducted at TGen or by reputable CROs. Measures will be taken to ensure the validity and reproducibility of purchased products. All studies will include appropriate controls and quality assessments.

In Vivo: Aim 1 covers all mouse models in the research proposal. Mice (Balb/c) are maintained directly by the vendor (Crown Bio and Covance) and are responsible for genetic testing to prevent genetic drift. Murine cell lines used in these models are sourced and validated by each vendor.

Drugs and chemicals: We have already synthesized GMP and GLP grade SR-8541A from Laxai to be used for mouse models and GLP toxicology. Checkpoint inhibitors (anti-mCTLA-4 and anti-mPD-1) will be purchased by the mouse model CRO from Bio X Cell. Olaparib and Cisplatin will be sourced and validated by the corresponding CRO.

Antibodies: Ex vivo analysis of specimens procured in Aim 1 will be completed using antibodies from reputable commercial sources such as: BD Biosciences, Cell Signaling Technologies, Abcam, and Bio X Cell.

Dogs: Aim 2 covers the IND-enabling dog GLP toxicology to be completed at Charles River Laboratories. Dogs will be sourced directly by the vendor. The vendor will follow the study design guidelines of the Committee for Human Medicinal Products for Human Use (CHMP), OECD Guideline 417, and ICH Harmonized Tripartite Guidelines M3 (R2), S3A, and S7B.