

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

		3. DATE RECEIVED BY STATE	State Application Identifier
1. TYPE OF SUBMISSION*		4.a. Federal Identifier HL164131-A1	
<input type="radio"/> Pre-application <input checked="" type="radio"/> Application <input type="radio"/> Changed/Corrected Application		b. Agency Routing Number	
2. DATE SUBMITTED	Application Identifier	c. Previous Grants.gov Tracking Number	
5. APPLICANT INFORMATION			UEI* : EKE1RJKQSGL9
Legal Name*: LIGHTSEED, INC. Department: Division: Street1*: 2845 NE 9TH ST APT 604 Street2: City*: FORT LAUDERDALE County: State*: FL: Florida Province: Country*: USA: UNITED STATES ZIP / Postal Code*: 333043650			
Person to be contacted on matters involving this application Prefix: First Name*: Richard Middle Name: Last Name*: Pestell Suffix: Position>Title: Professor Street1*: 2845 NE 9TH ST APT 604 Street2: City*: FORT LAUDERDALE County: State*: FL: Florida Province: Country*: USA: UNITED STATES ZIP / Postal Code*: 333043650 Phone Number*: 2674020545 Fax Number: Email: richard.pestell@gmail.com			
6. EMPLOYER IDENTIFICATION NUMBER (EIN) or (TIN)*		14-1995891	
7. TYPE OF APPLICANT*		R: Small Business	
Other (Specify): <input type="checkbox"/> Small Business Organization Type <input type="radio"/> Women Owned <input type="radio"/> Socially and Economically Disadvantaged			
8. TYPE OF APPLICATION*		If Revision, mark appropriate box(es). <input type="radio"/> New <input type="radio"/> Resubmission <input checked="" type="radio"/> Renewal <input type="radio"/> Continuation <input type="radio"/> Revision <input type="radio"/> A. Increase Award <input type="radio"/> B. Decrease Award <input type="radio"/> C. Increase Duration <input type="radio"/> D. Decrease Duration <input type="radio"/> E. Other (specify):	
Is this application being submitted to other agencies?* <input type="radio"/> Yes <input checked="" type="radio"/> No		What other Agencies?	
9. NAME OF FEDERAL AGENCY*		10. CATALOG OF FEDERAL DOMESTIC ASSISTANCE NUMBER	
National Institutes of Health		TITLE:	
11. DESCRIPTIVE TITLE OF APPLICANT'S PROJECT*			
Improving outcomes in cancer treatment-related cardiotoxicity.			
12. PROPOSED PROJECT		13. CONGRESSIONAL DISTRICTS OF APPLICANT	
Start Date* 12/01/2024		Ending Date* 11/30/2026	
		FL-023	

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

Prefix:	First Name*:	Xuanmao	Middle Name:		Last Name*:	Jiao	Suffix:
Position/Title:	Senior Researcher						
Organization Name*:	LIGHTSEED, INC.						
Department:							
Division:							
Street1*:	2845 NE 9TH ST APT 604						
Street2:							
City*:	FORT LAUDERDALE						
County:							
State*:	FL: Florida						
Province:							
Country*:	USA: UNITED STATES						
ZIP / Postal Code*:	333043650						
Phone Number*:	2404861421	Fax Number:	Email*: xuanmao.jiao@bblumberg.org				

15. ESTIMATED PROJECT FUNDING

a. Total Federal Funds Requested*	\$2,029,100.00
b. Total Non-Federal Funds*	\$0.00
c. Total Federal & Non-Federal Funds*	\$2,029,100.00
d. Estimated Program Income*	\$0.00

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

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

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

I agree*

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

18. SFLLL or OTHER EXPLANATORY DOCUMENTATION

File Name:

19. AUTHORIZED REPRESENTATIVE

Prefix:	First Name*:	Richard	Middle Name:		Last Name*:	Pestell	Suffix:
Position/Title*:	Professor						
Organization Name*:	LIGHTSEED, INC.						
Department:							
Division:							
Street1*:	2845 NE 9TH ST APT 604						
Street2:							
City*:	FORT LAUDERDALE						
County:							
State*:	FL: Florida						
Province:							
Country*:	USA: UNITED STATES						
ZIP / Postal Code*:	333043650						
Phone Number*:	2674020545	Fax Number:	Email*: richard.pestell@gmail.com				

Signature of Authorized Representative*

Completed on submission to Grants.gov

Date Signed*

03/28/2024

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

424 R&R and PHS-398 Specific

Table Of Contents

SF 424 R&R Cover Page.....	1
Table of Contents.....	3
Performance Sites.....	4
Research & Related Other Project Information.....	6
Project Summary/Abstract(Description).....	7
Project Narrative.....	8
Facilities & Other Resources.....	9
Equipment.....	19
Research & Related Senior/Key Person.....	21
Research & Related Budget Year - 1.....	81
Research & Related Budget Year - 2.....	84
Budget Justification.....	87
Research & Related Cumulative Budget.....	89
Research & Related Budget - Consortium Budget (Subaward 1).....	91
Research & Related Budget - Consortium Budget (Subaward 2).....	100
Total Direct Costs Less Consortium F&A.....	109
SBIR STTR Information.....	110
Commercialization Plan.....	112
PHS398 Cover Page Supplement.....	124
PHS 398 Research Plan.....	126
Specific Aims.....	127
Research Strategy.....	128
Progress Report Publication List.....	140
PHS Human Subjects and Clinical Trials Information.....	141
Vertebrate Animals.....	143
Bibliography & References Cited.....	146
Consortium/Contractual Arrangements.....	153
Letters of Support.....	156
Resource Sharing Plan(s).....	172
Other Plan(s).....	173
Authentication of Key Biological and/or Chemical Resources.....	174

Project/Performance Site Location(s)

Project/Performance Site Primary Location

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

Organization Name: LIGHTSEED, INC.
UEI: EKE1RJKQSGL9
Street1*: 2845 NE 9TH ST APT 604
Street2:
City*: FORT LAUDERDALE
County:
State*: FL: Florida
Province:
Country*: USA: UNITED STATES
Zip / Postal Code*: 333043650
Project/Performance Site Congressional District*: FL-023

Project/Performance Site Location 1

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: Baruch S. Blumberg Institute
UEI: NAYCKSJ7F68
Street1*: 100 Lancaster Avenue
Street2:
City*: Wynnewood
County:
State*: PA: Pennsylvania
Province:
Country*: USA: UNITED STATES
Zip / Postal Code*: 19096-3450
Project/Performance Site Congressional District*: PA-005

Project/Performance Site Location 2

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: Lankenau Institute for Medical Research

UEI: X7Y6RNVA4NR6

Street1*: 100 Lancaster Avenue

Street2:

City*: Wynnewood

County:

State*: PA: Pennsylvania

Province:

Country*: USA: UNITED STATES

Zip / Postal Code*: 19096-3450

Project/Performance Site Congressional District*: PA-005

Additional Location(s)

File Name:

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

1.a. If YES to Human Subjects

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

IRB Approval Date:

Human Subject Assurance Number

2. Are Vertebrate Animals Used?* Yes No

2.a. If YES to Vertebrate Animals

Is the IACUC review Pending? Yes No

IACUC Approval Date:

Animal Welfare Assurance Number none

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

4.b. If yes, please explain:

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

4.d. If yes, please explain:

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

5.a. If yes, please explain:

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

6.a. If yes, identify countries:

6.b. Optional Explanation:

Filename

7. Project Summary/Abstract* Summary_LightSeed_20240329.pdf**8. Project Narrative*** Narrative_LightSeed_20240329.pdf**9. Bibliography & References Cited** References_LightSeed_20240329.pdf**10. Facilities & Other Resources** Facilities_LightSeed_20240328.pdf**11. Equipment** Equipment_LightSeed_20240328.pdf

PROJECT SUMMARY

Anthracyclines, such as doxorubicin (DOX), are currently used to treat cancers (breast, stomach, uterus, ovary, bladder, lung), leukemia, and lymphoma. Chemotherapy-induced cardiac toxicities, most significantly cardiomyopathy, have substantial morbidity and mortality. The only FDA-approved preventive medication, dexrazoxane, reduces the anti-cancer efficacy of DOX-based chemotherapeutic agents, exhibits myelotoxicity as a concerning side effect, and is relatively ineffective in preventing DOX-induced toxicities. Given the current morbidity and mortality from DOX induced cardiotoxicity, there is an urgent unmet need to develop novel approaches to protect from DOX-induced cardiotoxicity. Several companies have been developing alternative formulations of Dox to reduce cardiotoxicity, some of which are now approved for use in Europe. Partially funded by a Phase I award (5R43HL164131-01A1), LightSeed sought to identify FDA-approved and novel drugs with a “dual function” 1) to minimize DOX-induced cardiotoxicity, while 2) enhancing cancer cell killing by DOX. We identified several novel cardio protectants, including two antagonists of the G-protein coupled receptor cysteine-cysteine chemokine receptor 5 (CCR5), maraviroc and vicriviroc, as well as ten additional compounds that protect cardiac cells from DOX-induced apoptosis. In preclinical mouse studies, maraviroc enhanced DOX induced cancer cell killing in adult and pediatric malignancies. Our preliminary studies demonstrate that 1) Maraviroc provides substantial cardiac protection in cultured cardiac myocytes (human cell lines, and canine myocytes) and in a commonly used mouse model of chronic DOX cardiotoxicity; 2) Maraviroc enhanced cancer cell killing; 3) Maraviroc enhanced survival in a murine model of triple negative breast cancer; 3) Novel compounds provide cardioprotection in human cardiomyocyte cell lines. In line with the commendation of the NIH needs assessment and our freedom to operate IP analysis this Phase II proposal will first test the cardioprotective activity of the CCR5 inhibitors in combination with four different DOX formulations which have shown partial reduction in cardiotoxicity. The impact of CCR5i on **(a)** PEGylated liposomes, Doxil; **(b)** non-pegylated liposomes, Myocet; **(c)** a polymeric micelle (PM), SP1049C; and **(d)** the polymeric nanoparticle (PNP) LivaTag will be compared in an accepted mouse model of DOX-induced cardiotoxicity. We will determine the most effective sequence and dose of administration. We will then use the optimal DOX formulation/sequence of administration and test the combination with the additional cardio protectants identified during our Phase I proposal. We will thereby identify the most effective cardio protectant/DOX formulation combination with dual function. Aim 2 will assess the antitumor efficacy of this combination in C57BL/6 mice implanted with orthotopic Py8119 tumors. Aim 3 will perform preliminary safety / toxicology studies with the cardio protectant/DOX combination in Sprague Dawley rats. This novel drug combination will have an impact in the treatment of cancer by simultaneously enhancing the antitumor efficacy of DOX while protecting from DOX-induced cardiotoxicity.

PROJECT NARRATIVE

The improved survival of cancer patients, with more than one-third surviving at least five years after their initial diagnosis, is partly due to chemo- (doxorubicin—DOX) and radiation therapies; however, such treatments cause significant cardiotoxicity. We have shown that the FDA-approved inhibitor of the CCR5 receptor maraviroc mitigates DOX-induced cardiotoxicity while enhancing DOX-induced breast cancer cell killing. The studies proposed in this Phase II renewal will assess the combination of various cardio protectants with different DOX reformulations to identify the most potent combination and the optimal sequencing of administration that achieves strong antitumor efficacy and cardio protection, followed by preliminary safety to support future IND-enabling studies in preparation for human clinical trials.

FACILITIES AND OTHER RESOURCES

LightSeed is renting space at the Lankenau Institute for Medical Research (LIMR). The PI of this application, Dr. Xuanmao Jiao, Principal Scientist at LightSeed, is also an Associate Professor at the Baruch S. Blumberg Institute and occupies laboratory space together with Drs. Pestell and Jiao at LIMR. Dr. Pestell is also a member of the Wistar Cancer Center. As employees of the Baruch S. Blumberg Institute, they all have access to facilities and shared resources at all three research institutions, located within close distance of each other.

Biohazards Handling and Disposal. All work involving biological agents/materials including cancer cell lines will be carried out in compliance with NIH Guidelines and the CDC/NIH Biosafety in Microbiological and Biomedical Laboratories (BMBL) practices. LightSeed scientists involved in this proposal will be responsible for the safe practices of biohazard handling and waste disposal as defined in the policies and procedures established in LightSeed's Biosafety Manual, which is available to all personnel. Each Institution participating have their own Biosafety Manual and BMBL policies, and all laboratory personnel is required to attend annual safety training; strict adherence to safety practices and personal protective equipment requirements are implemented at each institution.

Intellectual Property. LightSeed has a pending patent around the use case of drugs to reduce cardiotoxicity with cancer therapies (US20230035491A1, filed in December 15, 2020). A patent entitled "Use of modulators of CCR5 in the treatment of cancer" (US 9,453,836) was issued in 2016. LightSeed is currently expanding its IP protection strategy, with the ten new compounds, and plans to submit additional protections for current and future applications of this technology.

PESTELL LABORATORY

Dr Pestell has access to facilities at three Institutes, expanding the repertoire of capabilities. The geographical separation of the additional facilities at Wistar and Blumberg has not been a barrier attested by the publications since the laboratory located at LIMR.

1. The Lankenau Institute for Medical Research (LIMR)
2. The Blumberg Institute
3. The Wistar Institute

Dr. Pestell has appointments as Distinguished Professor at the Blumberg Institute, his laboratory is physically located at LIMR with access to its facilities and has a formal Adjunct Appointment at the Wistar Institute, and is a Wistar Cancer Center Member, allowing access to their facilities. EI PI Dr. Xuanmao Jiao is also an Associate Professor at The Blumberg Institute.

Laboratory: Drs. Pestell and Jiao occupy very well-equipped laboratories for approximately 1,100 square feet of usable laboratory space at the Lankenau Institute for Medical Research. The Facilities that are used extensively by the lab are located on the same floor. The services of these facilities are provided on a charge-back basis.

Clinical: The PI has access to clinical material through a Core Tumor repository with matched normal and malignant tissues.

Animal: The Lankenau Institute for Medical Research has a Central Animal Facility accredited by AAALAC. The transgenic and knockout mice used in this project have been generated and well characterized in the preliminary studies.

Computer: The PI has a Macintosh Power PC computer which is networked and a Hewlett Packard Laser Jet IV printer. The laboratories are also equipped with computers which are networked and can converse by high- speed modem. A Digital computer is dedicated to literature searching in a separate room.

Office: The PI has an office in close proximity to the laboratory.

Other: The Proteomics Facility used for the preliminary studies is located at the Wistar Institute and accessible by user fees. This Facility consists of multiple sections, for protein sequencing, amino acid analysis, protein/peptide purification by HPLC or electrophoretic techniques (e.g. electro blotting), peptide synthesis, and mass spectrometry. Other facilities are located at the Lankenau Institute for Medical Research (LIMR) and include Bio imaging, Biostatistics, Cytogenetics, Flow Cytometry, Pathology, and X-Ray Crystallography and other facilities located at the Wistar Institute. The services of the facilities are provided on a charge-back basis. All the

LIMR facilities are located on the same floor or within two floors from Dr. Pestell's laboratory in the same building. The administrative office for LIMR is located on the floor below the PI's office. There is a conference room and library that contains key journals. The transgenic mice are housed on the same floor as the laboratory providing easy access for the proposed experiments. The laboratories are well equipped for molecular biology experiments. The investigators of LIMR, the Blumberg Institute and the Wistar Institute, are focused on cancer research and represent a substantial collective group of approximately funded laboratories. Many laboratories are actively involved in studies of transcriptional control, innovative transgenic mouse modeling and translational research initiatives. This Institution provides a rich research environment that fosters the exchange of ideas and provides a number of relevant seminars, symposia, and journal clubs.

LANKENAU INSTITUTE FOR MEDICAL RESEARCH

Shared Resource Summaries

The proposed project work will be conducted at The Lankenau Institute for Medical Research. The laboratories LIMR have all the resources and facilities to do the proposed research.

LIMR (affiliated with Thomas Jefferson University) is a state-of-the-art research facility located in a modern, 53,000-square-foot three-story building contiguous with the Lankenau Medical Center, which contains a 320-bed tertiary care teaching hospital in Wynnewood, PA. LIMR and the Medical Center have a vibrant Infectious Disease group. All equipment required for the project is available at LIMR.

Laboratory: Within LIMR are sixteen 1,137-square-foot laboratories located on the perimeter of the first and second floors that are designed and equipped to support research utilizing the latest molecular and biochemical techniques. All laboratories are equipped with at least one fume hood and one to two laminar flow hoods serviced with vacuum and gas. CO₂ incubators are supplied by a central CO₂ generating system located in the loading dock adjacent to the ground floor. All the laboratories are well equipped with basic equipment necessary to perform molecular and biochemical techniques, including micro-centrifuges, balances, shakers, water baths, SDS PAGE equipment, transblot apparatus, agarose gel electrophoresis equipment, PCR machines, real time qPCR, power supplies, gel dryers, pH meters, warm/stir plates, vortexes, a variety of pipeting devices, lyophilizers, rotoevaporators, ultrafiltration apparatus, 4 hybridization ovens, deli-style refrigerators, microwaves, ELISA plate readers, and spectroflurometers. Low temperature freezers are in a central location as are liquid nitrogen storage units supported from a central tank.

Animal: The Animal Care and Use Program at LIMR has been fully accredited by AAALAC International since 1987 and has the distinction of being given Emeritus status by AAALAC International in 2006. All the laboratory animals are housed in the Research Annex. The Annex encompasses 6,400 gross square feet and is designed to accommodate the housing of rodents and rabbits. It contains (a) six animal rooms, (b) a cage wash area, (c) rooms for storage, animal receiving, and feed, and (d) men's and women's locker rooms, a janitor closet, and an office. A Class II Type B2 hood is available for work with athymic mice. All mice are housed in micro-isolator cage units. An IVIS bioluminescence imager, MicroCT scanner, and ultrasound imager are located in the Research Annex. Immunocompromised mice are housed under SPF conditions in the Opti Mice cage units. Veterinary care programs are overseen by a Veterinary Consultant who is on call 24 hours a day. A supervisor and two animal care technicians are employed by LIMR to manage the facility.

Computer: All the laboratories have personal computers having full access to both internet and intranet service via the Main Line Health System Network. Wireless internet service is also available throughout the Institute.

Other: A centralized Editorial Office provides assistance with the preparation of grant applications, manuscripts, progress reports, posters for scientific meetings, etc. In addition, a biomedical engineer and a part-time technician are on staff to perform minor repairs of laboratory equipment, routine maintenance of common use equipment, and to facilitate and/or design specialized equipment. The state-of-the art Annenberg Conference Center for Medical Education is also located on the Lankenau campus. This center houses meeting rooms with video-conferencing capabilities, medical simulation laboratories, a large auditorium, and is the home of the Lankenau Medical Center Library.

LIMR faculty are members of the Kimmel Cancer Center (Thomas Jefferson University), an NCI-designated

cancer center, located in Center City Philadelphia, and have available to them the following additional facilities: Bioimaging Facility, Biostatistics Facility, Clinical Trials Support Office, Flow Cytometry Facility, Glasswashing and Media Preparation Facility, Microarray Facility, Molecular Interaction Facility, Nucleic Acid Facility, Pathology Facility, Proteomics and Mass Spectrometry Facility, Transgenic and Gene Targeting Facility. LIMR researchers have also access to the core laboratories of University of Pennsylvania, the Wistar Institute, and Drexel University.

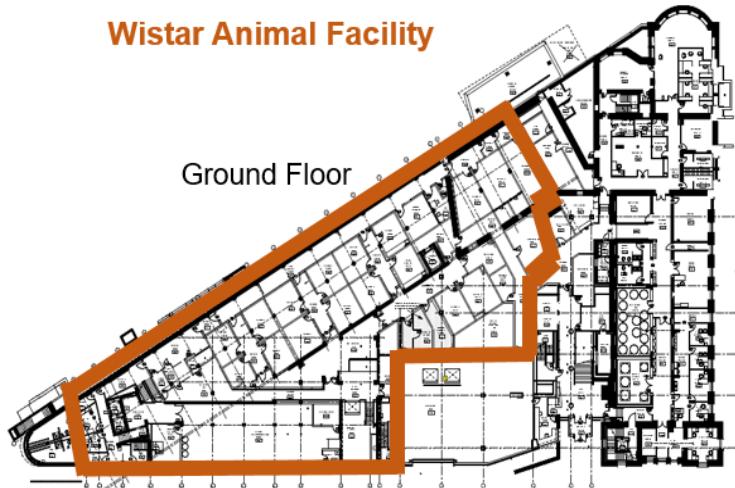
THE WISTAR INSTITUTE

Shared Resources Summaries:

Animal Facility:

The **Animal Facility** provides services in laboratory animal medicine and husbandry, routine animal procurement, animal health surveillance, veterinary care, inventory monitoring, quarantine housing, routine technical support and technical training for all Wistar scientists using animals in their research. The aim is to facilitate research through humane and efficient management of animal populations. The Wistar Animal Care and Use Program (ACUP) has been fully accredited by AAALAC International since 1998. Full accreditation status was most recently renewed in November 2015. In addition, the Wistar ACUP has an assurance on file with the Office of Lab Animal Welfare at the NIH and is registered with the USDA as a research institution.

The Animal Facility maintains an NSG (NOD.Cg-*Prkdc*^{scid} *Il2rg*^{tm1Wjl}/SzJ) breeding colony producing immunodeficient mice for use by Wistar investigators. In addition, Wistar maintains an inter-institutional agreement with Fox Chase Cancer Center to generate transgenic and genetically modified mice utilizing services and expertise of both Fox Chase Transgenic Mouse Facility staff and Wistar Animal Facility staff. The Wistar Cancer Center also provides access to the services of a full-time mouse pathologist.



The new Wistar vivarium (Figure 1) is located on the ground floor of the Cancer Research Building at Wistar. In this facility, 15,360 net sq. ft. (NSF) are dedicated to animal care and vivarium support. The facility operates as a modified barrier facility, has rack space for about 6000 ventilated mouse cages, and is fully equipped including a quarantine room, a procedure room, holding rooms with biosafety cabinets, an imaging / holding room equipped with a Xenogen IVIS 200 imager (overseen by the Wistar Imaging Facility), and required support areas. An irradiator is also available for Wistar vivarium users.

Figure 1. The Wistar Institute Animal Facility. Construction blueprint of the new 15,360 NSF Wistar vivarium.

Ventilated sterile disposable caging is used for mice. Additional holding space for mice is available and space for limited numbers of other small animal species may be arranged upon request. Rack and cage wash facilities, operated by Wistar animal facility staff, include a rack washer, autoclaves, and a gas decontamination chamber and are available for sanitizing and sterilizing all types of cages systems and equipment.

The Wistar Institutional Animal Care and Use Committee (IACUC) is responsible for overseeing the Animal Care and Use Program at Wistar and ensures that it complies with ethical and regulatory standards for the care, use, and treatment of animals. The IACUC ensures this by conducting regular reviews of animal use protocols, regular program reviews, post approval monitoring, investigator training, and animal user and caretaker training. Two attending veterinarians oversee all animal health concerns in Wistar's facility.

Bioinformatics:

The functions of the **Bioinformatics Facility** reflect the research requirements of the three Cancer Center research programs and are broadly divided into three areas: (i) statistical analyses and computational modeling;

(ii) development of advanced bioinformatics tools for integrative cancer biology; and (iii) data-management. Facility personnel are highly trained and experienced to support these goals. Typical data analyses include large scale information datasets (omics data), generated by high-throughput technologies addressing the following complex areas:

- Genome and transcriptome sequencing (alternate splicing, RNA editing, mutation detection, CNV)
- Gene regulation (ChIP-chip, ChIP-seq, epigenetic profiling, promoter methylation arrays)
- Biomarkers (e.g. mRNA, miRNA microarray and protein expression data)
- Proteomic analyses (mass spectrometry-based spectra, LCMS, DIGE, etc.)
- Polymorphism genotyping (e.g. Single Nucleotide [SNP] and Copy Number variations [CGH], LOH)
- Pathway and network analysis
- Integration of multi-platform data
- Other customized data analysis projects

The Bioinformatics facility has a wide array of computational resources to support bioinformatics, computational chemistry, database, and web services. Available computing power consists of multiple items, including eleven high-performance Dell PowerEdge R servers and total of over 20 machines dedicated for different specific needs. Virtual machine servers are used for a variety of intensively used applications, supported by Dell PowerEdge R7xx servers with Xeon Dual or Quadra cores and up to 128GB RAM. Every computer has on average 2-3TB local storage and additionally the facility stores immediately accessible critical data for ongoing analyses on fiber channel raid disk system (33TB combined). Backups of all facility computers are performed with Symantec Backup EXEC software which drives a Promise E610 storage unit with 40TB for backup data. The sequencing data is archived and backed up monthly to dedicated 20TB storage, then backed up quarterly to tape storage of 100TB capacity. Additionally, BF takes advantage of computational resources provided by Center for Systems and Computational Biology (CSCB) and uses 18-nodes (432 cores total) high performance computational cluster (HPCC) with 2.40-2.67GHz dual Intel® Xeon® CPUs and 144GB RAM per node. The HPCC is backed up by an Isilon storage cluster with a total of 660TB available.

Data sharing solutions are implemented in several ways. All facility computers are connected by Gigabit Ethernet, protected by a firewall on the Wistar network, which is in turn connected to the University of Pennsylvania network, PennNet. This provides T1 access speed to national and international networks. While internal institutional data can be shared through commonly accessible network file folders, the secure ftp server is used to deliver data to outside collaborators and customers. Additionally, users can share large files through secure FileTransfer server. The BF staff constantly evaluates needs for tools that can be installed and maintained locally in order to improve upload/download speeds for applications that require large data transfers or allow big batch analyses to be performed. Local versions of UCSC Genome Browser, BLAST tool and Galaxy server are currently deployed and available for users.

The following services are performed by the BF on a regular basis:

- **Project design and consultation:** recommendations on experimental design, statistical considerations and possible pitfalls of the proposed experiment
- **Analysis of High-dimensional data:** qualified PhD staff performs preprocessing and high-level analysis of data, with the most experience in large multi-dimensional datasets analysis (omics data), generated by high-throughput technologies such as genome sequencing (Illumina HiSeq/NextSeq), RNA/miRNA-seq studies, gene regulation (ChIP-chip, ChIP-seq, epigenetic profiling), mRNA and miRNA microarray expression data and microarray promoter methylation surveys, proteomics (mass spectrometry-based spectra, LCMS, DIGE, etc.), polymorphism genotyping (e.g. Single Nucleotide [SNP] and Copy Number variations [CGH], LOH)
- **Application management:** assessment of the need for an existing application, preparing computational resources for the software installation (system administration), deployment and maintenance of the application
- **Systems development:** assessment of the need for a custom application, gathering of requirements, implementation. Database-driven Web applications are developed using standard platforms such as Python/Django, Java/J2EE + Eclipse/Maven IDE. Desktop software development is Java-oriented for portability between different platforms
- **Algorithms development:** custom algorithm implementation using R or Matlab and custom programming on Perl/Python with port to C if high performance is required
- **Data visualization:** data analysis results visualization in plots for presentations, posters, publications, grants

- **Education and training:** general data handling and management, Excel, caBIG (caArray, caTissue, GenePattern), Ingenuity
- **Writing:** analysis-specific sections for Grant application, journal paper methods sections, etc.

Flow Cytometry:

The **Flow Cytometry Facility** provides flow cytometric services and supports the use of flow cytometric techniques by Wistar Institute investigators. The facility's aims are to: 1) provide the technological capability for high quality, single and multi-parameter analyses and/or cell sorting of many types of biological cells from homogeneous or mixed cell populations; 2) provide training and expertise to assist investigators in choosing experimental conditions and reagents that optimize the use of the facility's instrumentation for their experimental needs; and 3) advise and provide technical support for analysis of flow cytometry/cell sorting data for publication, presentation, and inclusion in grant applications, along with storing, archiving and retrieving flow cytometric data.

The facility operates **two cell sorters**, a BD Biosciences **FACSAria II** (4 laser, 16 colors, 4-way sorting), and a new Beckman Coulter **MoFlo Astrios EQ** High-speed sorter (7 lasers, up to 47 parameters, 6-way mixed mode sorting including the ability to sort into plates). Facility staff operates these instruments on behalf of investigators. Both the FACSAria II and the MoFlo Astrios utilize air management units, with the Astrios installed in a biosafety cabinet. Both are prepared to sort BSL-2+ level samples, including infected or uncharacterized murine, human, or non-human primate cell lines, blood, or dissociated tissue samples.

Three user friendly flow cytometry analysis instruments are available in the facility for use by researchers or facility staff for data acquisition and analysis. These include two BD Biosciences **LSR II** instruments (one 14 color and one 18 color), a BD **FACSCelesta** (12 color) and a BD **FACSCalibur** each with a High- Throughput Samplers / plate adaptors for high throughput acquisition/analysis.

An **Amnis ImageStream^x** Multispectral Imaging System is also part of the facility. This one- camera, six-color instrument combines high-speed, high-resolution image capture and detailed quantitation and statistical analysis for a wide range of cell analysis applications.

Genomics:

The **Genomics Facility** (GF), which supports the complete Illumina Genomics platforms is led by Dr. Louise Showe, Scientific Director and Dr. Celia Chang, Managing Director. The GF provides routine and state-of-the-art technologies to support Wistar genomics research, including massively parallel sequencing on the Illumina NextSeq500. The GF has a Nanostring nCounter Analysis platform with single cell gene expression capabilities, an ABI 3130xl Genetic Analyzer, a PerkinElmer Plate Reader Victor X3, a Covaris S2 High Performance Ultrasonicator, and a GE Healthcare Bioscience ImageQuant LAS4010. Facility personnel are highly trained and have extensive experience with all platforms. They also have several years of experience processing a variety of cell and tissue samples including PAXgene blood samples and have the capacity to process large numbers of samples for Capillary sequencing or Next Generation sequencing. The GF provides full-service Next Generation Sequencing (NGS) support from sample preparation, library generation, and quality control with sequencing data being transferred to the Wistar Bioinformatics Facility using a well-established pipeline. Sequencing is presently being carried out using the newly purchased Illumina NextSeq500 and offers 3' RNA sequencing as a replacement for the soon to be discontinued Illumina microarray platforms at similar costs. The NGS service is highly recommended to outside users by Illumina representatives and boasts many repeat users.

Specific Services

- Illumina NextSeq 500, single and paired-end sequencing are available with or without multiplexing and sequencing library construction
- DNA Sequencing including ChIP-seq and ATAC-seq
- RNA Sequencing including RIP-seq and Gro-seq
- Methylation Seq
- Gene Expression Analysis: The Lexogen QuantSeq 3' mRNA-Seq library prep kit is used to generate Illumina compatible libraries. It produces one fragment per transcript, which allows more accurate determination of gene expression and makes it the best alternative to Microarrays.
- NanoString nCounter Analysis System, both custom and commercial gene sets
- WaferGen iCell8 Single Cell Analysis System

- Digital PCR using a RainDrop System
- Capillary DNA sequencing and microsatellite analysis on ABI 3130xl
- 16-panel microsatellite services
- Other services:
 - C.bovis detection
 - SNP Genotyping analysis using Tagman assay
 - RNA and DNA preparation for all services
 - Sequencing library preparation
 - qRT-PCR from primer selection through data extraction
 - Pre-experiment consultation and project troubleshooting
 - Training for all sample preparation protocols, utilization of ABI 7900HT, Covaris, Victor X3 for fluorescence-based assays, Bioanalyzer, etc.

Histotechnology:

The **Histotechnology Facility** provides basic histology services, including the fixation, processing and paraffin embedding of all types of tissues for light microscopy (i.e. routine stains, immunohistochemistry or in-situ hybridization). Routine hematoxylin and eosin staining as well as special staining is performed in the laboratory. Sections are cut from paraffin or frozen blocks and slides are prepared for immunohistochemistry and/or in-situ hybridization. Researchers are advised to contact the facility in advance about freezing and fixing techniques so the best sections can be obtained.

Instrumentation in the laboratory includes: Tissue-Tek VIP Processor, Zeiss HMS Slide Stainer, Hacker RCM-3655 Coverslipping Machine, TissueTek Embedding center, Reichert-Jung 2065 rotary microtome, and Shandon cryostat E.

Imaging:

The **Imaging Facility** provides access to specialized equipment and services, which allow researchers to visualize how the temporal and spatial organization of regulatory events within cells, tissues and organisms impact both normal and pathological processes. Current capabilities include: manual and automated widefield microscopy for brightfield and fluorescence imaging, scanning confocal microscopy, live-cell time lapse imaging, 2 Photon intravital microscopy, small animal whole body luminescence and fluorescence imaging, low magnification photomacrography and customized quantitative image analysis. Full service assistance by facility staff is available for all image acquisition and analysis or users may be trained for unassisted use of all core assets. Other services include assistance with the operation and maintenance of microscopes in individual laboratories.

The following equipment and services are available in the facility:

Widefield Microscopy: Multiple upright and inverted microscopes are available for standard techniques in brightfield, darkfield, fluorescence, phase contrast and differential interference contrast imaging. These systems are available with individual image capture workstations networked to the institute server for ease of transfer and data backup. Instruments include: a Nikon 80i upright, a Nikon E600 upright, a Zeiss Axioskop 2 upright, a Nikon TE2000 inverted, and a Nikon SMZ1500 Fluorescence stereoscope.

Automated and Live-Cell Time-Lapse Microscopy: A Nikon TE300 inverted microscope with custom environmental chamber, fluorescence filter wheels, motorized XY stage and a Z axis controller is available to capture 2D, multipoint, time-lapse image sequences of brightfield and fluorescent samples. The system is also used for automated full slide scanning, tiling and stitching at low to high magnifications.

Scanning Confocal Microscopy: Two Leica confocal systems are available, including a Leica TCS SP8X confocal microscope and a Leica TCS SP5 II system. Both advanced instruments are configured with high-speed resonant scanners, highly sensitive HyD detectors, AOTF and AOBS spectral separation, and incubation systems for live cell applications. The SP8X is equipped with a 405nm laser for DAPI excitation and a white light laser with continuous excitation from 470-670nm in 1nm increments with up to 8 simultaneous lines available. This system also features automatic focus control to counteract Z-drift and a Tokai-Hit stage-top incubation system capable of supporting hypoxia experiments. The SP5 II confocal microscope has 9 excitation laser lines, including a 405nm for DAPI, and a custom environmental chamber that can support larger sample containers. Both confocal systems allow investigators to carry out high-resolution, three-dimensional, single cell observations, photobleaching experiments, spectral separations, thick specimen analysis and co-localization studies in fixed and live samples for single point or multi-dimensional time-lapse investigations.

2 Photon Microscopy: A Leica SP8 MP Spectral system with 4 channels is available for intravital imaging of live mice, analysis of explanted specimens, and second harmonic generation microscopy for the visualization of extracellular matrix proteins such as collagen. It is configured on a fixed-stage upright platform and includes a Chameleon XR Ti-Sapphire laser with excitation ranging from 705-980nm, standard and high-speed resonant scanners, HyD NDD detectors and a 25X/1.0 W objective with Motorized Correction Collar.

Small Animal Whole Body Bioluminescence and Fluorescence Imaging: A PerkinElmer IVIS 200 imager is available for real-time, non-invasive studies of *in vivo* tumor growth, regression, and metastasis. System software provides the capability to standardize measurements for quantitative longitudinal analysis. This system is housed in the Institute's barrier mouse facility.

Custom image capture: The facility maintains equipment to provide researchers with "traditional" photographic support including location and studio photography of small animals, such as mice, experimental set-ups, gels, plates and gross specimens. A Nikon D200 digital camera has a variety of interchangeable lenses, an assortment of portable and studio lighting options, and a wide selection of customized specimen platforms.

Quantitative image capture and analysis: The facility provides customized services to develop quantitative analysis packages for individual experimental protocols. Users are first guided in accurate imaging techniques, then provided with objective analysis algorithms to create standardized, quantitative data from image-based experiments. Custom workstations are available for use in the main facility.

Proteomics and Metabolomics:

The **Proteomics and Metabolomics Facility** provides high sensitivity proteomics and metabolomics analyses using state-of-the-art mass spectrometry (MS) instruments and methods. Consultation with facility staff concerning experimental design and sample preparation is recommended prior to sample preparation to ensure optimal experimental design. Proteomics services include identifications of either purified proteins or complex protein mixtures, such as sub-proteomes, complete proteomes, secretomes, or formaldehyde fixed-paraffin embedded tissues, using electrospray ionization tandem mass spectrometry (ESI MS/MS). Typically, either individual bands are excised from 1-D SDS gels, or the entire gel lane is analyzed by slicing it into uniform fractions followed by trypsin digestion and nanocapillary LC-MS/MS analysis (Gel/LC-MS/MS). While colloidal Commassie stained gels are preferred, simple protein identifications can be obtained from barely detectable silver-stained bands. Protein samples can also be digested in-solution for LC-MS/MS analysis. Data are searched against appropriate sequence databases to identify peptides and the corresponding proteins, and results are filtered to produce low false-positive rates. Complementary services include reverse-phase microbore HPLC peptide mapping, MALDI MS analysis of intact proteins and purified peptides, and ESI MS analysis of intact proteins. Posttranslational modification (PTM) analyses including identifications of specific modified residues in purified proteins or global phosphoproteome or ubiquitome analyses. Investigators should recognize that in most cases these studies are quite complex and require substantially larger amounts of sample than simple protein identifications.

Another major application of proteomics is the quantitative comparison of two or more proteomes. Several options for quantitation are available. Label-free quantitation (LFQ) can be conducted on multiple global proteomes by measuring MS ion current signal intensities. This method is simple and can be quite accurate, particularly if biological triplicates are analyzed at the same time. When metabolic labeling is feasible, a SILAC experiment will yield the most accurate quantitative comparisons, particularly if multiple steps are involved between sample collection and LC-MS/MS analysis. Stable isotope tagging using TMT reagent is preferred for higher throughput concurrent MS quantitation of up to 10 different samples. Alternatively, for small scale experiments, selected peptides of interest can be manually quantitated based on the MS ion current signal. Finally, multiple reaction monitoring (MRM) either in a label-free mode or with isotopically coded internal standards involves substantial initial assay development time but is a high throughput method for accurately quantitating specific previously identified proteins and can include quantitation of known PTM levels on these proteins.

Comprehensive characterization of the metabolome is challenging due to the enormous variety of chemical and physical properties of metabolites and the large dynamic range of concentrations. Metabolomics services currently offered are targeted assays for mostly polar metabolites from cells, biological fluids, conditioned media and tissues. Metabolites are analyzed using a Sciex 5500 QTRAP hybrid triple quadrupole linear ion trap mass spectrometer operating via MRM mode. Metabolites are separated by LC prior to MRM analysis. Multiple types of LC separations (reversed-phase with and without ion pairing; HILIC pH 3 or pH9) are used to separate the

various classes of metabolites, and they are detected using both negative and positive ion modes in the mass spectrometer. A high-flow HPLC system is used to improve reproducibility and throughput of the MRM analysis. Approximately 200 metabolites spanning 32 different classes can currently be detected, including metabolites involved in central carbon metabolism, tryptophan catabolism, and amino acid metabolism.

Instrumentation used by the facility include:

- One ThermoFisher Scientific Q Exactive™ HF Mass Spectrometer with online Waters NanoACQUITY® Nano-capillary UHPLC
- One ThermoFisher Scientific Q Exactive™ Plus Mass Spectrometer with online Waters NanoACQUITY® Nano-capillary UHPLC
- One SCIEX QTRAP® 5500 Mass Spectrometer with online Waters NanoACQUITY® Nano-capillary UHPLC and Shimadzu Nexera XR HPLC
- Applied Biosystems Voyager-DE® PRO MALDI TOF Mass Spectrometer
- Agilent 1100 Microbore HPLC System
- Multiple PC Servers and associated hardware with software for protein/peptide and metabolite identification and analysis

Biomedical Research Support Core:

The **Biomedical Research Support Core** supports Wistar investigators in planning, conducting and reporting ethical and innovative research based on human subjects by supporting the development of research partnerships between Wistar investigators and clinical care providers. The BRSC designed and administers the CDETweb® toolset to facilitate and standardize the exchange of clinical and research-derived information at Wistar. Serving as a centralized resource for human subjects research at Wistar, the BRSC facilitates research compliance with ethical and regulatory mandates. Consultation with BRSC staff is recommended prior to initiating a project to utilize human subject samples from a clinical site for your research. Services include:

- **Clinical study data management** - account (CDETweb® tool) set-up and user training
- **Sample accrual for clinical studies** - initiate projects between clinicians and Wistar researchers to conduct molecular validation studies of predictive/prognostic biomarkers and new cancer targets. These services include patient identifying and informed consent procedures, sample tracking, triaging and cataloging of clinically-annotated, patient-derived samples such as blood, biopsies, metastatic tumors, BALs, etc.
- **Phlebotomy** - collection of human blood samples from donors for use in Wistar research projects.
- **Tissue microarrays** - creation of customized tissue microarrays using a library of tissue blocks from over 3,200 patients and including 58 tissue sites (e.g. ovary, skin, pancreas, prostat, bronchus and lung, colon, breast, etc.)

Clinical study data management:

The Translational Research core facility provides Wistar investigators and their clinical partners with:

- Web-based tools for secure data reporting in compliance with 21-CFR, HIPPA and other regulatory mandates, through our proprietary electronic data management system CDETweb.org*.
- A CDETweb® core toolset for the centralized collection of limited demographics, clinical and sample-related information.
- Web-based, on-demand activity reports for individual investigators, projects, IRB protocols, etc.
- Support for the preparation of clinical study protocols and data management plans.
- Protocol management tools for IRB personnel

Wistar researchers access clinical data for patient-derived samples, request blood draws from phlebotomy, and create/request custom tissue microarrays through their CDETweb® account. Customized CDETweb® extensions are provided to address the needs of individual investigators (e.g., sample tracking, file management, protocol-based eCRFs, study schedules, progress tracking, etc.). CDETweb® was most recently audited for compliance in 2015 by Coalfire, Inc.

Sample accrual for clinical studies:

The Wistar Institute has partnered with the Helen F. Graham Cancer Center and Research Institute (HFGCCRI) at Christiana Care Health System, a member institution of the National Community Cancer Center Program (NCCCP). Recognized nationally for outstanding cancer care in the region, the HFGCCRI captures more than 3,000 new oncology cases a year (74% of all cancer cases in the state of Delaware). As configured, the Wistar-HFGCCRI partnership aims to ensure access to Wistar investigators to clinically-annotated, patient-derived primary tumor samples (i), develop a portfolio of inter-disciplinary collaborations between Wistar scientists and

HFGCCRI clinicians on disease site-specific translational cancer research (ii), and provide access to more treatment-naïve patients for molecular validation studies of predictive/prognostic biomarkers and new cancer targets (iii). Through this agreement, the first in the US to involve an NCI-designated “basic research” Cancer Center and a community Cancer Center, Wistar investigators gain access to a large, 4,000 specimen clinically-annotated cancer tissue bank at the HFGCCRI for collaborative molecular validation studies. Under the leadership of Dr. Nicholas Petrelli, HFGCCRI Medical Director, and Associate Director for Translational Research at the Wistar Cancer Center, the HFGCCRI has developed an impressive infrastructure for clinical oncology research, with academically-oriented specialty multidisciplinary clinics in the main organ sites, a tissue bank of more than 4,000 clinically-annotated patient specimens, active contribution to The Cancer Gene Atlas (TCGA) program (76% approval of specimens submitted after quality control), and one of the highest patient accrual rates on NCI-sponsored oncology clinical trials (24%, compared to a national average of 4%).

A process is in place to allow protocols for Wistar investigators, utilizing patient specimens from HFGCCRI, to be reviewed and approved by the Christiana IRB in a single step. In addition, a research nurse is exclusively dedicated to supporting patient consent, sample tracking, triaging and cataloging of tissues collected for collaborative projects with Wistar scientists. The Wistar-HFGCCRI agreement currently supports various IRB-approved protocols for collection of fresh biospecimens, including fine-needle aspirate, biopsies, surgical resections, cord blood and biological fluids (plasma, serum) from patients with melanoma (Dr. Herlyn), lung cancer (Drs. Showe, Huang and Gabrilovich), ovarian cancer (Drs. Conejo-Garcia, Speicher and Zhang), breast and head and neck cancer (Dr. Gabrilovich). Blood drawing and specimens handling facilities are present within the Clinical Research Unit at HFGCC. Data collection and assurance of compliance are performed by the Clinical Research Office and all regulatory compliance documents for work at HFGCCRI are handled centrally by this office. An internal audit of randomly selected cases for clinical trials at HFGCCRI is conducted by staff of the Clinical Research Office on a regular basis.

Phlebotomy:

The Wistar Phlebotomy Service solicits and collects human blood samples from donors for use in Wistar research. Coordination of donor outreach, pre-screening, donor consent, and collection of 10-500 ml samples in response to requests made by authorized research staff is performed by an experienced phlebotomist.

Donor recruitment, informed consent processes and blood collection are performed under protocols approved by the Wistar Institutional Review Board (IRB), administered by the Office of Science Administration.

Tissue microarrays:

Customized tissue microarrays (TMA) are available through the Wistar-Christiana partnership. The TMA laboratory in the Helen F. Graham Cancer Center and Research Institute (HFGCCRI) is equipped with the Quick-Ray Tissue Microarray System, water baths and a library of tissue blocks. Upon request staff will create customized TMAs from a library of tissue blocks representing 58 tissue sites, including ovary, skin, pancreas, prostate, bronchus and lung, colon, breast, etc., from over 3,200 patients. Through their CDETweb account Wistar researchers may explore the entire library of tissue blocks, including corresponding clinical data for each patient tissue block, and select tissue blocks to include on a custom TMA.

After selected donor blocks and corresponding path reports are reviewed by a HFGCCRI pathologist, slides are cut, heated and stained per request. Recipient block(s) and corresponding slide(s) undergo final review by a pathologist and are then delivered to the requesting researcher at Wistar.

BARUCH S BLUMBERG INSTITUTE FACILITIES

Overall:

The Baruch S Blumberg Institute “owns” and operates the Pennsylvania Biotechnology Center, a 110,000 ft² research and instructional “incubator” for life sciences startup companies. The Center contains all of the equipment and infrastructure for state-of-the-art molecular biology, drug discovery, and life sciences research, including NMR, DNA sequencing, protein peptide identification, flow cytometry. Faculty of the Blumberg Institute have access to these resources either by “right” (as members of the Blumberg Institute) or through vendor relationships (where Center companies provide fee for services access. In addition, of course, Blumberg scientists have access for animal resources, genomics, arraying, DNA sequencing, and numerous other

services, by way of institutional affiliations (with, for example, The Commonwealth Medical School), and institutionally arranged relationships with commercial vendors.

For its exclusive use, The Baruch S Blumberg Institute reserves ~16,000 ft² of laboratory, office and instructional space at The Center, as described below, here:

Laboratory: The labs are modern, “molecular” and “tissue culture” biology-oriented labs, remodeled in 2007, with laminar flow hoods, chemical fume hood, incubators for culture, inverted (Nikon) and direct (Olympus) microscopes, access to direct and inverted imaging microscopes, including a Leitz De-convolution microscope; FACs (FACs Caliber, and FACs Arias), and several mass spectrometry and one NMR system. There is equipment for ELISA (plate washer-reader system) and glycan assays (Dionex HPLC system) and development, which are within our lab, and immediate access to a ThermoFinnigan LTQ.

Institutional environment: We are located at The Pennsylvania Biotechnology Center of Bucks County (PA Biotech Center), in which we are co-located in a 3 building, 110,000 sq ft complex with The Hepatitis B Foundation and Baruch S. Blumberg Institute as well as 40 small start-up companies. There is thus a concentration of drug discovery-oriented scientists, here, along with a strong “commercialization” theme. There are two companies on-site that provide CRO type contract services, for medicinal chemistry, and flow cytometry.

Computers: Personal and on-line lab equipment computers (MAC and PC) are served by a password protected, secure network linked by 100BaseT and Gig-ethernet and secure WiFi, with central network storage. There are several networked computers handling research needs in the PI's laboratory and offices. Several printers, plotters, scanners and computer-linked instruments are located throughout the laboratories. A full-time IT Manager oversees the hardware, software, with over 20 years of experience.

Office: The PI has a private office with a personal computer (MAC), a printer and shared offices for his research staff, each whom has their own computers and shared printer. There is a centralized administrative office located adjacent to the laboratories and research offices. There is also access to 1 large lecture hall and several classrooms and conference rooms (within Blumberg Institute) as well as small library and tele- and video-conferencing equipment for meetings, lectures and classes.

Administrative Support: A central administrative office is staffed with a full-time Office Manager, a Grants Administrator equipped to handle all matters relating to this project, including compliance, budgeting, and staffing as well as a Scientific Facilities Manager equipped to handle all regulatory and compliance matters related to the scientific laboratories and equipment. The admin office has a fax machine, scanner, color printer, and coordinates all shipping and receiving for the entire building, along with full mail services.

Drug Discovery An unusual feature of the Blumberg is its resources for therapeutic drug discovery and development. In addition to its own diverse compound library of small molecules (~120,000), and its natural products collection (one of the largest and most diverse in the world, the result of a gift of the entire US Collection of Merck and Schering Plough's collection (representing ~70% of all plant, bacteria and fungi species on earth), the Center has an “industry standard” robotics and bioinformatics screening unit, managed by a former Director of High Through Put Screening, from Johnson and Johnson. Biomek and EPIC robot systems for screening, and individuals with significant drug discovery and development experience are on site to collaborate and assist an investigators' research.

EQUIPMENT

The laboratory is fully equipped to perform cellular and molecular biology experiments. In addition to the equipment in the lab, LightSeed scientists have access to all major and shared equipment from all three institutes.

LANKENAU INSTITUTE FOR MEDICAL RESEARCH

Major Equipment: LIMR has all the equipments required to run the proposed project. The Institute has an assortment of microscopes for performing dark phase and light field and fluorescent microscopy, including 3 Wild M3Z dissecting microscopes, a Leitz Labovert inverted compound microscope and a Nikon Diaphot inverted compound microscope, which are both equipped with micromanipulators, Axioplan and IMT-2 photomicroscopes, a Zeiss Axioplan Phase Contrast Fluorescent microscope, a Zeiss Axioscop 20 Fluorescent microscope equipped with a Optronics DEI 750 video camera and a Video Sony UP-D 5600MD printer, an Olympus BX60 fluorescent/brightfield microscope dedicated for image analysis, an Olympus BH2 fluorescent/bright field microscope with fully automated Prior stage for cell counting, a Canon BX60 fluorescent microscope, a Nikon SMZ1500 fluorescent dissecting microscope and Nikon Confocal microscope. Available on a shared basis are a Varian HPLC system equipped with a IN/US bRAM Model 2 flow-through detector, Vickers M-85 scanning densitometer, Speed-Vac, Bio-Rad Gene Pulser with capacitance extender for electroporation of eukaryotic cells, a BioMag Magnetic separator for magnetic cell sorting, a Turner Model 20e luminometer, and 2 Coulter counters. In addition to the basic laboratory equipment, a central service laboratory has a BD FACSCanto II flow cytometer containing 488nm solid state and 633nm HeNe lasers with 6-color capability, two Cruachem oligonucleotide synthesizers, two ABI Prism 310 Genetic Analyzer for automatic DNA sequence analysis, 3 Bio-Rad DNA sequencing apparatus, ABI Prism 7700 Sequence Detector, and a Molecular Dynamics Phosphoimager/ Densitometer. The Institute also has Biotek ELISA readers. The instruments are available to the entire scientific staff.

Spacious scientific support areas (1005 ft²) are designed into the central core area on each floor: The central glasswash area equipped with three automatic washers, a Getinge/Castle Biohazard autoclave, and two large capacity forced air ovens is located in the core of the ground floor. Installed in the central glasswash is a constant re-circulating high purity reverse osmosis water system (Culligan) that supplies 18 megohm water and steam to the washers and autoclave, respectively. A full-time glasswash technician is employed by the Institute to operate this facility. Common rooms for equipment shared by the entire scientific staff are located on the first and second floor central core areas where there are 4 Beckman Optima ultra-high speed centrifuges, 4 Sorvall plus 1 Beckman high speed centrifuges, 3 Packard liquid scintillation counters, 1 Packard Cobra gamma counter, 2 Virtis freeze dryers, 2 Scotsman ice machines, a Gilford 2600 recording spectrometer, a Beckman DU 7500 UV/visible spectrophotometer, 2 floor mounted environmental shakers, a Branson ultrasonic cell disrupter, and in each common service area, a Culligan recirculating water system that supplies the laboratories with high purity water as well as the source of high purity steam for the Getinge/Castle Biohazard autoclave on each floor. A refrigerator/freezer room adjoins the common equipment room on each floor. On each of these floors is a dark room equipped with a copy stand, UV light box, and a film processor (Kodak Xomat in one of the dark rooms and a Konica XRX in the other) and a BioRad Chemidoc for gel and blot documentation and analysis. In addition, a walk-in cold room and warm room are located adjacent to the common equipment room on both of these floors. A Histology Laboratory, Liquid Nitrogen Freezer Room, mechanical space and a Storage Room are located on the perimeter of the Ground floor. A fully equipped histology laboratory is equipped with a RMC tissue processor, 2 Leica/Reichert microtomes and 1 Bright Instruments Cryo-microtome.

In the Liquid Nitrogen Freezer Room, five liquid nitrogen freezers are attached to a manifold system specially designed to monitor and maintain a safe level of liquid nitrogen in each freezer. In addition to liquid nitrogen freezers, this room can accommodate -20°C and -80°C freezers. A large liquid nitrogen tank is located exterior to the Freezer Room as the source. Also located on the ground floor is a room specifically designed to safely operate a J. L. Shepherd Mark I, Model 68A Cesium Irradiator.

LIMR has a number of Cores in place to support the research programs of all investigators. The Cores include a Histology Core, which prepares and sections frozen and paraffin embedded tissues; an Imaging Core to analyze microscopic specimens; and a Transgenic Mouse Core, which generates mutant mice. LIMR investigators also have access to the core facilities at Thomas Jefferson University, Wistar Institute and

University of Pennsylvania.

BARUCH S BLUMBERG INSTITUTE FACILITIES

Shared Facilities/Major Equipment

(Relevant to the project, and accessible):

Autoclaves, glassware/kitchens/ tissue culture rooms, dark rooms, microscope dark rooms and Nikon digital microscopy, with IF attachments; liquid scintillators, FACS caliber, Guava, LTQ Mass Spec (with on line HPLC); ABI QTrap, multiple HPLC systems, FPLC, ABI array printer, confocal microscope, NMR, Biomek 200 Robot and Epic high throughput sequencing systems and a FACS Aria (via Flowmetric, on site) are all housed within the same building and are available within a 1 minute walk from the PI's laboratory BSL-3 work, but currently used as BSL-2+), equipped with a biosafety cabinet and two CO₂ incubators, a microscope, a refrigerator, -20 and -80 freezers.

Waters Alliance 2690 HPLC systems with enhanced and Fluorescent detectors ABI Voyager DE Pro MALDI-TOF Mass Spectrometer; 1 Agilent LS Mass Spec (LCMS6120) ThermoFinnigan LTQ linear ion trap Mass Spec; ThermoFinnigan LCQ Mass Spec w/ a splitter ABI Q-trap Mass Spectrometer LI-COR Odyssey Imaging systems for detection of proteins.

Real Time PCR Systems – (Applied Biosystems-7000 Prism, 7500 Fast, Roche-LightCycler LC-480, LightCycler II). Olympus Fluorescent Microscopes (models BX60, IX70, IX81)

Nikon Deconvolution Fluorescent Microscope – Eclipse TE 2000-U, Guava Flow Cytometer – EasyCyte Plus; 1 Becton Dickinson Flow Cytometer – FACSCalibur

Gel Imaging systems (Alpha Innotech-Chem Imager, Kodak-Gel Logic 500, Bio Rad- Molecular Imager FX (2); Hope Film Processor – Micro Max; Waters HPLC systems for protein purification.; Liquid Handling Robot – Beckman Coulter – BioMek NX MC 2 Liquid Nitrogen Freezers – MVE – 1500 Series-19; Liquid Scintillation Counter – Wallac – 1409 DSA 2 Plate Readers - BioTek-Synergy2, AID-EliSpot

Plate Readers (Scintillation and Luminescence) – Wallac-1450 Microbeta Trilux, Perkin Elmer

Sonicators – Misonix-XL2020; Spectrophotometers – Thermo Scientific – NanoDrop 1000, NanoDrop Speed Centrifuges Sorvall IRC5B; 2 Ultra Centrifuges – Beckman – L8-70, L8-70M Thermocyclers, Eppendorf-MasterCycler Pro S, MasterCycler Gradient (2), Biometra- Thermocycler: Techne-GeneE Ultra-Pure Waters – Millipore-MilliQ Synthesis 1 300MHZ NMR

RESEARCH & RELATED Senior/Key Person Profile (Expanded)

PROFILE - Project Director/Principal Investigator						
Prefix:	First Name*:	Xuanmao	Middle Name	Last Name*:	Jiao	Suffix:
Position/Title*:	Senior Researcher					
Organization Name*:	LIGHTSEED, INC.					
Department:						
Division:						
Street1*:	2845 NE 9TH ST APT 604					
Street2:						
City*:	FORT LAUDERDALE					
County:						
State*:	FL: Florida					
Province:						
Country*:	USA: UNITED STATES					
Zip / Postal Code*:	333043650					
Phone Number*:	2404861421		Fax Number:			
E-Mail*:	xuanmao.jiao@bblumberg.org					
Credential, e.g., agency login: xxj007						
Project Role*:	PD/PI		Other Project Role Category:			
Degree Type:	1997, 1984		Degree Year: PhD, BS			
Attach Biographical Sketch*:	File Name:		1_Biosketch_Jiao_Final_20240329.pdf			
Attach Current & Pending Support:	File Name:					

PROFILE - Senior/Key Person				
Prefix:	First Name*: RICHARD	Middle Name G	Last Name*: PESTELL	Suffix:
Position/Title*:	Professor			
Organization Name*:	Baruch S. Blumberg Institute			
Department:	PA Cancer & Regenerative Med C			
Division:				
Street1*:	100 East Lancaster Ave Suite 222			
Street2:				
City*:	Wynnewood			
County:				
State*:	PA: Pennsylvania			
Province:				
Country*:	USA: UNITED STATES			
Zip / Postal Code*:	190960000			
Phone Number*:	267-402-0545		Fax Number:	
E-Mail*:	richard.pestell@gmail.com			
Credential, e.g., agency login: RPESTELL				
Project Role*:	Other (Specify)		Other Project Role Category: Subaward PI	
Degree Type:	MBA, MD, PHD, MB/BS		Degree Year: 2011, 1997, 1991, 1981	
Attach Biographical Sketch*:	File Name:		2_Biosketch_Pestell_Final_20240329.pdf	
Attach Current & Pending Support:	File Name:			

PROFILE - Senior/Key Person				
Prefix:	First Name*: Zhiping	Middle Name	Last Name*: Li	Suffix:
Position/Title*:	Chief Scientific Officer			
Organization Name*:	LIGHTSEED, INC.			
Department:				
Division:				
Street1*:	2845 NE 9th ST APT 604			
Street2:				
City*:	FORT LAUDERDALE			
County:				
State*:	FL: Florida			
Province:				
Country*:	USA: UNITED STATES			
Zip / Postal Code*:	333043650			
Phone Number*:	2155036976		Fax Number: 2155032365	
E-Mail*:	Zhiping.Li@jefferson.edu			
Credential, e.g., agency login: ZXL108				
Project Role*:	Co-Investigator		Other Project Role Category:	
Degree Type:	PhD, MS, BSc		Degree Year: 1999, 1990, 1987	
Attach Biographical Sketch*:	File Name:		3_Biosketch_Li_Final_20240329.pdf	
Attach Current & Pending Support:	File Name:			

PROFILE - Senior/Key Person				
Prefix:	First Name*: Richard	Middle Name N	Last Name*: Kitsis	Suffix:
Position/Title*:	Professor			
Organization Name*:	Albert Einstein College of Medicine			
Department:				
Division:				
Street1*:	1300 Morris Park Avenue			
Street2:				
City*:	Bronx			
County:				
State*:	NY: New York			
Province:				
Country*:	USA: UNITED STATES			
Zip / Postal Code*:	104611975			
Phone Number*:	7184302465		Fax Number:	
E-Mail*:	richard.kitsis@einsteinmed.edu			
Credential, e.g., agency login: rkitsis				
Project Role*:	Other (Specify)		Other Project Role Category: Other Significant Contributor	
Degree Type:	MD,AB			
Degree Year:	1980,1976			
Attach Biographical Sketch*:	File Name: 4_Biosketch_Kitsis_Final_20240329.pdf			
Attach Current & Pending Support:	File Name:			

PROFILE - Senior/Key Person				
Prefix:	First Name*: Anthony	Middle Name W	Last Name*: Ashton	Suffix:
Position/Title*:	Associate Professor			
Organization Name*:	Lankenau Institute for Medical Research			
Department:				
Division:				
Street1*:	100 W Lancaster Ave, Rm 128			
Street2:				
City*:	Wynnewood			
County:				
State*:	PA: Pennsylvania			
Province:				
Country*:	USA: UNITED STATES			
Zip / Postal Code*:	190960000			
Phone Number*:	4844762888		Fax Number:	
E-Mail*:	AshtonA@MLHS.org			
Credential, e.g., agency login: AWASHTON				
Project Role*:	Other (Specify)		Other Project Role Category: Subaward PI	
Degree Type:	PHD, BS, BSC HONS			
Degree Year:	1997,1992,1991			
Attach Biographical Sketch*:	File Name: 5_Biosketch_Ashton_Final_20240328.pdf			
Attach Current & Pending Support:	File Name:			

PROFILE - Senior/Key Person				
Prefix:	First Name*: Taimoor	Middle Name	Last Name*: Khan	Suffix:
Position/Title*:	Postdoctoral Research Fellow			
Organization Name*:	BARUCH S BLUMBERG INSTITUTE			
Department:				
Division:				
Street1*:	3805 Old Easton Road Doylestown			
Street2:				
City*:	Philadelphia			
County:				
State*:	PA: Pennsylvania			
Province:				
Country*:	USA: UNITED STATES			
Zip / Postal Code*:	189020000			
Phone Number*:	215-489-4911		Fax Number:	
E-Mail*:	patti.mcaloon@bblumberg.org			
Credential, e.g., agency login: TAIMOORKHAN				
Project Role*:	Other (Specify)		Other Project Role Category: Other Significant Contributor	
Degree Type:	PHD, MS, BS		Degree Year: 2021, 2013, 2011	
Attach Biographical Sketch*:	File Name:		6_Biosketch_Khan_Final_20240329.pdf	
Attach Current & Pending Support:	File Name:			

PROFILE - Senior/Key Person				
Prefix:	First Name*: Danni	Middle Name	Last Name*: Li	Suffix:
Position/Title*:	Postdoctoral Research Fellow			
Organization Name*:	Baruch S. Blumberg Institute			
Department:				
Division:				
Street1*:	100 Lancaster Avenue			
Street2:				
City*:	Wynnewood			
County:				
State*:	PA: Pennsylvania			
Province:				
Country*:	USA: UNITED STATES			
Zip / Postal Code*:	190963450			
Phone Number*:	000-000-0000		Fax Number:	
E-Mail*:	danni.li@bblumberg.org			
Credential, e.g., agency login: DANNILI				
Project Role*:	Other (Specify)		Other Project Role Category: Other Significant Contributor	
Degree Type:	PhD, MD, BS		Degree Year: 2023, 2019, 2015	
Attach Biographical Sketch*:	File Name:		7_Biosketch_Danni_Li_Final_20240329.pdf	
Attach Current & Pending Support:	File Name:			

PROFILE - Senior/Key Person				
Prefix:	First Name*: Sean	Middle Name	Last Name*: Lal	Suffix:
Position/Title*:	Associate Professor of Medicine			
Organization Name*:	The University of Sydney			
Department:				
Division:				
Street1*:	Anderson Stuart Building, Eastern Ave			
Street2:				
City*:	Camperdown			
County:				
State*:				
Province:				
Country*:	AUS: AUSTRALIA			
Zip / Postal Code*:	NSW 2050			
Phone Number*:	000-000-0000		Fax Number:	
E-Mail*:	sean.lal@sydney.edu.au			
Credential, e.g., agency login: SEANLAL				
Project Role*:	Other (Specify)		Other Project Role Category: Other Significant Contributor	
Degree Type:	PhD, FRACP, MPhil, MB/BS		Degree Year: 2017, 2014, 2009, 2006	
Attach Biographical Sketch*:	File Name:		9_Biosketch_Lal_Final_20240328.pdf	
Attach Current & Pending Support:	File Name:			

PROFILE - Senior/Key Person				
Prefix:	First Name*: Alexander	Middle Name V	Last Name*: Kabanov	Suffix:
Position/Title*:	MESCAL S. FERGUSON DISTINGUISHED PROFESSOR			
Organization Name*:	UNC Eschelman School of Pharmacy			
Department:				
Division:				
Street1*:	120 Mason Farm Road			
Street2:	Genetic Medicine Building, room 1094, Box 7362			
City*:	Chapel Hill			
County:				
State*:	NC: North Carolina			
Province:				
Country*:	USA: UNITED STATES			
Zip / Postal Code*:	275997362			
Phone Number*:	+1 (919) 537-3800		Fax Number:	
E-Mail*:	kabanov@email.unc.edu			
Credential, e.g., agency login: KABANOV.ALEXANDER				
Project Role*:	Other (Specify)		Other Project Role Category: Other Significant Contributor	
Degree Type:	DSC,PHD,MS		Degree Year: 1990,1987,1984	
Attach Biographical Sketch*:	File Name:		10_Biosketch_Kabanov_Final_20240328.pdf	
Attach Current & Pending Support:	File Name:			

PROFILE - Senior/Key Person				
Prefix:	First Name*: Dawn	Middle Name	Last Name*: Bowles	Suffix:
Position/Title*:	Assist Professor			
Organization Name*:	Duke University Medical Center			
Department:				
Division:				
Street1*:	DUMC 2642			
Street2:	Research Drive			
City*:	Durham			
County:				
State*:	NC: North Carolina			
Province:				
Country*:	USA: UNITED STATES			
Zip / Postal Code*:	277100000			
Phone Number*:	919-668-1947		Fax Number:	
E-Mail*:	dawn.bowles@duke.edu			
Credential, e.g., agency login: bowle009				
Project Role*:	Other (Specify)		Other Project Role Category: Other Significant Contributor	
Degree Type:	PHD, BS			
Degree Year:	1998, 1990			
Attach Biographical Sketch*:	File Name: 11_Biosketch_Bowles_Final_20240328.pdf			
Attach Current & Pending Support:	File Name:			

PROFILE - Senior/Key Person				
Prefix:	First Name*: EDWARD	Middle Name T.H.	Last Name*: YEH	Suffix:
Position/Title*:	Chairman, Department of Internal Medicin			
Organization Name*:	University of Arkansas for Medical Sciences			
Department:				
Division:				
Street1*:	4301 W. Markham St. 3/08			
Street2:				
City*:	Little Rock			
County:				
State*:	AR: Arkansas			
Province:				
Country*:	USA: UNITED STATES			
Zip / Postal Code*:	722050000			
Phone Number*:	573-639-3461		Fax Number:	
E-Mail*:	eyeh@uams.edu			
Credential, e.g., agency login: EYEH01				
Project Role*:	Other (Specify)		Other Project Role Category: Other Significant Contributor	
Degree Type:	MD,AB			
Degree Year:	1980,1976			
Attach Biographical Sketch*:	File Name: 12_Biosketch_Yeh_Final_20240328.pdf			
Attach Current & Pending Support:	File Name:			

PROFILE - Senior/Key Person				
Prefix:	First Name*: Javid	Middle Name J	Last Name*: Moslehi	Suffix:
Position/Title*:	Associate Professor in Residence, UCSF			
Organization Name*:	University of California San Francisco			
Department:				
Division:				
Street1*:	Smith Cardiovascular Research Building			
Street2:	555 Mission Bay Blvd., South, Mail Code 3118			
City*:	San Francisco			
County:				
State*:	CA: California			
Province:				
Country*:	USA: UNITED STATES			
Zip / Postal Code*:	941433118			
Phone Number*:	415-502-8778		Fax Number:	
E-Mail*:	javid.moslehi@ucsf.edu			
Credential, e.g., agency login: jmoslehi1				
Project Role*:	Other (Specify)		Other Project Role Category: Other Significant Contributor	
Degree Type:	MD, BA			
Degree Year:	2001, 1996			
Attach Biographical Sketch*:	File Name: 8_Biosketch_Moslehi_Final_20240329.pdf			
Attach Current & Pending Support:	File Name:			

PROFILE - Senior/Key Person				
Prefix:	First Name*: Dario	Middle Name C	Last Name*: Altieri	Suffix:
Position/Title*:	Director, Cancer Center			
Organization Name*:	The Wistar Institute			
Department:				
Division:				
Street1*:	3601 Spruce Street			
Street2:				
City*:	Philadelphia			
County:				
State*:	PA: Pennsylvania			
Province:				
Country*:	USA: UNITED STATES			
Zip / Postal Code*:	191040000			
Phone Number*:	215-495-6970		Fax Number:	
E-Mail*:	daltieri@wistar.org			
Credential, e.g., agency login: DALTIERI				
Project Role*:	Other (Specify)		Other Project Role Category: Other Significant Contributor	
Degree Type:	MD			
Degree Year:	1982			
Attach Biographical Sketch*:	File Name: 13_Biosketch_Altieri_Final_20240328.pdf			
Attach Current & Pending Support:	File Name:			

BIOGRAPHICAL SKETCH

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

NAME: XUANMAO JIAO, PH.D.

eRA COMMONS USER NAME (credential, e.g., agency login): xxj007

POSITION TITLE: Associate Professor

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
Peking University	BSc	05/1984	Biochemistry
The Chinese Academy of Sciences	PhD	05/1997	Cell Biology
CC, NIH	Visiting Fellow	05/2002	Immunology
NIAID, NIH	Research Fellow	05/2004	Immunology
Georgetown University	PostDoc	05/2005	Cancer Biology

A. Personal Statement

My expertise in CCR5 signaling, stem cells and transgenics is well matched with this proposal entitled "*Improving Outcomes in Cancer Treatment-Related Cardiotoxicity*". I have been involved with many studies identifying key molecular mechanisms governing stem cell function, including *NFkB*, *p21Cip1*, *Akt*, *c-Jun*, *Dach1* and *cyclin D1*. I have been working on the role of stem cell regulatory factors in cancer, in particular secreted factors, and characterized these factors in the preliminary studies for the current grant. primarily breast cancer including *c-Jun* and its related signal pathways including *CCL5/CCR5*, *cyclin D1* and *Dach1*. I have more than 45 original research papers published in peer-reviewed journals. Before this, I worked on chemotaxis, vaccine development, liposome technology, membrane fusion and mitochondria functions. I have extensive expertise and understanding of the mechanisms thought to govern tumor metastasis, tumorigenesis, cancer stem cell expansion, and chemotaxis. My specific interest is using both living cell imaging and traditional immunofluorescent staining technique to visualize biological process in single cell level which are well suited to the studies outlined.

Patents

- Pestell RG, Wu K, **Jiao X**, Katiyar S, Popov VM, Casmiro M, Methods and compositions for the diagnosis, prognosis and treatment of Cancer. Jan. 10, 2013. Pub. No.: US2013/0011411.A1

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

1. 1R43HL164131-01A1 (PI) "Improving Outcomes in Cancer Treatment-Related Cardiotoxicity". 8/18/22-8/17/2024. (drug screening for cardio protectants).

B. Positions, Scientific Appointments, and Honor

2023 – present	Principal Scientist, LightSeed, Fort Lauderdale, FL
2016 – present	Associate Professor, Pennsylvania Cancer and Regenerative Medicine Center, Baruch S. Bloomberg Institute, Doylestown, PA
2005-2016	Instructor, Department of Cancer Biology, Thomas Jefferson University, Kimmel Cancer Center, Philadelphia, PA

1994-1998	Research Associate Professor, the State Key Laboratory of Biomembrane and Membrane Biotechnology, Institute of Zoology, Chinese Academy of Sciences, Beijing 100080, China
1989-1994	Research Associate, State Key Laboratory of Biomembrane and Membrane Biotechnology, Institute of Zoology, Chinese Academy of Sciences, Beijing 100080, China
1984-1989	Research Assistant, Department of Cell Biology, Institute of Zoology, Chinese Academy of Sciences, Beijing 100080, China

Honors

2003	Fellows Award for Research Excellence 2004, National Institutes of Health, Bethesda, Maryland, USA
1996	Third-grade Prize for the Progress in Science and Technology, Heilongjiang Province, China
1996	First-grade Prize for the Progress in Science and Technology, the Education Committee of Heilongjiang Province, China
1994 – 1998	Award for Young Scientist Outstanding Achievements, Institute of Zoology, Chinese Academy of Sciences, Beijing

C. Contributions to Science

1.DNA damage/repair, cell migration, EMT and stem cell expansion. I contributed to a body of work that first described several novel non-canonical functions of cyclin D1. Using gene knockout mice with a castration-estrogen replacement paradigm I showed that cyclin D1 was required for the induction of a subset of estrogen responsive genes, primarily those genes that are involved in growth factor receptor expression and activation of proliferative receptor ligands. Using cyclin D1 and Notch deletion mice I showed a key requirement for cyclin D1 in the activation of Notch processing and signaling.

1. Li Z, Chen K, **Jiao X**, Wang C, Willmarth NE, Casimiro MC, Li W, Ju X, Kim SH, Lisanti MP, Katzenellenbogen JA, Pestell RG. Cyclin D1 integrates estrogen-mediated DNA damage repair signaling. *Cancer Res.* 2014, 74(14):3959-70. Epub 2014 May 15 PMID: 24830723 PMCID: PMC4102655
2. Ju X, Casimiro MC, Gormley M, Meng H, **Jiao X**, Katiyar S, Crosariol M, Chen K, Wang M, Quong AA, Lisanti MP, Ertel A, Pestell RG. Identification of a cyclin D1 network in prostate cancer that antagonizes epithelial-mesenchymal restraint. *Cancer Res.* 2014, 74(2):508-19. Epub 2013 Nov 26. PMID: 24282282 PMCID: PMC3914674
3. Fu M, Wang C, Rao M, Wu X, Bouras T, Zhang X, Li Z, **Jiao X**, Yang J, Li A, Perkins ND, Thimmapaya B, Kung AL, Munoz A, Giordano A, Lisanti MP, Pestell RG, Cyclin D1 represses p300 transactivation through a cyclin-dependent kinase-independent mechanism, *J. Biol. Chem.*, 2005, 280(33): 29728-42 PMID: 15951563
4. Lindsay J, **Jiao X**, Sakamaki T, Casimiro MC, Shirley LA, Tran TH, Ju X, Liu M, Li Z, Wang C, Katiya S, Rao M, Allen KG, Glazer RI, Ge C, Stanley P, Lisanti MP, Rui H, Richard RG, ErbB2 induces Notch1 activity and function in breast cancer cells, *Clinical and Translational Science*, 2008, 1(2):107-115 PMID: 20443831 PMCID: PMC3590841

2. Induction of stem cell expansion and breast cancer metastasis. I was the first to show c-Jun induced mammary tumor cellular invasion and breast cancer stem cell expansion. Disruption of c-Jun reduced cellular migration and invasion through inhibition of c-Src and hyperactivation of ROCK II Kinase. c-Jun was also required for TGF- β -mediated cellular migration via nuclear Ca²⁺ signaling. I showed the novel finding that c-Jun determines alternative splicing and that this alternate splicing is also found in subsets of human breast cancer.

1. Katiyar S, **Jiao X**, Addya S, Ertel A, Rose V, Casimiro MC, Zhou J, Lisanti MP, Nasim T, Fortina P, Pestell RG. Mammary gland selective excision of c-jun identifies its role in mRNA splicing. *Cancer Res.* 2012, 72(4):1023-342011, Epub 2011 Dec 15. (equal first author) PMID: 22174367 PMCID: PMC3288968
2. Janowski E, **Jiao X**, Katiyar S, Lisanti MP, Liu M, Pestell RG, Morad M. c-Jun is required for TGF- β -mediated cellular migration via nuclear Ca²⁺ signaling. *Int J Biochem Cell Biol.* 2011, 43(8):1104-13. Epub 2011 Apr 5. (equal first author) PMID: 21447400
3. **Jiao X**, Katiyar S, Willmarth NE, Liu M, Ma X, Flomenberg N, Lisanti MP, Pestell RG. C-Jun induces mammary epithelial cellular invasion and breast cancer stem cell expansion. *J Biol Chem.* 2010, 285(11):8218-8226. Epub 2010 Jan 6 PMID: 20053993 PMCID: PMC2832973
4. **Jiao X**, Katiyar S, Liu M, Mueller SC, Lisanti MP, Li A, Pestell TG, Wu K, Ju X, Li Z, Wagner EF, Takeya

T, Wang C, Pestell RG, Disruption of c-Jun Reduces Cellular Migration and Invasion through Inhibition of c-Src and Hyperactivation of ROCK II Kinase, Mol. Biol. Cell, 2008, 19(4):1378-1390 PMID: 18216279 PMCID: PMC2291431

3. The analysis of cell-fate factors in breast and prostate cancer stem cells and tumorigenesis. I contributed to studies that demonstrated the cell fate determination factor Dach1, which participates in organismal development, governs EMT through a phosphorylation-dependent induction of EMT gene translation. I also showed that Dach1 governs stem cell function through transcriptional regulation of a subset of stem cells genes (Sox, Oct, Nanog).

1. Chen K, Wu K, **Jiao X**, Wang L, Ju X, Wang M, Di Sante G, Xu S, Wang Q, Li K, Sun X, Xu C, Li Z, Casimiro MC, Ertel A, Addya S, McCue PA, Lisanti MP, Wang C, Davis RJ, Mardon G, Pestell RG. The endogenous cell-fate factor dachshund restrains prostate epithelial cell migration via repression of cytokine secretion via a cxcl signaling module. Cancer Res. 2015, 75(10):1992-2004. Epub 2015 Mar 13. PMID: 25769723 PMCID: PMC4433595
2. Wu K, Chen K, Wang C, **Jiao X**, Wang L, Zhou J, Wang J, Li Z, Addya S, Sorensen PH, Lisanti MP, Quong A, Ertel A, Pestell RG. Cell fate factor DACH1 represses YB-1-mediated oncogenic transcription and translation. Cancer Res. 2014, 74(3):829-39. Epub 2013 Dec 12. PMID: 24335958 PMCID: PMC3933065
3. Wu K, **Jiao X**, Li A, Katiyar S, Casimiro MC, Yang W, Zhang Q, Willmarth NE, Chepelev I, Crosariol M, Wei Z, Hu J, Zhao K, Pestell RG. Cell fate determination factor Dachshund reprograms breast cancer stem cell function. J Biol Chem. 2011, 286(3):2132-42. Epub 2010 Oct 11 PMID: 20937839 PMCID: PMC3023510
4. Wu K, Katiya S, Li A, Liu M, Ju X, Popov V, **Jiao X**, Lisanti MP, Antonella, C, Pestell RG, Dachshund inhibits oncogene-induced breast cancer cellular migration and invasion through suppression of interleukin-8, Proc. Nat. Acad. Sci. USA, 2008, 105(19):6924-9. Epub 2008 May 8. PMID: 18467491 PMCID: PMC2374551

4. Heterotypic signaling in cell-survival and autophagy. I contributed to studies in the Pestell Laboratory that led to an understanding of the mechanisms by which the stroma associated with tumors contributes to metabolic substrates and heterotypic signals. His laboratory demonstrated that cyclin D1 conveys distinct functions to either restrain or induce autophagy in a cellular compartment specific manner. I showed that the induction of cyclin D1 found in tumor stroma is sufficient to induce a tumor promoting immune response.

1. Pestell, TG, **Jiao, X.**, Kumar, M, Peck, AR, Prisco, M, Deng, S., Ertel, A., Casimiro, MC, Ju, X, Di Rocco, A, Di Sante, G., Katiyar, S, Shupp, A. , Lisanti, MP, Jain, P, Wu, K, Rui, H, Hooper, DC, Yu, Z, Goldman, AR, Speicher, DS, Laury-Kleintop, LL, Pestell, RG, Stromal cyclin D1 promotes heterotypic immune signaling and breast cancer growth. Oncotarget 2017, August 4, 2017
2. Li, Z., **Jiao, X.**, Di Sante, G*, Adam Ertel, A, Casimiro, MC, Min Wang, M, Sanjay Katiyar, S, Xiaoming Ju, X., Klopfenstein, DV, Tozeren, Dampier, W, Chepelev, I, Jeltsch, A, Pestell, RG *Cyclin D1 integrates G9a-Mediated Histone Methylation.*, Oncogene. Feb 4.
3. Chen, K, **Jiao X.**, Ashton, A., Di Rocco, A., Pestell, TG., Sun, Y., Zhao, J, Casimiro, MC, Li, Z., Lisanti, MP, McCue., PA, Shen, D., Achilefu, A Rui, H, Pestell, RG. The membrane-associated form of cyclin D1 enhances cellular invasion. 2020., Oncogenesis. 2020 Sep 18;9(9):83PMID: 32948740).
4. Chen K., **Jiao, X.**, Di Rocco A., Xu, S, Ertel, A, Yu, Z., Di Sante, G, Wang, M, Li Z, Pestell T, Casimiro, MC, Skordalakes, E, Shen, D, Achilefu, A, Pestell, RG. Endogenous cyclin D1 promotes the rate of onset and magnitude of mitogenic signaling via Akt1 Ser473 phosphorylation. 2020. Cell Reports 2020 Sep 15;32(11):108151.PMID: 32937140.

5. The Role of chemokine and chemokine receptor in cell migration and tumor metastasis. CCR5 antagonists, originally developed as HIV entry inhibitors, were found reducing invasiveness and metastatic capability of breast and prostate cancer cells both in vitro and in vivo, with immediate clinical implications for evaluation as anti-metastatic drugs. Ligand (IL-8)-induced partitioning of human CXCR1 chemokine receptors to lipid raft facilitates G-protein-dependent signaling. The role of PI3 kinase in chemoattractant gradient sensing.

1. Velasco-Velazquez M, **Jiao X**, De La Fuente M, Pestell TG, Ertel A, Lisanti MP, Pestell RG. CCR5

- antagonist blocks metastasis of basal breast cancer cells. *Cancer Res.* 2012, 72(15):3839-50, Epub 2012 May 25. (equal first author) PMID:22637726.
- 2. **Jiao, X.**, Velasco-Velázquez, M., Li, Z., Xu, S., Sicoli, D., Mu, Z., Cristofanilli, M., Rui, H., Den, R.B., Fatatis, A., Pestell, R.G. CCR5 governs breast cancer stem cell expansion and DNA damage repair. *Cancer Res.* 2018. 2018 Apr 1;78(7):1657-1671. Epub 2018 Jan 22. PMID:29358169.
 - 3. **Jiao, X.**, Patel, T., Jaeger, D., Pestell, R.G. Recent Advances with Therapeutic targeting of CCR5 for Cancer and its Role in Immuno Oncology. *Cancer Res.*, 2019, Jul 10. pii: canres.1167.2019.
 - 4. **Jiao, X.**, Wang, M., Zhang, Z., Li, Z., Ni, D., Ashton, A.W., Tang, H-Y., Speicher, D.W., Pestell, R.G. Leronlimab, a humanized monoclonal antibody to CCR5, blocks breast cancer cellular metastasis and enhances cell death induced by DNA damaging chemotherapies. *Breast Cancer Res.* 2021, In Press.

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: RICHARD G. PESTELL

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

POSITION TITLE: Professor

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 Western Australia, Perth, Australia	MB BS (MD)	1981	Medicine
University of Melbourne, Melbourne, Australia	PhD	1991	Molecular Biology
University of Melbourne, Melbourne, Australia	MD (Thesis)	1997	Molecular Biology
Harvard Univ. Medical School, Cambridge, MA	Postdoctoral	1993	Molecular Biology
New York University, New York, NY	MBA	2011	Biotech Entrepreneurship

A. Personal Statement

I have made many original discoveries in the areas of targeted therapies for cancer including the discovery of CCR5 re expression in cancer. Since my *patented* discovery, the field of targeting CCR5 for cancer metastasis has dramatically developed with three active studies currently accruing patients. The proposed studies entitled “Improving Outcomes in Cancer Treatment-Related Cardiotoxicity” are therefore well matched with my expertise.

My research has focused on abnormalities of cell-cycle control and ways of treating cancer based on these pathways. My laboratory has contributed to an understanding of the mechanisms governing the onset and progression of tumorigenesis and metastasis and has been continuously funded by the NIH since my Postdoctoral Fellowship at Harvard in 1993. I have a proven track record of high impact novel scientific discoveries on the mechanism governing hormone responsive **cancers and therapy resistance**. My research served as the preclinical reference citation in the IND (investigational new drug) application to the FDA for the initial clinical research programs (palbociclib, NCT02947685), for what is now the standard of care for the treatment of breast cancer subsets. Of the 484 publications (1996-2023), 161 have been cited in 677 patents (SciVal).

I have published on the molecular mechanisms governing **cardiomyopathy** in human and murine models of *Trypanosoma cruzi* infection (Chagas' disease). These studies characterized the cell survival and death pathways and the role of both cell intrinsic (cardiac myocyte-derived endothelin-1) and cell extrinsic (immune) factors driving the cardiomyopathy (representative examples refs 22-26).

In recent studies we have identified novel heterotypic signals that both promote tumor growth and worsen doxorubicin-induced cardiac damage. Having made many of the original discoveries of CCR5 function in breast and prostate cancer and discovered many key genetic regulators of cancer stem cells, my expertise is well matched with the proposed studies.

CCR5 and cancer metastasis. I was the first to show that oncogenes induce expression of CCR5 in breast cancer, that CCR5 is overexpressed primarily in triple negative breast cancer, that CCR5 serves to promote homing of breast cancer cells to bone and that inhibition of CCR5 with small molecule inhibitors block cancer metastasis *in vivo* in immune competent and immune deficient mice.

1. Velasco-Velázquez M, Jiao X, De La Fuente M, Pestell TG, Ertel A, Lisanti MP, **Pestell RG**. CCR5 antagonist blocks metastasis of basal breast cancer cells. *Cancer Res.* 2012 Aug 1;72(15):3839-50. PMID:22637726.
2. Sicoli D, Jiao X, Ju X, Velasco-Velazquez M, Ertel A, Addya S, Li Z, Andò S, Fatatis A, Paudyal B, Cristofanilli M, Thakur ML, Lisanti MP, **Pestell RG**. CCR5 receptor antagonists block metastasis to bone of v-Src oncogene-transformed metastatic prostate cancer cell lines. *Cancer Res.* 2014 Dec 1;74(23):7103-14. PMID:25452256
3. Jiao X, Velasco-Velázquez MA, Wang M, Li Z, Rui H, Peck AR, Korkola JE, Chen X, Xu S, DuHadaway JB, Guerrero-Rodriguez S, Addya S, Sicoli D, Mu Z, Zhang G, Stucky A, Zhang X, Cristofanilli M, Fatatis A, Gray JW, Zhong JF, Prendergast GC, **Pestell RG**. CCR5 governs DNA damage repair and breast cancer stem cell expansion. *Cancer Res.* 2018 Apr 1;78(7):1657-1671. doi: 10.1158/0008-5472.CAN-17-0915. Epub 2018 Jan 22. PMID:29358169.
4. Jiao, X., Wang, M., Zhang, Z., Li, Z., Ni, D., Ashton, A.W., Tang., H-Y., Speicher, DW, **Pestell, R.G.** Leronlimab, a humanized monoclonal antibody to CCR5, blocks breast cancer cellular metastasis and enhances cell death induced by DNA damaging chemotherapies. *Breast Cancer Res.* 2021, 23;1,1-15.

B. Positions, Scientific Appointments, and Honors

Positions and Employment

2017-present	President, Distinguished Professor, Pennsylvania Cancer and Regenerative Medicine Center. 3805 Old Easton Rd, Doylestown, PA 18902.
2008-2010	Founding Director, Delaware Valley Institute for Clinical & Translational Science.
12/2005-2015	Director of the Sidney Kimmel Cancer Center, Executive Vice President Thomas Jefferson University, Chairman of the Dept. of Cancer Biology, Thomas Jefferson University, Philadelphia, PA.
2002-11/2005	Director, Lombardi Comprehensive Cancer Center, Associate Vice President, Georgetown University Medical Center, Chairman, Dept. of Oncology, Professor of Medicine, Georgetown University, Washington, DC.
2000-2002	Chairman, Division of Hormone-Dependent Tumor Biology & Director, Program in Hormone Dependent Cancers, AECOM.
1996-2001	Associate Prof. and Prof., Dept. of Med., Developmental and Mol. Biol., Albert Einstein College of Medicine (AECOM), Yeshiva University, New York, NY.
1993-1996	Assistant Prof., Dept. of Med. Northwestern Univ. Med. School, Chicago, IL.
1991-1993	Clin. & Res. Fellow, Mass General Hospital, Harvard Univ. Medical School, Cambridge, MA.
1982-1991	Intern, Registrar, (Endocrin. and Oncol.) Royal Perth & St. Vincent's Hospital, Melbourne; NHMRC Scholar, University of Melbourne, (Dept. of Medicine), Australia.

Other Positions **1)** President, International Network for Cancer Treatment and Research, Inc. (USA) (2005-2010); **2)** Board Member, American Australian Association (USA) (2010-Present); **3)** Board Member, Cancer Research and Prevention Foundation (2007-Present); **4)** Board Member, American Association for Cancer Research (Finance and Scientific Meeting Committees) (2004-2009); **5)** External Advisory Board (EAB) for 7 NCI designated Cancer Centers and Shemyakin & Ovchinnikov Institute of Bioorganic Chemistry, Moscow, Russia. **6)** Reviewer for NIH RO1, P30, R21, Dept. of Defense, Susan Komen and 15 other State, Federal (NIH, prior member REN, TCB, BCE), and International funding agencies (Israel, France, Poland, UK, Ireland, Australia). **7)** Expert Testimony, United States Senate, Committee on Appropriations, Subcommittee on Departments of Labor, Health and Human Services, Education, and Related Agencies on NIH funding (2009) **8)** Member of 21 Academic societies. **9)** Founder and CEO of 6 biotechnology companies.

Honors

2019	Order of Australia (AO) for distinguished contribution "in the field of endocrinology and oncology".
2016	Doctor of Medical Sciences, <i>Honoris Causa</i> , University of Melbourne Australia.
2016	Jamie Brooke Lieberman Remembrance Award, Susan G. Komen.
2015	Eric Susman Prize (award by Royal College of Physicians for major advance in medicine).
2014	Advance Global Australian Award (Biotechnology/Innovation).
2011	Fellow, American Association for the Advancement of Science.
2010	Susan Komen Light of Life Award.

- 2008 Doctoris *Honoris Causa* (UWA).
- 2007 Fellow of Royal Society of Medicine.
- 2005 Endocrine Society of Australia's Harrison Award.
- 2002 Gragnani Endowed Chair.
- 2000 Member of American Society Clinical Investigators.
- 1999 Chair of Susan Komen Study Section.
- 1998 Pfeiffer Award.
- 1998 Irma T. Hirsch Weil Caulier Career Award.
- 1996 Neil Hamilton Fairley NHMRC Fellowship.
- 1996 NIH FIRST Award.
- 1996 NIH Shannon Award.
- 1991 Fellow of Royal Australian College of Physicians.
- 1991 Australian Genome Conference Prize.
- 1990 Winthrop Travelling Fellowship.
- 1988 NHMRC Postgraduate and HECS Scholarships.
- 1975 University Commonwealth Scholarship.
- 1975 Sandoz Medical Research Prize.

C. Contributions to Science

1. Tumor suppressors and signal transduction in breast cancer. My laboratory contributed to the early understanding that tumor suppressors have direct effects on hormone signaling. We showed that cyclin D1 serves as a direct transcriptional target of oncogenic signals. Using gene deletion mice we demonstrated the requirement for *Akt1* in tumor growth and metastasis via heterotypic signals.

1. Bromberg, J.F., Wrzeszczynska, M.H., Devgan, G., Zhao, Y., **Pestell, R.G.**, Albanese, C., and Darnell, J.E., Jr., Stat3 as an oncogene. *Cell*. 1999 Aug 6; 98(3): p. 295-303. PMID: 10458605
2. Shtutman, M., Zhurinsky, J., Simcha, I., Albanese, C., D'Amico, M., **Pestell, R.G.**, and Ben-Ze'ev, A., The cyclin D1 gene is a target of the beta-catenin/LEF-1 pathway. *Proc Natl Acad Sci USA*. 1999 May 11; 96(10): p. 5522-7. PMID: 10318916 PMCID: PMC21892
3. Tanaka, H., Matsumura, I., Ezoe, S., Satoh, Y., Sakamaki, T., Albanese, C., Machii, T., **Pestell, R.G.**, and Kanakura, Y. E2F1 and c-Myc potentiate apoptosis through inhibition of NF- κ B activity that facilitates MnSOD-mediated ROS elimination. *Mol Cell*. 2002 May; 9(5): p. 1017-29. PMID: 12049738
4. Ju, X., Katiyar, S., Wang, C., Liu, M., Jiao, X., Li, S., Zhou, J., Turner, J., Lisanti, M.P., Russell, R.G., Mueller, S.C., Ojeifo, J., Chen, W.S., Hay, N., and **Pestell, R.G.**, *Akt1* governs breast cancer progression *in vivo*. *Proc Natl Acad Sci USA*. 2007 May 1; 104(18): p. 7438-43. PMID: 17460049 PMCID: PMC1863437 DOI: 10.1073/pnas0605874104

2. Cyclin D1 non-canonical functions governing gene expression. Dr. Pestell has been a pioneer in the understanding of cell-cycle control in cancer. He was the first to show that: 1) cyclins are direct transcriptional targets of oncogenic and tumor suppressor signals; 2) cyclin D1 expression is rate-limiting for oncogene-induced breast tumor and colon growth *in vivo*; 3) cyclin D1 binds DNA to regulate gene expression and chromosomal instability; 4) cyclins interact with nuclear receptors and tumor suppressors; 5) cyclins regulate mitochondrial metabolism, cellular migration, the non-coding genome and its biogenesis;

1. Wang C, Li Z, Lu Y, Du R, Katiyar S, Yang J, Fu M, Leader JE, Quong A, Novikoff PM, **Pestell RG**. Cyclin D1 repression of nuclear respiratory factor 1 integrates nuclear DNA synthesis and mitochondrial function. *Proc Natl Acad Sci USA*. 2006 Aug 1;103(31):11567-72. Epub 2006 Jul 24. PMCID: PMC1518800 DOI: 10.1073/pnas0603363103
2. Casimiro MC, Crosariol M, Loro E, Ertel A, Yu Z, Dampier W, Saria E, Papanikolaou A, Li Z, Wang C, Fortina P, Addya A, Tozeren A, Knudsen ES, Arnold A, **Pestell RG**. ChIP sequencing of cyclin D1 reveals a transcriptional role in chromosomal instability in mice. *J Clin Invest*. 2012 Mar 1;122(3):833-43. doi: 10.1172/JCI60256. Epub 2012 Feb 6 PMCID: PMC3287228 DOI: 10.1172/JC160256
3. Yu, Z., Willmarth, N.E., Zhou, J., Katiyar, S., Wang, M., Liu, Y., McCue, P.A., Quong, A.A., Lisanti, M.P., and **Pestell, R.G.**, microRNA 17/20 inhibits cellular invasion and tumor metastasis in breast cancer by heterotypic signaling. *Proc Natl Acad Sci USA*. 2010 May 4;107(18):8231-6. Epub 2010 Apr 20 PMCID: PMC2889540 DOI: 10.1073/pnas1002080107

4. Yu Z, Wang L, Wang C, Ju X, Wang M, Chen K, Loro E, Wu K, Casimiro MC, Gormley M, Ertel A, Fortina P, Tozeren A, Liu Z, Chen Y, **Pestell RG**. Cyclin D1 Induction of Dicer Governs MicroRNA Processing and Expression in Breast Cancer. *Nat. Commun.* 2013 Nov 29;4:2812. PMCID: PMC3874416 DOI: 10.1038/ncomms3812.

3. Cancer Stem cells. The Pestell lab has defined the requirement for specific target genes in the formation of breast and prostate epithelial cancer stems cells using transgenic or inducible knockout mice (NFkB, p21^{CIP1}, c-jun) and have defined distinct roles for cyclin D1 in polarity vs stem cell function. These transgenic animals have been shared widely with the research community.

1. Liu, M., Casimiro, M.C., Wang, C., Shirley, L.A., Jiao, X., Katiyar, S., Ju, X., Li, Z., Yu, Z., Zhou, J., Johnson, M., Fortina, P., Hyslop, T., Windle, J.J., and **Pestell, R.G.**, p21CIP1 attenuates Ras- and c-Myc-dependent breast tumor epithelial mesenchymal transition and cancer stem cell-like gene expression in vivo. *Proc Natl Acad Sci USA.* 2009 Nov 10; 106(45): p. 19035-9. PMCID: PMC2776463 DOI:10.1073/pnas0910009106
2. Genander, M., Halford, M.M., Xu, N.J., Eriksson, M., Yu, Z., Qiu, Z., Martling, A., Greicius, G., Thakar, S., Catchpole, T., Chumley, M.J., Zdunek, S., Wang, C., Holm, T., Goff, S.P., Pettersson, S., **Pestell, R.G.**, Henkemeyer, M., and Frisen, J., Dissociation of EphB2 signaling pathways mediating progenitor cell proliferation and tumor suppression. *Cell.* 2009 Nov 13; 139(4): p. 679-92. PMCID: PMC2786256 DOI:10.1016/j.cell.2009.08.048
3. Liu, M., Sakamaki, T., Casimiro, M., Willmarth, N., Quong, A., Ju, X., Ojeifo, J., Jiao, X., Yeow, W-S., Wang, C. Katiyar, S., Shirley, L., Albanese, C., Joyce, D., **Pestell, R.G.** The canonical NF- κ B pathway governs mammary tumorigenesis in transgenic mice via tumor stem cell expansion. *Cancer Res.* 2010 Dec 15;70(24):10464-10473. PMCID: PMC3010731 NIHMSID: NIHMS247137 DOI:10.1158/0008.5472.CAN-10-0732
4. Jiao , X., Katiyar, S., Willmarth, N.E., Liu, M., Ma, X., Flomenberg, N., Lisanti, M.P., and **Pestell, R.G.**, c-Jun induces mammary epithelial cellular invasion and breast cancer stem cell expansion. *J Biol Chem.* 2010 Mar 12; 285(11): p. 8218-26. PMCID: PMC2832973 DOI:10.1074/jbc.M110.100792

4. Nuclear receptor acetylation. Dr. Pestell was the first to show nuclear receptors including the estrogen and androgen receptor, are acetylated, that acetylation regulates contact-independent growth, and that this event is rate-limiting in hormone signaling and that acetylation is a general mechanism conserved among diverse nuclear receptors regulating diverse biological processes. Dr. Pestell proved that a single residue acetylated in the nuclear receptor, converted a growth suppressor into a growth activator. There have been >19,300 publications on nuclear receptor acetylation since our original discovery.

1. Fu M, Wang C, Reutens AT, Wang J, Angeletti RH, Siconolfi-Baez L, Ogryzko V, Avantaggiati ML, **Pestell RG**. p300 and p300/cAMP-response element-binding protein-associated factor acetylate the androgen receptor at sites governing hormone-dependent transactivation. *J Biol Chem.* 2000 Jul 7;275(27):20853-60. PMID:10779504 DOI: 10.1074/jbc.M000660200
2. Wang, C., Fu, M., Angeletti, R.H., Siconolfi-Baez, L., Reutens, A.T., Albanese, C., Lisanti, M.P., Katzenellenbogen, B.S., Kato, S., Hopp, T., Fuqua, S.A., Lopez, G.N., Kushner, P.J., and **Pestell, R.G.**, Direct acetylation of the estrogen receptor alpha hinge region by p300 regulates transactivation and hormone sensitivity. *J Biol Chem.* 2001 May 25; 276(21): p. 18375-83. PMID: 11279135 DOI:10.1074/jbc.M100800200
3. Fu M, Wang C, Wang J, Zhang X, Sakamaki T, Yeung YG, Chang C, Hopp T, Fuqua SA, Jaffray E, Hay RT, Palvimo JJ, Jänne OA, **Pestell RG**. Androgen Receptor Acetylation Governs Transactivation and MEKK1-Induced Apoptosis Without Affecting In Vitro Sumoylation and transrepression Function. *Mol Cell Biol.* 2002 May;22(10):3373-88 PMID:11971970,PMCID:PMC133781 DOI:10.1128/MCB.22.10.3373.3388.2002
4. Fu M, Rao M, Wang C, Sakamaki T, Wang J, Di Vizio D, Zhang X, Albanese C, Balk S, Chang C, Fan S, Rosen E, Palvimo JJ, Jänne OA, Muratoglu S, Avantaggiati ML, Pestell RG. Acetylation of the androgen receptor enhances coactivator binding and promotes prostate cancer cell growth. *Mol Cell Biol.* 2003 Dec;23(23):8563-75. PMID: 14612401 PMCID: PMC262657

5. Cardiac signaling and cell death. Dr. Pestell has published on the molecular mechanisms governing cardiomyopathy in human and murine models of *Trypanosoma cruzi* infection (Chagas' disease). These studies

characterized the cell survival and death pathways and the role of both cell intrinsic (cardiac myocyte-derived endothelin-1) and cell extrinsic (immune) factors driving the cardiomyopathy.

1. Petkova, S.B., Ashton, A., Bouzahzah, B., Huang, H., **Pestell, R.G.**, and Tanowitz, H.B., Cell cycle molecules and diseases of the cardiovascular system. *Front Biosci.* 2000 Apr 1; 5: p. D452-60.
2. Petkova, S.B., Tanowitz, H.B., Magazine, H.I., Factor, S.M., Chan, J., **Pestell, R.G.**, Bouzahzah, B., Douglas, S.A., Shtutin, V., Morris, S.A., Tsang, E., Weiss, L.M., Christ, G.J., Wittner, M., and Huang, H., Myocardial expression of endothelin-1 in murine *Trypanosoma cruzi* infection. *Cardiovasc Pathol.* 2000 Sep-Oct; 9(5): p. 257-65.
3. Huang, H., Yanagisawa, M., Kisanuki, Y.Y., Jelicks, L.A., Chandra, M., Factor, S.M., Wittner, M., Weiss, L.M., **Pestell, R.G.**, Shtutin, V., Shirani, J., and Tanowitz, H.B., Role of cardiac myocyte-derived endothelin-1 in chagasic cardiomyopathy: molecular genetic evidence. *Clin Sci (Lond)*. 2002 Aug; 103 Suppl 48: p. 263S-266S.
4. Huang, H., Petkova, S.B., Cohen, A.W., Bouzahzah, B., Chan, J., Zhou, J.N., Factor, S.M., Weiss, L.M., Krishnamachary, M., Mukherjee, S., Wittner, M., Kitsis, R.N., **Pestell, R.G.**, Lisanti, M.P., Albanese, C., and Tanowitz, H.B., Activation of transcription factors AP-1 and NF-κ B in murine Chagasic myocarditis. *Infect Immun.* 2003 May; 71(5): p. 2859-67.

Reagents generated in my laboratory have been shared widely with the research community. More than 300 national and international laboratories have received clones, transgenic lines, and inducible mice from my laboratory since 10/2006.

Summary of Published Work (>615 published works with ~90,500 citations):

<https://pubmed.ncbi.nlm.nih.gov/?term=Pestell+R&sort=date>

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: Zhiping Li

ERA COMMONS USER NAME (credential, e.g., agency login): zxl108

POSITION TITLE: Associate Professor

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
Shanxi Medical University. Shanxi, China	B.Sc. (M.D.)	06/1987	Clinical Medicine
Shanxi Medical University. Shanxi, China	MS	06/1990	Internal Medicine (Hematology)
Beijing Medical University. Beijing, China	Ph. D.	06/1999	Hematology
Albert Einstein College of Medicine. Bronx, NY	Post-doc	12/2002	Hematology/Oncology
Georgetown University. Washington, DC	Post-doc	12/2005	Oncology

A. Personal Statement

My expertise in molecular biology, cell analysis including ChIP studies and transgenic animal analysis is well matched with the proposed study for the proposed grant *entitled* "Improving Outcomes in Cancer Treatment-Related Cardiotoxicity". Relevant to these studies I have extensive experience with studying tumor growth and metastasis. I have published in the area of EMT and DNA damage repair showing cyclin D1 governs DNA damage repair and EMT. Currently I am an Associate Professor at the Baruch S. Blumberg Institute in Pennsylvania. I have worked with Dr. Pestell since 2002. Previously I received my M.D. from Shanxi Medical University in China and my Ph.D. from Beijing Medical University in China. Since December 2002 my first-author papers are focused on cancer including DNA damage/repair, cellular migration and transcription, themes of direct relevance to this proposal.

Mechanisms governing the DNA damage repair response and hormone responsive cancers and role of CCR5. I contributed to the novel finding that cyclin D1 governs hormone signaling include estrogen signaling in the mammary gland *in vivo*. I showed cyclin D1 delays the DNA damage response represents yet another non-nuclear feature of this cancer gene contributing to estrogen-mediated breast tumorigenesis (*Cancer Research*. 2014; 74(14): 3959-70).

1. Wang C, Fan S, **Li Z**, Fu M, Rao M, Ma Y, Lisanti MP, Albanese C, Katzenellenbogen BS, Kushner PJ, Weber B, Rosen EM, Pestell RG. Cyclin D1 Antagonizes BRCA1 Repression of Estrogen Receptor {alpha} Activity. *Cancer Research*. 65(15): 6557-67, 2005. PMID: 16061635. PMCID: Identifier absent.
2. Casimiro MC, Wang C, **Li Z**, Di Sante G, Willmarth NE, Addya S, Chen L, Liu Y, Lisanti MP, and Pestell RG. Cyclin D1 Determines Estrogen Signaling in the Mammary Gland *In Vivo*. *Molecular Endocrinology* 2013; 27(9): 1415-28. PMCID: PMC3753428.
3. **Li Z**, Chen K, Jiao X, Wang C, Willmarth NE, Casimiro MC, Li W, JU X, Kim SH, Lisanti MP, Katzenellenbogen JA, Pestell RG. Cyclin D1 integrates estrogen-mediated DNA damage repair signaling. *Cancer Res*. 74(14): 3959-70: 2014. PMCID: PMC4102655.
4. Jiao, X., Wang, M., Zhang, Z., **Li, Z.**, Ni, D, Ashton, A.W., Tang., H-Y., Speicher, DW, Pestell, R.G. Leronlimab, a humanized monoclonal antibody to CCR5, blocks breast cancer cellular metastasis and enhances cell death induced by DNA damaging chemotherapies. *Breast Cancer Res*. 2021, In Press.

B. Positions, Scientific Appointments, and Honors

- 2018 – Present Associate Professor, Baruch. S. Blumberg Institute, Pennsylvania, USA
2005 – 2017 Research Assistant then Associate Professor, Department of Cancer Biology, Sidney Kimmel Cancer Center, Thomas Jefferson University. Philadelphia, PA.
1990 – 1996 Resident Physician and Instructor, Institute of Hematology, Shanxi Medical University. Shanxi, China

C. Selected Peer-reviewed Publications

1. Regulation of DNA damage response and chromosomal instability.

DNA damage can initiate cancer. Radiological and chemical agents used to treat cancer patients often cause DNA damage. My studies indicate that **alternative cyclin D1 splice forms differentially regulate the DNA damage response** (*Cancer Research*. 2010; 70(21): 8802-11). I contributed to the novel finding that cyclin D1 governs BRCA1 function, and the specific isoforms of cyclin D1 govern DNA repair, which paved the way for the current understanding that cyclin D1 is involved in DNA repair function.

1. Casimiro MC, Crosariol M, Loro E, Ertel A, Yu Z, Dampier W, Saria EA, Papanikolaou A, Stanek TJ, **Li Z.**, Wang C, Fortina P, Addya S, Tozeren A, Knudsen ES, Arnold A, Pestell RG. ChIP sequencing of cyclin D1 reveals a transcriptional role in chromosomal instability in mice. *J Clin Invest*. 122(3): 833-43; 2012. PMCID: PMC3287228.
2. Myklebust MP, **Li Z**, Tran TH, Rui H, Knudsen ES, Elsaleh H, Fluge Ø, Vonen B, Myrvold HE, Leh S, Tveit KM, Pestell RG, Dahl O. Expression cyclin D1a and D1b as predictive factors for treatment response in colorectal cancer. *British Journal of Cancer* 107, 1684–169, 2012. PMCID: PMC3493874.
3. Casimiro MC, Di Sante G, Crosariol M, Loro E, Dampier W, Ertel A, Yu Z, Saria EA, Papanikolaou A, **Li Z**, Wang C, Addya S, Lisanti MP, Fortina P, Cardiff RD, Tozeren A, Knudsen ES, Arnold A, Pestell RG. Kinase-independent role of cyclin D1 in chromosomal instability and mammary tumorigenesis. *Oncotarget*. 2015; 6(11): 8525-38. PMCID: PMC4496164.
4. **Li Z**, Jiao X, Di Sante G, Ertel A, Casimiro MC, Wang M, Katiyar S, Ju X, Klopfenstein DV, Tozeren A, Dampier W, Chepelev I, Jeltsch A, Pestell RG. Cyclin D1 integrates G9a-mediated histone methylation. *Oncogene*. 2019 May;38(22):4232-4249. Epub 2019 Feb 4. PMID: 30718920.

2. Cancer cellular invasion and metastasis.

Since December 2002 when I joined in Dr. Richard Pestell's lab I had worked on the role of cyclin D1 in breast cancer. Cyclin D1 is best known as the regulatory subunit of a dimeric holoenzyme including the cell cycle-dependent kinase CDK4, which phosphorylates and inactivates the retinoblastoma protein Rb to promote progression through the G1-S phase of the cell cycle. The role of cyclin D1 in processes outside of its well-known function in cell cycle is relatively under-explored. My studies reveal that **cyclin D1 acts to promote cellular migration by inhibiting Rho/ROCK signaling and expression of thrombospondin-1 (TSP-1)**, an extracellular matrix protein that regulates cell migration in many settings including cancer (*Molecular and Cellular Biology*. 2006; 26(11): 4240-56). It has been shown that p27^{KIP1} has a pro-migratory function through inhibiting RhoA activity. My studies showed that **cyclin D1 promotes cellular migration via up-regulating p27^{KIP1} abundance and physical interaction with p27^{KIP1}** (*Cancer Research*. 2006; 66(20): 9986-94). **Alternate cyclin D1 mRNA splicing modulates p27^{KIP1} binding and cell migration** (*Journal of Biological Chemistry* 2008; 283(11): 7007-15).

1. **Li Z**, Wang C, Jiao X, Lu Y, Fu M, Andrew A, Quong AA, Dye C, Yang J, Dai M, Ju X, Zhang X, Li A, Burbelo P, Stanley ER, Pestell RG. Cyclin D1 regulates cellular migration through the inhibition of thrombospondin-1 and ROCK signaling. *Mol Cell Biol.*, 26(11): 4240-56, 2006. PMID: 16705174. PMCID: PMC1489104.
2. **Li Z**, Jiao X, Wang C, Ju X, Lu Y, Yuan L, Lisanti MP, Katiyar S, and Pestell RG. Cyclin D1 induction of cellular migration requires p27^{KIP1}. *Cancer Res*. 66 (20): 4459-62, 2006. PMID: 17047061. PMCID: Identifier absent.
3. **Li Z**, Wang C, Jiao X, Katiyar S, Casimiro MC, Prendergast GC, Powell MJ and Pestell RG. Alternate cyclin D1 mRNA splicing modulates p27^{KIP1} binding and cell migration. *J Biol Chem*. 283(11):7007-15, 2008. PMID: 18180298. PMCID: Identifier absent.

4. Casimiro MC, Di Sante G, Ju X, **Li Z**, Chen K, Crosariol M, Yaman I, Gormley M, Meng H, Lisanti MP, Pestell RG. Cyclin D1 promotes androgen-dependent DNA damage repair in prostate cancer cells. *Cancer Res.* 2016 Jan 15;76(2):329-38. Epub 2015 Nov 18. PMID:26582866

3. miRNA expression and biogenesis in breast invasion and heterotypic signaling: I have contributed to publications that showed for the first time that cyclin D1 governs miRNA via transcriptional effects of cyclin D1 in the context of chromatin. I contributed to the original studies showing a key role for cyclin D1 in cytosolic metabolism and in the regulation of fat metabolism via PPAR γ .

1. Wang C, **Li Z**, Lu Y, Du R, Katiyar S, Yang J, Fu M, Leader JE, Quong AA, Phyllis M, Novikoff PM, Pestell RG. Cyclin D1 repression of nuclear respiratory factor 1 integrates nuclear DNA synthesis and mitochondrial function. *Proc Natl Acad Sci U S A.* 103(31): 11567-72, 2006. PMID: 16864783.
2. Yu Z, Wang C, Wang M, **Li Z**, Casimiro MC, Liu M, Wu K, Whittle J, Ju X, Hyslop T, McCue P, and Pestell RG. A cyclin D1/microRNA 17/20 regulatory feedback loop in control of breast cancer cell proliferation. *Journal of Cell Biology.* 182(3): 509-17; 2008. PMCID: PMC2500136.
3. Yu Z, Wang L, Wang C, Ju X, Wang M, Chen K, Loro E, **Li Z**, Zhang Y, Wu K, Casimiro MC, Gormley M, Ertel A, Fortina P, Chen Y, Tozeren A, Liu Z, Pestell RG. Cyclin D1 induction of Dicer governs microRNA processing and expression in breast cancer. *Nat Commun.* 4:2812, 2013. PMCID: PMC3874416.
4. Pestell TG, Jiao X, Kumar M, Peck AR, Prisco M, Deng S, **Li Z**, Ertel A, Casimiro MC, Ju X, Di Rocco A, Di Sante G, Katiyar S, Shupp A, Lisanti MP, Jain P, Wu K, Rui H, Hooper DC, Yu Z, Goldman AR, Speicher DW, Laury-Kleintop L, Pestell RG. Stromal cyclin D1 promotes heterotypic immune signaling and breast cancer growth. *Oncotarget.* 2017 Aug 4;8(47):81754-81775. eCollection 2017 Oct 10. PMID:29137220.

4. Stem cells in Cancer.

Dr. Pestell was the first to show that cell-fate determination factor, Dach1, restrains tumor growth. The abundance of Dach1 is reduced in human cancers. My research in Dr. Richard Pestell's lab also involved in studying the role of Dach1 in breast, prostate, and lung cancer.

1. Chen K, Wu K, Gormley M, Ertel A, Wang J, Zhang W, Zhou J, Di Sante G, **Li Z**, Rui H, Quong AA, McMahon SB, Deng H, Lisanti MP, Wang C, Pestell RG. Acetylation of the cell-fate factor dachshund determines p53 binding and signaling modules in breast cancer. *Oncotarget* 2013; 4(6): 923-35. PMCID: PMC3757249.
2. Wu K, Chen K, Wang C, Jiao X, Wang L, Zhou J, Wang J, **Li Z**, Addya S, Sorensen PH, Lisanti MP, Quong A, Ertel A, and Pestell RG. Cell Fate Factor DACH1 Represses YB-1-Mediated Oncogenic Transcription and Translation. *Cancer Research* 2014; 74(3): 829-39. PMCID: PMC3933065.
3. Chen K, Wu K, Jiao X, Wang L, Ju X, Wang M, Di Sante G, Xu S, Wang Q, Li K, Sun X, Xu C, **Li Z**, Casimiro MC, Ertel A, Addya S, McCue PA, Lisanti MP, Wang C, Davis RJ, Mardon G, and Pestell RG. The Endogenous Cell-Fate Factor Dachshund Restrains Prostate Epithelial Cell Migration via Repression of Cytokine Secretion via a CXCL Signaling Module. *Cancer Research* 2015; 75(10): 1992-2004. PMCID: PMC4433595.
4. Jiao X, **Li Z**, Wang M, Katiyar S, Di Sante G, Farshchian M, South AP, Cocola C, Colombo D, Reinbold R, Zucchi I, Wu K, Tabas I, Spike BT, Pestell RG. Dachshund depletion disrupts mammary gland development and diverts the composition of the mammary gland progenitor pool. *Stem Cell Reports.* 2019 Jan 8;12(1):135-151. Epub 2018 Dec 13. PMID:30554919.

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: Richard N. Kitsis

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

POSITION TITLE: Professor of Medicine

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
Harvard College, Cambridge, MA	A.B.	03/1976	Chemistry
University of California, San Francisco, CA	M.D.	06/1980	Medicine
Massachusetts General Hospital, Boston, MA	Intern	06/1981	Medicine
Boston VA Medical Center, Boston, MA	Res & Chief Res	06/1987	Medicine
Albert Einstein College of Medicine, Bronx, NY	Clinical Fellow	06/1989	Cardiology
Albert Einstein College of Medicine, Bronx, NY	Postdoctoral	06/1993	Molecular/Cellular Biol

A. Personal Statement

Over the past 30 years, my lab has studied (a) fundamental mechanisms of cell death; and (b) roles of cell death in human disease. We have elucidated basic aspects of cell death biology including mechanisms that mediate apoptosis and regulated forms of necrosis, molecular interconnections among these programs, and connections with other aspects of mitochondrial biology. We have also investigated roles of cell death in heart disease, including Doxorubicin induced cardiotoxicity. My expertise is thus very well matched with the proposed application entitled “Improving Outcomes in Cancer Treatment-Related Cardiotoxicity”. When I set up my lab in 1991, I was still engaged in the practice of cardiology and eventually served as Chief of Cardiology at Albert Einstein College of Medicine and Montefiore Medical Center, a ~1500-bed hospital. But, as my interests shifted to more fundamental questions, I elected to give up clinical activities and to focus completely on basic and translational research. I am also very involved in mentoring. I have served as Ph.D. thesis advisor to >25 individuals and have mentored ~75 postdoctoral and cardiology fellows. I take great pride in my trainees' accomplishments, a significant proportion of whom have gone on to independent scientific careers. Additionally, trainees include many women and some under-represented minorities. I also serve as MPI of an NIH T32 cardiovascular training grant that includes predoctoral and postdoctoral positions.

Current research in the lab includes:

1. A novel role for procaspase-9 in necrosis during myocardial infarction
We have identified a new pathway of caspase-9-mediated necrosis that operates during myocardial infarction and in other paradigms and are delineating mechanisms.
2. Interrelationships between ER/SR and mitochondria in regulating necrotic cell death and metabolism
We have created novel peptides and small molecules that conformationally active or inhibit mitofusins (*Nature*, 2016; *Science*, 2018 below) and are using these to alter the physical distance between ER/SR and mitochondria. We are testing whether these reagents can be used to limit cardiac damage during myocardial infarction and, conversely, to augment myocardial energetics in heart failure.
3. Mechanisms of cancer therapy-induced cardiomyopathies
We are developing small molecules to inhibit the cardiotoxic effects of several cancer therapies (*Nature Chemical Biology*, 2019; *Nature Cancer*, 2020 below).

4. Function of the mitochondrial ATP synthase *in vivo*

We have created mice that lack the 29-subunit mitochondrial ATP synthase and are exploring mitochondrial processes that are regulated directly and indirectly by this complex.

5. Interrelationships between cellular senescence and cell death in aging

Through genome wide CRISPR screening and other approaches, we are attempting to understand the mechanisms that direct a stressed cell toward a senescent versus cell death fate.

6. Regulation of ferroptosis by mitochondria

We have identified unexpected connections between mediators of oxidative phosphorylation and mitochondrial dynamics and ferroptotic cell death and are delineating mechanisms.

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

R01HL138475

Kitsis (PI)

07/15/18-06/30/22

Chaperone-mediated autophagy in normal cardiac biology and heart failure

Department of Defense PR191593

Kitsis (PI)

07/01/20-06/30/23

Development of small molecule BAX inhibitors to prevent cancer therapy-induced cardiomyopathy

R01HL159062

Kitsis (PI)

08/01/2021-07/31/2025

Modulation of mitofusin activity to treat heart disease

R01HL128071

Kitsis (MPI)

04/01/15-05/31/19

Linking cell death and mitochondrial quality control mechanisms in heart failure

Citations:

1. Franco A*, **Kitsis RN***, Fleischer JA, Gavathiotis E, Kornfeld OS, Gong G, Biris N, Benz A, Qvit N, Donnelly SK, Chen Y, Mennerick S, Hodgson L, Mochly-Rosen D, Dorn GW (2016). Correcting mitochondrial fusion by manipulating mitofusin conformations *Nature*, 540:74-79. PMID27775718. PMC5315023.
2. Garner TP, Amgalan D, Reyna DE, Li S, **Kitsis RN**, Gavathiotis E (2019). Small-molecule allosteric inhibitors of BAX. *Nat Chem Biol*, 15:322-330. PMID30718816. PMC6430685.
3. Amgalan D, Garner TP, Pekson R, Jia XF, Yanamandala M, Paulino V, Liang FG, Corbalan JJ, Lee J, Chen Y, Karagiannis G, Sanchez LR, Liang H, Narayananagari SR, Mitchell K, Lopez A, Margulets V, Scarlata M, Santulli G, Asnani A, Peterson RT, Hazan RB, Condeelis JS, Oktay MH, Steidl U, Kirshenbaum LA, Gavathiotis E, **Kitsis RN** (2020). A small molecule allosteric inhibitor of BAX protects against doxorubicin-induced cardiomyopathy. *Nat Cancer*, 1:315-328. PMID32776015. PMC7413180.
4. McKimpson WM, Chen Y, Irving JA, Zheng M, Weinberger J, Tan WLW, Tiang Z, Jagger AM, Chua Jr SC, Pessin JE, Foo RSY, Lomas DA, **Kitsis RN** (2021). Conversion of the death inhibitor ARC to a killer activates pancreatic β-cell death in diabetes. *Developmental Cell*, 56:747-760. PMID33667344. PMC8146085.

B. Positions, Scientific Appointments, and Honors

Positions and Scientific Appointments

- 2010- Founding Director, Wilf Family Cardiovascular Research Institute Albert Einstein College of Medicine
2005-10 Chief, Division of Cardiology, Albert Einstein College of Medicine and Montefiore Medical Center
2003- The Dr. Gerald and Myra Dorros Chair, Cardiovascular Disease Albert Einstein College of Medicine
2003- Professor, Departments of Medicine and Cell Biology, Einstein
1998-03 Associate Professor, Departments of Medicine and Cell Biology, Einstein
1993-98 Assistant Professor, Department of Cell Biology, Albert Einstein College of Medicine
1991-98 Assistant Professor, Department of Medicine, Albert Einstein College of Medicine

Honors

2020	The Borun Lecture, University of California, Los Angeles
2017	Chair, Scientific Committee, Sarnoff Cardiovascular Research Foundation
2016	Keynote Lecture, University of Alabama, Department of Biomedical Engineering Retreat
2015	Organizer, Keystone Symposium "Mitochondria, Metabolism, and Heart Failure"
2015	Keynote Lecture, Medical College of Wisconsin, Cardiovascular Research Center Retreat
2015	Keynote Lecture, Vanderbilt University Cardiovascular Center Retreat
2015	Member, Scientific Committee, Sarnoff Cardiovascular Research Foundation
2015	Elected, Fellowship American Association for the Advancement of Science (AAAS)
2015	Keynote Lecture, Case Western Reserve School of Medicine Cardiovascular Center Retreat
2015	Keynote Lecture, Columbia University, Department of Physiology and Cellular Biophysics Retreat
2013	President's Distinguished Lecture Award, International Society of Heart Research World Meeting
2012	Keynote Lecture, Society of Heart and Vascular Metabolism, Oxford University, UK
2011	Organizer, Keystone Symposium "Mechanisms of Cardiac Growth, Death and Regeneration"
2009	Keynote Lecture, Japanese Circulation Society
2009	Member and Chair, NIH Myocardial Ischemia and Metabolism Study Section (MIM)
2009	Keynote Lecture, Indiana University School of Medicine, Riley Heart Research Center Retreat
2006	Member, NIH/NHLBI Strategic Planning Working Group on Heart Failure and Cardiomyopathy
2006	Keynote Lecture, Department of Pathology Symposium, University of British Columbia
2006	Chair, AHA Council on Basic Cardiovascular Sciences (BCVS)
2005	Elected, Association of American Physicians (AAP)
2004	Elected, Association of University Cardiologists (AUC)
2003	Organizer, AHA Basic Cardiovascular Science (BCVS) Meeting
2003	Plenary Lecture, Heart Failure Society of America National Meeting
2002	The Blount Lecture, University of Colorado
2002	Plenary Lecture, American Heart Association National Meeting
2002	Landmark Lecture, International Society of Heart Research
2001	Keynote Lecture, British Cardiac Society
2000	Monique Weill-Caulier Scholar Award
2000	State-of-the-Science Lecture, Heart Failure Society of America National Meeting
1998	Elected, American Society of Clinical Investigation (ASCI)
1998	State-of-the-Art Lecture, American Heart Association National Meeting
1993-	Associate Editor: Cell Death and Differentiation. Past Associate Editor: Circulation Heart Fail; Editorial Boards: Circulation, Circ Res, J Mol Cell Cardiol, Cardiovasc Res, J Clin Invest. Reviewer: Nature, Nat Med, Nat Immunol, Nat Comm, Science, Science Advances, Science Signaling, Molecular Cell, Cell Metab, Cell Reports, J Clin Invest, New Engl J Med, Proc Natl Acad Sci USA, Mol Cell Biol, J Biol Chem, J Cell Biol, Cell Death Differ, Circulation, Circ Res, J Mol Cell Cardiol, Cardiovasc Res, Cancer Res, Cell Cycle
1992	NIH Clinical Investigator Award
1979	First Place, Dean's Prize for Research, University of California, San Francisco
1979	Elected, Alpha Omega Alpha, University of California, San Francisco
1976	Summa Cum Laude, Department of Chemistry, Harvard University

C. Contributions to Science

1. ARC, an inhibitor of multiple cell death pathways.

The decision of a cell to die is tightly controlled by cell death activators and inhibitors. Most inhibitors target a single or limited repertoire of cell death mechanisms. In contrast, the cell death inhibitor ARC antagonizes multiple apoptosis and necrosis mechanisms, some of which we have delineated. For example, ARC blocks death receptor-mediated apoptosis and necrosis (the latter referred to as necroptosis) through direct binding with the death domains of death receptors and their adaptor molecules, which precludes formation of signaling complexes. Interestingly, we found that this binding defines a new class of interactions involving non-homotypic "death-fold" motifs. ARC also blocks mitochondria-mediated apoptosis through direct interactions with BAX resulting in inhibition of BAX conformational activation and translocation to mitochondria. A second mechanism by which ARC opposes the mitochondrial death pathway is through its interaction with p53 in the nucleus resulting in inhibition of p53 tetramerization (important for p53 function as a transcription factor) and exposure of a p53 nuclear export sequence that relocates p53 to the cytoplasm. Further, we have demonstrated that ARC

plays important roles in the pathogenesis of human diseases including myocardial infarction, heart failure, pulmonary hypertension, cancer, diabetes, and muscular dystrophy.

- a. Nam Y-J, Mani K, Ashton AW, Peng C-F, Krishnamurthy B, Hayakawa Y, Lee P, Korsmeyer SJ, **Kitsis RN**. Inhibition of both the extrinsic and intrinsic death pathways through nonhomotypic death-fold interactions. *Molecular Cell*, 2004. 15:901-912. PMID: 15383280. PMCID: none.
- b. Foo RS-Y, Nam Y-J, Ostreicher MJ, Metzl MD, Whelan RS, Peng C-F, Ashton AW, Fu W, Mani K, Chin S-F, Provenzano E, Ellis I, Figg N, Pinder S, Bennett MR, Caldas C, **Kitsis RN**. Regulation of p53 tetramerization and nuclear export by ARC. *Proc Natl Acad Sci (USA)*, 2007. 104:20826-20831. PMID: 18087040. PMCID: PMC2409226.
- c. McKimpson WM, Zheng M, Chua SC, Pessin JE, **Kitsis RN**. ARC is essential for maintaining pancreatic islet structure and β -cell viability during type 2 diabetes. *Sci Rep*, 2017. 7:7019. PMID: 28765602. PMCID: PMC5539143.
- d. McKimpson WM, Chen Y, Irving JA, Zheng M, Weinberger J, Tan WLW, Tiang Z, Jagger AM, Chua Jr SC, Pessin JE, Foo RSY, Lomas DA, **Kitsis RN**. Conversion of the death inhibitor ARC to a killer activates pancreatic β -cell death in diabetes. *Developmental Cell*, 2021. 56:747-760. PMID: 33667344. PMCID: in progress.

2. Connections between cell death programs and related mitochondrial and ER functions.

Initially, apoptosis was believed to be the only regulated form of cell death. But, work over the past two decades has identified up to 11 other regulated cell death programs. These findings have posed an important new challenge to the cell death field: to understand molecular connections among cell death programs and their links to other fundamental biological processes in the organelles in which cell death signaling is taking place. We have approached this in the context of the central cell death regulator BAX, which also mediates mitochondrial fusion through impacting the function of mitofusins (MFNs). First, we discovered that BAX is critical not only for apoptosis, which has long been known, but also for necrotic cell death, and this occurs through a distinct mechanism involving mitochondrial fusion. Second, we have discovered the first peptides and small molecules that allosterically regulate the activities of MFN1 and MFN2. Third, we have begun to dissect connections between ER stress, cell death, and senescence.

- a. Whelan, RS, Konstantinidis K, Wei A-C, Chun Y, Reyna DE, Jha S, Yang Y, Calvert JW, Lindsten T, Thompson CB, Crow MT, Gavathiotis E, Dorn II GW, O'Rourke B, **Kitsis RN**. Bax regulates primary necrosis through mitochondrial dynamics. *Proc Natl Acad Sci (USA)*, 2012. 109:6566-6571. PMID: 22493254. PMCID: PMC3340068
- b. Chen H, Ruiz PD, McKimpson WM, Novikov L, **Kitsis RN**, Gamble MJ. MacroH2A1 and ATM play opposing roles in paracrine senescence and the senescence-associated secretory phenotype. *Molecular Cell*, 2015. 59: 719-731. PMID: 26300260. PMCID: PMC4548812.
- c. Franco A*, **Kitsis RN***, Fleischer JA, Gavathiotis E, Kornfeld OS, Gong G, Biris N, Benz A, Qvit N, Donnelly SK, Chen Y, Mennerick S, Hodgson L, Mochly-Rosen D, Dorn GW. Correcting mitochondrial fusion by manipulating mitofusin conformations. *Nature*, 2016. 540:74-79. PMID: 27775718. PMCID: 5315023 * equal contributions.
- d. Rocha AG, Franco A, Krezel AM, Rumsey JM, Alberti JM, Knight WC, Biris N, Zacharioudakis E, Janetka JW, Baloh RH, **Kitsis RN**, Mochly-Rosen D, Townsend RR, Gavathiotis E, Dorn GW. Rationally designed mitofusin agonists reverse *in vitro* and *in vivo* CMT2A mitochondrial defects. *Science*, 2018. 360:336-341. PMID: 29674596. PMCID: PMC6109362.

3. Translational biology: Roles of regulated cell death in heart disease.

My lab helped pioneer the cardiac cell death field. This includes the initial work showing that regulated forms of cardiomyocyte death play critical roles in the two major cardiac syndromes: myocardial infarction and heart failure. As described above, we have extended this work over the past several years to develop small molecule drug prototypes to manipulate cell death in a variety of cardiac syndromes including cancer therapy-induced cardiomyopathies. These approaches have included both screening of large chemical libraries and structure-based rational design.

- a. Bialik S, Geenen DL, Sasson IE, Cheng R, Horner JW, Evans SM, Lord EM, Koch CJ, **Kitsis RN**. Myocyte apoptosis during acute myocardial infarction in the mouse localizes to hypoxic regions but occurs independently of p53. *J Clin Invest*, 1997. 100:1363-1372. PMID: 9294101. PMCID: PMC508314.
- b. Wencker D, Chandra M, Nguyen KT, Miao W, Garantziotis S, Factor SM, Shirani J, Armstrong RC, **Kitsis**

RN. A mechanistic role for cardiac myocyte apoptosis in heart failure. *J Clin Invest*, 2003. 111:1497-1504. PMID: 12750399. PMCID: PMC155051.

- c. Garner TP, Amgalan D, Reyna DE, Li S, **Kitsis RN**, Gavathiotis E. Small-molecule allosteric inhibitors of BAX. *Nat Chem Biol*, 2019. 15:322-330. PMID: 30718816. PMCID: PMC6430685.
- d. Amgalan D, Garner TP, Pekson R, Jia XF, Yanamandala M, Paulino V, Liang FG, Corbalan JJ, Lee J, Chen Y, Karagiannis G, Sanchez LR, Liang H, Narayananagari SR, Mitchell K, Lopez A, Margulets V, Scarlata M, Santulli G, Asnani A, Peterson RT, Hazan RB, Condeelis JS, Oktay MH, Steidl U, Kirshenbaum LA, Gavathiotis E, **Kitsis RN**. A small molecule allosteric inhibitor of BAX protects against doxorubicin-induced cardiomyopathy. *Nat Cancer*, 2020. 1:315-328. PMID: 32776015. PMCID: PMC7413180.

Complete List of Published Work in My Bibliography

<https://www.ncbi.nlm.nih.gov/myncbi/richard.kitsis.2/bibliography/public/>

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: Ashton, Anthony Wayne

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

POSITION TITLE: Professor, Division of Cardiovascular Medicine, Lankenau Institute for Medical Research

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)	MM/YY	FIELD OF STUDY
University of New South Wales, Sydney, Australia	BSc	1989-1991	Anatomy and Physiology
University of New South Wales, Sydney, Australia	BSc (Hons.) Class 1	1992-1992	Bone Metabolism
University of New South Wales, Sydney, Australia	PhD	1993-1997	Vascular Biology
The Albert Einstein College of Medicine, Bronx, NY	Postdoctoral period 1	1997-2001	Angiogenesis
	Postdoctoral period 2	2001-2002	Cardiovascular biology

A. Personal Statement

My expertise in immune adaptations, angiogenesis, mouse models, mechanisms of cardiac cell death and signal transduction from G protein coupled receptors are well matched with the proposed SBIR phase 2 grant application entitled "*Improving Outcomes in Cancer Treatment-Related Cardiotoxicity*". My laboratory has studied molecular regulation of cardiovascular remodeling for ~22 years, elucidated the role for multiple molecular regulators of angiogenesis and myocardial survival, and expanded these concepts to multiple disease states including myocardial infarction and Chagas' cardiomyopathy, pregnancy induced heart failure, chemotherapy-induced cardiomyopathy, cancer and asthma.

I am currently Professor, Division of Cardiovascular Medicine, at The Lankenau Institute for Medical Research, working in a laboratory 1 floor below the laboratory of Dr Xuanmao Jiao (LightSeed LLC). I have published extensively over the last few years with Drs. Jiao and Pestell (the two other PIs on this proposal) and I have developed substantial preliminary data for the enclosed grant application. My laboratory has studied molecular mechanisms of cardiac remodeling and cell death and I will be examining the mechanisms of cell death in the proposed studies. I have been supervisor to 14 undergraduate and 22 post-graduate students doing degrees by research and over 25 post-doctoral fellows, many of whom have gone on to independent academic faculty positions.

B. Positions and Honors**Positions and Employment**

02/1996-11/1996	Research Assistant Department of Pathology, University of NSW, Sydney, Australia
05/1997-11/2002	Post-doctoral Fellow, Department of Medicine/Cardiology, Albert Einstein College of Medicine (AECOM), Bronx, NY
12/2002-08/2005	Instructor of Medicine, Department of Medicine/Cardiology, AECOM, Bronx, NY
5/2004-08/2005	Instructor, Department of Pathology, AECOM, Bronx, NY.
08/2005-12/2006	Assistant Professor, Departments of Cardiothoracic Surgery & Pathology, AECOM.
01/2007-04/2020	Scientific Director and Senior Research Fellow, Division of Perinatal Research, Kolling Institute of Medical Research, University of Sydney.
01/2007-present	Visiting Assistant Professor, Department of Pathology, AECOM, Bronx NY.
08/2019-present	Visiting Associate Professor, Baruch S. Blumberg Institute, Pennsylvania Cancer and

07/2020-present Regenerative Medicine Center, Philadelphia, PA.
 Professor, Division of Cardiovascular Medicine, Lankenau Institute for Medical Research.

Honors

1995	Young Investigator of the Year, Australian Vascular Biology Society Annual Meeting
2000-2002	Postdoctoral Fellowship, American Heart Association (Heritage Affiliate)
2001-2004	Consultant, Cardiovascular Division, Eli Lilly and Company
2001-present	<u>Editorial Boards:</u> <i>Scientific Reports</i> , <i>Frontiers in Oncology/Pharmacology</i> , <i>Cells</i> <u>Reviewer:</u> <i>Circ Res</i> , <i>J Biol Chem</i> , <i>J Clin Invest</i> , <i>Thromb Res</i> , <i>Prost Leuko Essential Fatty Acids</i> , <i>Am J Path</i> , <i>PLoS Path/ONE/NTD</i> , <i>Hyperten Res</i> , <i>Parasit Res</i> , <i>Cancer Letters</i>
2006-2011	R.D. Wright Biomedical Career Development Award, NHMRC of Australia
2006-present	Consultant, Mechanisms of Infectious Diseases NIH Training Grant, AECOM
2007	PaLMS International Travel Award, Scientific Staff Council, RNSH
2007	Ludwig Institute for Cancer Research Invited Seminar Series
2007	Ramsay Health Care International Study Fellowship, Scientific Staff Council, RNSH
2007	Cayman Chemical Travel Award; 10th International Conference on Eicosanoids and Other Bioactive Lipids in Cancer, Inflammation and Related Diseases
2008	International Exchange Program Award, Australian Academy of Science.
2009	Young Investigator of The Year Award, Cancer Research; 11th International Conference on Bioactive Lipids in Cancer, Inflammation and Related Diseases
2009	Plenary Lecture, Succeeding in Research Translation, Cancer Research Network
2010	Bridging Support for Senior Scientists, RNSH
2011	Colin I Johnston Award and Lecture, High Blood Pressure Research Council of Australia
2011	Plenary Lecture, 15 th Symposium of the Australia and New Zealand Microcirculation Society
2013	DVC (Research) Bridging Support, University of Sydney
2013	Plenary Lecture, 3 rd International Conference on Clinical and Experimental Cardiology
2013	Plenary Address and Session Chair; 13th International Conference on Bioactive Lipids in Cancer, Inflammation, and Related Diseases
2013	Plenary Lecture, Hematology and Oncology Targeted Therapies symposium
2013-2014	Member of Organising Committee, International Society of Hypertension Satellite Meeting
2013-2016	State Representative to the Board, Australia and New Zealand Microcirculation Society
2014	DVC (Research) Bridging Support, University of Sydney
2014	Plenary Address; Cardiology 2014
2014	Organizing Committee, Cardiology 2015
2015, 2017	DVC (Research) Bridging Support, University of Sydney
2015	Conference Convenor and Chair Organising Committee, Australia and New Zealand Microcirculation Society Biennial Meeting
2016-2019	Vice President, Australia and New Zealand Microcirculation Society
2017	Invited Lecture, Lipid Interest Group, Department of Medicine, Stony Brook University
2022	POLM Investigator Award, 17th International Conference on Bioactive Lipids in Cancer, Inflammation, and Related Diseases
2023	Dr. Louis Plzak Award for Cardiovascular Research; Sharpe-Strumia Research Foundation

C. Contribution to Science (Selected from 91 peer-reviewed publications; 25 are in biomedical journals with impact factors ≥ 5 ; H Factor: 27; Mean citations/publication: 39)

Collaborative research studies with Drs. Jiao and Pestell. These studies included elucidation of the molecular mechanisms governing G protein coupled receptor signaling, cellular migration, invasion and molecular mechanisms of tumor formation *in vitro* and *in vivo* (representative publications from 13 total).

1. Ashton AW, Dhanjal HK, Rossner B, Mahmood H, Patel VI, Nadim M, Lota M, Shahid F, Li Z, Joyce D, Pajkos M, Dosztányi Z, Jiao X, Pestell RG (2022). Acetylation of nuclear receptors in health and disease: an update. *FEBS J.* 2022 Dec 5. doi: 10.1111/febs.16695
2. Chen, K, Jiao X., **Ashton, AW.**, Di Rocco, A., Pestell, TG., Sun, Y., Zhao, J, Casimiro, MC, Li, Z., Lisanti, MP, McCue., PA, Shen, D., Achilefu, A. Rui, H, **Pestell, RG** (2020). *The membrane-associated form of cyclin D1 enhances cellular invasion.* *Oncogenesis*; 9(9):83.
3. Jiao, X., Wang, M., Zhang, Z., Li, Z., Ni, D, **Ashton, A.W.**, Tang., H-Y., Speicher, DW, **Pestell, R.G.** *Leronlimab, a humanized monoclonal antibody to CCR5, blocks breast cancer cellular metastasis and*

- enhances cell death induced by DNA damaging chemotherapies.* **Breast Cancer Res.** 2021 Jan 23;23(1):11.
4. Jiao X, Tian L, Zhang Z, Balceruk J, Kossenkov AV, Casimiro MC, Wang C, Liu Y, Ertel A, Soccio RE, Chen ER, Liu Q, **Ashton AW**, Tong W, **Pestell RG** (2021). *Ppary1 Facilitates ErbB2-Mammary Adenocarcinoma in Mice.* **Cancers** (Basel); 13(9):2171
 5. Li Z, Jiao X, Robertson AG, Di Sante G, **Ashton AW**, DiRocco A, Wang M, Zhao J, Addya S, Wang C, McCue PA, South AP, Cordon-Cardo C, Liu R, Patel K, Hamid R, Parmar J, DuHadaway JB, Jones SJM, Casimiro MC, Schultz N, Kossenkov A, Phoon LY, Chen H, Lan L, Sun Y, Iczkowski KA, Rui H, **Pestell RG**. (2023). *The DACH1 gene is frequently deleted in prostate cancer, restrains prostatic intraepithelial neoplasia, decreases DNA damage repair, and predicts therapy responses.* **Oncogene**; 42(22):1857-1873.

Transcriptomic Analysis of heart failure: We recently published our first transcriptomic assessment of non-failing and diseased cardiac tissues. The analysis used RNAseq to analyze the transcriptomic profile of left ventricular tissue from non-failing donor hearts, and patients with end-stage heart failure (either PPCM or DCM). We demonstrated similarities (metabolic pathways and extracellular matrix remodeling) and distinct differences (golgi vesicle biogenesis and budding, T-cell responses) between PPCM and DCM in multiple pathogenic pathways. Importantly, these data provided the means to identify the molecular subtypes of PPCM upon which the current proposal is based.

1. Taylor J, Yeung ACY, Ashton AW, Faiz A, Guryev V, Fang B, Lal S, Grosser M, Dos Remedios CG, Braet F, McLachlan CS, Li A (2023). Transcriptomic comparison of human peripartum and dilated cardiomyopathy identifies differences in key disease pathways. **J Cardiovasc Develop Disease**, 10(5):188.

Molecular regulation of cardiac remodeling: The decision of a cell to die is tightly controlled. We have characterized the role of multiple stimuli in determining cardiac remodeling. Our work on the mineralocorticoid receptor (MR) was the first to identify its role in post-infarction remodeling. Low-dose MR antagonists reduce cardiomyocyte death via prevention of proteasomal targeting of ARC after reperfusion injury. Our previous work defined ARC as a unique anti-apoptotic protein that blocks both intrinsic and extrinsic pathways of apoptosis. The propensity of MR activation to aggravate reperfusion injury was gender specific and, through computer modelling, suggest a potential agonist role for estrogen in MR activation and that testosterone likely functions to block MR ligand binding. This may explain the enhanced cardiovascular risk profile of older women receiving hormone replacement therapy and young women with metabolic syndrome.

1. Mihailidou, A, Tzakos, A, **Ashton, AW** (2019). Non-Genomic Effects of Aldosterone. **Vitamins and Hormones**, 109(133), 149-149
2. **Ashton AW**, Le TY, et al. (2015). Role of Nongenomic Signaling Pathways Activated by Aldosterone During Cardiac Reperfusion Injury. **Molecular Endocrinology**, 29:1144-55.
3. Le, TYL, **Ashton, AW**, et al. (2014) Role of androgens in sex differences in cardiac damage during myocardial infarction. **Endocrinology** 155(2):568-75
4. Le L, ..., **Ashton AW***, Mihailidou AS* (2012). Low dose spironolactone prevents ARC degradation during myocardial infarction. **Hypertension**. 59:1164-1169

Mechanisms of cell death. Dr Ashton has conducted studies on the mechanisms governing cell death including studies of ARC (apoptosis repressor with caspase recruitment domain) which is an endogenous inhibitor of apoptosis that antagonizes both central apoptosis pathways.

1. Nam YJ, Mani K, **Ashton AW**, Peng CF, Krishnamurthy B, Hayakawa Y, Lee P, Korsmeyer SJ, Kitsis RN. *Inhibition of both the extrinsic and intrinsic death pathways through nonhomotypic deathfold interactions.* **Mol Cell.** 2004;15(6):901-12. PMID:15383280.
2. Mercier I, Vuolo M, Madan R, Xue X, Levalley AJ, **Ashton AW**, Jasmin JF, Czaja MT, Lin EY, Armstrong RC, Pollard JW, Kitsis RN. *ARC, an apoptosis suppressor limited to terminally differentiated cells, is induced in human breast cancer and confers chemo- and radiation-resistance.* **Cell Death Differ.** 2005;12(6):682-6. PMID:15861191
3. Nam YJ, Mani K, Wu L, Peng CF, Calvert JW, Foo RS, Krishnamurthy B, Miao W, **Ashton AW**, Lefer DJ, Kitsis RN. *The apoptosis inhibitor ARC undergoes ubiquitin-proteasomal-mediated degradation in*

- response to death stimuli: identification of a degradation-resistant mutant.* J Biol Chem. 2007 282(8):5522-8. Epub 2006 Dec 1. PMID:17142452.
4. Foo RS, Nam YJ, Ostreicher MJ, Metzl MD, Whelan RS, Peng CF, **Ashton AW**, Fu W, Mani K, Chin SF, Provenzano E, Ellis I, Figg N, Pinder S, Bennett MR, Caldas C, Kitsis RN. *Regulation of p53 tetramerization and nuclear export by ARC.* Proc Natl Acad Sci U S A. 2007 104(52):20826-31. Epub 2007 Dec 17. PMID:18087040

Role of eicosanoids in Chagas cardiomyopathy: The role of eicosanoids in the pathogenesis of Chagas disease is an under-appreciated area of investigation. We were the first to identify a role for thromboxane A₂ in the pathogenesis of *T. cruzi* infection. Thromboxane A₂ in the *T. cruzi*-infected mice was of both parasite and host origins and the response to thromboxane A₂, irrespective of its source, was a signal that controlled acute mortality and parasite proliferation/density. Moreover, treatment of *T. cruzi* infected mice with aspirin (irreversibly inhibits COX1 activity) promoted mortality and parasitemia in murine models of Chagas disease. This work subsequently characterized the effects of parasite-derived thromboxane A₂ on host responses using transcriptomics and expanded the roles of eicosanoids in Chagas disease to include the pro-resolving mediators Resolvin D1/D5/E2. These results redefined the mediators that control host-parasite interactions to propagate parasitic infection/transmission and established eicosanoids as quorum sensors that control parasite intracellular growth.

1. Colas, RA*, **Ashton, AW***, et al. (2018). Trypanosoma cruzi produces the specialized pro-resolving mediators Resolvin D1, Resolvin D5 and Resolvin E2. *Infect. Immun.* 86(4). pii: e00688-17.
2. Mukherjee, S, Sadekar, N, **Ashton, AW**, et al., (2013). Identification of a functional prostanoid-like receptor in the protozoan parasite, *Trypanosoma cruzi*. *Parasitology Research*, 112: 1417-25.
3. Machado FS, Mukherjee S, Weiss LM, Tanowitz HB, **Ashton AW** (2013). Bioactive lipids in *Trypanosoma cruzi* Infection. *Advances in Parasitology*, 76: 1-31
4. Mukherjee, S, et al. **Ashton, AW** (2011). Aspirin treatment of mice infected with *T cruzi* and implications for the pathogenesis of Chagas disease. *PLoS ONE*, 6: e16959 (doi:10.1371/journal.pone.0016959).
5. **Ashton, AW**, et al. (2007). Thromboxane A₂ is a key regulator of pathogenesis during *Trypanosoma cruzi* infection. *J. Experimental Medicine*, 204:929-40. (Faculty of 1000 – top 100 articles of 2007).

Mechanisms of immune adaptation and G protein coupled receptors in pregnancy: We have characterized the alterations in immunologic function and GPCRs in models of placentation. Activation of thromboxane receptors (TP β) in transgenic mice retarded intrauterine growth of pups, and induced PE and in the dams. Dysregulation of TP splicing was confirmed in pathologic placentae from IUGR and PE affected pregnancies. These data identify TP β as a new risk factor for PPCM and PE and highlight the shared pathogenic mechanism for the two disorders. These data evolved into policy initiatives that altered guidelines around timing of birth and outcomes for development. This work identified human-specific regulators that co-evolved with, and provide a basis for the human origins of, pre-eclampsia - a disease without viable therapeutic options.

1. Powell, K, Carrozza; A, Stephens, AS, Tasevski, V, Morris, JM, **Ashton, AW**, Dona, AC (2018). *Utility of metabolic profiling of serum in the diagnosis of pregnancy complications.* Placenta: 66:65-73.
2. McKelvey, KJ, Yenson, VM, **Ashton, AW**, Morris, JM, McCracken, SA (2016). *Embryonic/fetal mortality and intrauterine growth restriction is not exclusive to the CBA/J sub-strain in the CBA × DBA model.* Scientific Reports, 6:35138 (DOI: 10.1038/srep35138).
3. Powell KL, Stevens V, Upton DH, McCracken SA, Simpson AM, Tasevski V, Morris JM, **Ashton AW** (2016). *Role for the thromboxane A₂ receptor β -isoform in the pathogenesis of intrauterine growth restriction.* Scientific Reports, 6:28811 (DOI: 10.1038/srep28811)..
4. McKelvey, KJ, Powell, KL, **Ashton, AW**, Morris, JM, McCracken, SA (2015). *Exosomes: Mechanisms of Uptake.* Journal of Circulating Biomarkers, DOI: 10.5772/61186
5. McCracken SA, Hadfield KA, Macfarlane M, **Ashton AW**, Morris JM (2016). *NF- κ B Regulation in T-cells in Pregnancy is Mediated via Fas/FasL Interactions: The Signal for which is Derived from Exosomes Present in Maternal Plasma.* Reproductive Immunology: Open Access, 1:1-8

Understanding molecular regulation of angiogenesis: The control of neovascularization is integral to understanding multiple diseases including well described (cancer and retinopathy) and esoteric (such as

asthma) angiopathies. We have defined multiple pathways (cyclin D, PKC) but identified alternative splicing as perhaps the most significant mechanism controlling angiogenesis. We were the first to describe the anti-angiogenic effects of a novel splice variant of the GPCR, thromboxane A₂ receptor (TP β), which stabilize vascular networks with age. We have also defined angiogenesis as an important part of airway remodeling in asthma. Indeed, angiostatic proteins derived from matrix proteins ameliorate airway remodeling and prevent onset of symptoms in preclinical models. We have identified alternative splicing of the CollV- α 3 and CollV- α 5 genes in asthmatic airways promotes neovascularization to perpetuate airway narrowing and lung function.

1. Wang, J, Faiz, A, Ge, Q, Vermeulen, CJ, Van der Velden, J, Snibson, KJ, van de Velde, R, Sawant, S, Xenaki, D, Oliver, B, Timens, W, ten Hacken, N, van den Berge, M, James, A, Elliot, JG, Dong, L, Burgess, JK*, **Ashton, AW*** (2018). *Unique mechanisms of CTGF regulation in airway smooth muscle in asthma*. J Cell Mol Med: 22(5):2826-2837. (doi: 10.1111/jcmm.13576)
2. Harkness, LM, Weckmann, M, Kopp, M, **Ashton, AW***, Burgess, JK* (2017). *Tumstatin regulates the angiogenic and inflammatory potential of airway smooth muscle extracellular matrix*. J Cell Mol Med, doi: 10.1111/jcmm.13232
3. Harkness LM, **Ashton AW***, Burgess JK* (2015). *Asthma is not only an airway disease, but also a vascular disease*. Pharmacology and therapeutics, 148:17-33
4. **Ashton, AW**, Ware, JA (2004). *Thromboxane A₂ receptor signaling inhibits vascular endothelial growth factor-induced endothelial cell differentiation and migration*. Circulation Research, 95:372-379.
5. **Ashton, AW**, Cheng, Y, Helisch, A, Ware, JA (2004). *Thromboxane A₂ receptor antagonists antagonizes the proangiogenic effects of FGF-2*. Circulation Research, 94:735-742.

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: Khan, Taimoor

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

POSITION TITLE: Postdoctoral Research Fellow

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 Peshawar, Peshawar, Pakistan.	BS	07/2011	Biotechnology
Comsats University, Islamabad, Pakistan.	MS	09/2013	Bio Sciences
Shanghai Jiao Tong University, Shanghai, China.	PhD	12/2021	Biology
University of California, San Francisco.	Postdoc	05/2023	Cancer Biology
Baruch S Blumberg Institute	Postdoc	Current	Cancer Biology

A. Personal Statement

My long term goal is to better challenge cancer malignancies by designing improved therapies and becoming an independent investigator in the field of cancer biology. I have the expertise necessary to successfully carry out the proposed research project entitled "*Improving Outcomes in Cancer Treatment-Related Cardiotoxicity*". by collaborating and working with top-notch scientists in the field of cancer research.

My prior studies include bio informatic analysis, development of novel biological databases, and currently studies of the mechanisms governing cell death and cancer therapy resistance.

After the successful completion of my one-year postdoc training at UCSF, I joined the team of world-renowned scientists in the laboratory of Dr. Pestell at the Baruch S. Blumberg Institute, who have made the seminal discoveries holds several patents in the field of CCR5 in cancer and cardio protection.

Citations:

1. Di Benedetto, C., **Khan, T.**, Serrano-Saenz, S., Rodriguez-Lemus, A., Klomsiri, C., Beutel, T.M., Thach, A., Walczak, H. and Betancur, P., 2023. Enhancer Clusters Drive Type I Interferon-Induced TRAIL overexpression in Cancer, and Its Intracellular Protein Accumulation Fails to Induce Apoptosis. *Cancers*, 15(3), p.967.
2. Khan, A., **Khan, T.**, Nasir, S.N., Ali, S.S., Suleman, M., Rizwan, M., Waseem, M., Ali, S., Zhao, X. and Wei, D.Q., 2021. BC-TFdb: a database of transcription factor drivers in breast cancer. *Database*, 2021.
3. Han, P., Zhang, L., Fu, Y., Fu, Y., Huang, J., He, J., Ni, P., **Khan, T.**, Jiao, Y., Yang, Z. and Zhou, R., 2023. A dual-response drug delivery system with X-ray and ROS to boost the anti-tumor efficiency of TPZ via enhancement of tumor hypoxia levels. *Nanoscale*, 15(1), pp.237-247.

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

- 2023 - Present Postdoctoral Research Fellow, Baruch S Blumberg Institute, Philadelphia, PA.
2022 - 2023 Postdoctoral Scholar, University of California, San Francisco.

2015 - 2021	CSC (Chinese Scholarship Council) PhD Scholar, School of Life Sciences and Biotechnology, Shanghai Jiao Tong University, China.
2014 - 2015	Researcher, National Institute for Biotechnology and Genetic Engineering, Faisalabad, Pakistan.
2013 - 2014	Director Health, Innovative Youth Forum, Swat, Pakistan.

Academic and Professional Honors/Memberships

2023 – Current	Associate Member of American Association for Cancer Research (AACR).
2022 – 2023	Member and participant of Immuno-X initiative at UCSF.
2015 - 2021	Chinese Government Fellowship for International PhD students in China.

C. Contributions to Science

1. Genetic control of cell cycle in breast cancer progression. I studied Rb pathway regulating tumor suppressor gene (critical gatekeeper) involved in the regulation of G1 to S transition during cell cycle. Rb2/p130 (member of Rb family) has a defined role in cellular proliferation and breast carcinogenesis. It is involved in the regulation of Rb pathway during cell cycle and it acts as a tumor suppressor in breast cancer. The ability of this gene to suppress neoplastic growth offers an attractive therapeutic target for treating disorders like abnormal cell growth in breast cancer. In my study, I correlated regulation of this important gene with prognostic parameters of breast cancer. I mainly utilized Quantitative Real Time PCR for determination of relative expression level of Rb2/p130 gene in human breast cancer tumors and compared it with the controls. I also investigated the correlation of expression of this gene with several clinico-pathological parameters. The study was aimed to provide baseline data for development of diagnostic and therapeutic approaches to better challenge breast cancer malignancy in Pakistani population. Furthermore, we also discovered a novel epigenetic mechanism regulating the RB2/p130 gene expression and found it correlated with higher promoter methylation status in breast cancer patients as compared to controls. The publication documented a novel regulatory mechanism of Rb2 expression in breast cancer where I served as a contributing author in the study.

:

1. Ullah, F., **Khan, T.**, Ali, N., Malik, F.A., Kayani, M.A., Shah, S.T.A. and Saeed, M., 2015. Promoter methylation status modulate the expression of tumor suppressor (RbL2/p130) gene in breast cancer. PLoS One, 10(8), p.e 0134687.
2. During my PhD research, I documented my doctoral thesis entitled as "*Development of biological databases and its application in designing therapeutics against human pathogenic viruses*". Herein, I developed two novel biological databases for emerging human pathogens with important data interpretations suggesting new ideas in viral therapeutics related biological research. The novel platforms that I developed provides easily available information which can be used to design immunology based therapeutic interventions and develop advanced detection techniques against emerging human pathogenic viruses. This includes genome and protein sequence information, proteins based immune related features, putative immunogenic epitopes, vaccine designs and RNA interference- related data. For each of the protein from all of the viral species i.e. "*Cytomegaloviruses*" and "*Mammarena Viruses*", specific vaccine epitopes were mapped using a cluster of immune-omics based algorithms and tools. The findings available in these freely accessible platforms provides advanced baseline data to develop novel therapeutics and better challenge viral pandemics in the future.
 1. **Khan, T.**, Khan, A., Nasir, S.N., Ahmad, S., Ali, S.S. and Wei, D.Q., 2021. CytomegalovirusDb: multi-Omics knowledge database for Cytomegaloviruses. Computers in Biology and Medicine, 135, p.104563.
 2. **Khan, T.**, Khan, A. and Wei, D.Q., 2021. MMV-db: vaccinomics and RNA-based therapeutics database for infectious hemorrhagic fever-causing mammarenaviruses. Database, 2021.
3. I documented **several therapeutic intervention** models for pathogens including SARS-COV-2. These studies mainly included important work on deciphering potential mechanisms involved in higher infectivity and progression of the COVID-19 Disease. The findings generated from these studies are crucial to better understand disease etiology and design novel therapeutics against these human pathogens.

1. Khan, A., Zia, T., Suleman, M., **Khan, T.**, Ali, S.S., Abbasi, A.A., Mohammad, A. and Wei, D.Q., 2021. Higher infectivity of the SARS-CoV-2 new variants is associated with K417N/T, E484K, and N501Y mutants: an insight from structural data. *Journal of cellular physiology*, 236(10), pp.7045-7057.
2. **Khan, T.**, Khan, A., Ansari, J.K., Najmi, M.H., Wei, D.Q., Muhammad, K. and Waheed, Y., 2022. Potential immunogenic activity of computationally designed mRNA-and Peptide-based prophylactic vaccines against MERS, SARS-CoV, and SARS-CoV-2: a reverse vaccinology approach. *Molecules*, 27(7), p.2375.
3. Khan, A., **Khan, T.**, Ali, S., Aftab, S., Wang, Y., Qiankun, W., Khan, M., Suleman, M., Ali, S., Heng, W. and Ali, S.S., 2021. SARS-CoV-2 new variants: Characteristic features and impact on the efficacy of different vaccines. *Biomedicine & Pharmacotherapy*, 143, p.112176.

Complete List of Published Work:

<https://scholar.google.com/citations?user=Tv6mZk8AAAAJ&hl=en>

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: Danni Li

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

POSITION TITLE: Postdoctoral Research Fellow

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
Guizhou medical university, Guizhou, China	BS	07/2015	Medicine
University of Tongji, Shanghai, China	MD	07/2019	Pathology and pathophysiology
University of Tongji, Shanghai, China	PhD	09/2023	Molecular Biology
Baruch S. Blumberg Institute, Philadelphia, PA	Postdoc	-	Molecular Biology

A. Personal Statement

After obtaining Ph.D degree from Tongji University Medical School in the field of breast cancer metastasis research, I recently proceed to the postdoctoral training in the Laboratory of Dr. Pestell. My research experience is well aligned with the proposed studies in the grant entitled "*Improving Outcomes in Cancer Treatment-Related Cardiotoxicity*". In particular I am familiar with assessing animal models of cancer in response to therapies, and analysing cardiac tissue and cell lines for cell survival as proposed herein. The Pestell laboratory has generated the discoveries that led to the current proposal and I have contributed to the preliminary data.

1. National Natural Science Foundation of China (81772810), 2018-2021, Regulation of Bmi-1 mediated CCAT2-miR-200c in triple negative breast cancer stem cells and tumors.
2. National Basic Research Program of China, 973 Program (20JC1410400), 2020- 2023, Study on the substructure distribution and function of long chain noncoding gene CCAT2 in breast cancer cells.
3. National Natural Science Foundation of China (81972476), 2020-2023, Study on the mechanism of regulation on ERα-miR-29a-Pten axis in breast cancer metastasis and targeted intervention of magnetic nanoparticles-mediated.

B. Positions

Present Postdoctoral Research Fellow, Baruch S. Blumberg Institute, Philadelphia, PA

C. Contributions to Science

1) Breast cancer. My Ph.D work was mainly focus on the field of development and progression in breast cancer. Our previously work identified circulating miRNAs as diagnostic biomarkers for early detection of breast cancer, and found that the targeted inhibition of miR-221/222 in MDA-MB-231 cells promoted cisplatin-induced cell apoptosis which showed a novel approach for the combination chemotherapy of cisplatin with small non-coding RNA such as miR-221/222 in treatment of human TNBC and indicated the importance of non-coding genome in mediating the mental stress-induced cancer regulation.

1. A circulating miR-19b-based model in diagnosis of human breast cancer. Qian Zhao, Lei Shen, Jinhui Lü, Heying Xie, **Danni Li**, Yuanyuan Shang, Liqun Huang, Lingyu Meng, Xuefeng An, Jieru Zhou, Jing

- Han, Zuoren Yu. *Front Mol Biosci*. 2022 Sep 16; 9:980841. doi: 10.3389/fmolb. 2022.980841. PMID: 36188229.
2. Targeted Inhibition of miR-221/222 Promotes Cell Sensitivity to Cisplatin in TripleNegative Breast Cancer MDA-MB-231 Cells. Li S, Li Q, Lü J, Zhao Q, **Li D**, Shen L, Wang Z, Liu J, Xie D, Cho WC, Xu S, Yu Z. *Front Genet*. 2020 Jan 14;10:1278. doi: 10.3389/fgene.2019.01278.
 3. Immune and nonimmune mechanisms mediate the mental stress-induced tumor growth in a xenograft model of breast cancer. Ma W, Liu P, Zheng J, Lü J, Zhao Q, **Li D**, Guo Y, Qian L, Wang Q, Miao X, Yu Z. *Cell Death Dis*. 2021 Oct 23;12(11):987. doi: 10.1038/s41419-021-04280-9.

2) Cancer stemness. Long non-coding RNAs have been demonstrated on its important roles in regulating tumor development and progression in breast cancer, we found a dual function of long non-coding RNA CCAT2 as a tumor suppressor in breast cancer depending upon its subcellular distribution for the first time and identified a miR-221/222 cluster as a novel regulator of Cancer stem cell in non-samll cell lung cancer.

1. Dual Function of CCAT2 in Regulating Luminal Subtype of Breast Cancer Depending on the Subcellular Distribution. Xie H, Guo Y, Xu Z, Wang Q, Wang T, Gu Y, **Li D**, Liu Y, Ma W, Liu P, Zhao Q, Lü J, Liu J, Yu Z. *Cancers (Basel)*. 2023 Jan 16;15(2):538. doi: 10.3390/cancers15020538.
2. Reck-Notch1 Signaling Mediates miR-221/222 Regulation of Lung Cancer Stem Cells in NSCLC. Guo Y, Wang G, Wang Z, Ding X, Qian L, Li Y, Ren Z, Liu P, Ma W, **Li D**, Li Y, Zhao Q, Lü J, Li Q, Wang Q, Yu Z. *Front Cell Dev Biol*. 2021 Apr 20;9:663279. doi: 10.3389/fcell.2021.663279.

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: LAL, SEAN, MD PHD

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

POSITION TITLE: Associate Professor of Medicine

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 Sydney, Sydney, Australia	MB BS (MD)	2006	Medicine
University of Sydney, Sydney, Australia	MPhil	2009	Molecular Biology
FRACP Cardiology specialty training, Australia	FRACP	2014	Clinical Cardiology
University of Sydney, Sydney, Australia	PhD	2017	Molecular Biology

A. Personal Statement

CI LAL completed an undergraduate degree in Medical Science with first class honours at the University of Sydney, receiving full academic scholarship. CI LAL pursued a graduate Medical Degree (MBBS) and a Master of Medicine by research (MPhil) at the University of Sydney, where CI LAL was awarded the Dean's Scholarship, the Medical Foundation Scholarship, and the University of Sydney Bercovici Medal for post-graduate research. CI LAL completed general and specialty clinical training at Royal Prince Alfred Hospital. During cardiology training, CI LAL was awarded a National Churchill Fellowship to study mechanisms of cardiac regeneration at Harvard Medical School. CI LAL is trained clinically and in research in heart failure. For CI LAL's PhD in this field, CI LAL was awarded a combined National Health and Medical Research Council (NHMRC) and National Heart Foundation (NHF) Scholarship, as well as the NHMRC and Royal Australasian College of Physicians (RACP) scholarship for research excellence. CI LAL was also awarded a Commonwealth Endeavour Postgraduate Fellowship to Harvard University and Massachusetts Institute of Technology (MIT), where CI LAL undertook proof of concept studies demonstrating the intrinsic regenerative capacity of the human heart, in addition to Attending duties in the CICU at The Brigham and Women's Hospital.

CI LAL is the elected Chair of the Heart Failure Council for Australia and New Zealand, which directs national clinical policies for the diagnosis and treatment of heart failure, including leading the Australian and NZ position statement on the effects of COVID-19 and heart failure. CI LAL is the Director of the Sydney Heart Bank at the University of Sydney, which is the largest (18,000 cryopreserved heart samples) human heart biobank in the world that was founded in collaboration with St Vincent's Hospital Sydney and has now expanded to include Royal Prince Alfred Hospital. It is completely not-for-profit and there are currently 58 international collaborators. CI LAL is the Head of the Cardiac Research Laboratory at the Charles Perkins Centre, which combines basic and translational research into human heart failure.

CI LAL is an Associate Professor of Medicine in the Faculty of Medicine and Health at the University of Sydney (tenured) and a Consultant Cardiologist at Royal Prince Alfred Hospital where CI LAL is Director of Heart Failure Services. CI LAL is 6.5 years post PHD and has over 75 publications (H-index 25) including **senior author** original research articles in top-ranking journals such as The European Heart Journal, Nature Communications, The Lancet Diabetes and Endocrinology, The European Journal of Heart Failure and JACC Basic to Translational. CI LAL has also been a contributing author for publications in Circulation, Circulation Heart Failure,

Circulation Research, Circulation: Genomics and Precision Medicine, PNAS, Nature Protocols, Nature Cardiovascular Research and JACC.

B. Positions, Scientific Appointments, and Honors

2022 - present	Tenured Associate Professor, School of Medical Sciences, Faculty of Medicine and Health, University of Sydney, Sydney, Australia
2021 - present	Director of Acute Heart Failure Services, Royal Prince Alfred Hospital Sydney, Australia,
2019 - present	Director of the Sydney Heart Bank, Head of Lab, Faculty of Medicine and Health, University of Sydney, Australia
2019 - present	Consultant (Attending) Cardiologist, Royal Prince Alfred Hospital, Sydney, Australia
2020 - 2021	Tenured Senior Lecturer, School of Medical Sciences, Faculty of Medicine and Health, University of Sydney, Sydney, Australia
2018 - 2019	Tenured Lecturer, School of Medical Sciences, Faculty of Medicine and Health, University of Sydney, Sydney, Australia
2015 - 2016	Australian Government Endeavour Post-Graduate Fellowship to Harvard Medical School and Brigham and Women's Hospital (Basic sciences, molecular biology)
2014 - 2017	Tenure-Track Lecturer, School of Medical Sciences, Faculty of Medicine and Health, University of Sydney, Sydney, Australia
2011 - 2013	Advanced Trainee Cardiology, Royal Prince Alfred Hospital, Sydney, Australia
2007 - 2010	Intern, Resident, Registrar, Royal Prince Alfred Hospital, Sydney, Australia

Honors Dux years 7-12, College Captain, First XI Captain & Tennis Captain, De La Salle College; Undergraduate Full Academic Scholarship, University of Sydney; Undergraduate Research Scholarship, National Heart Foundation; J. Stone Prize for 1st place in Honours Medical Sciences, University of Sydney; Ranked top 0.5% nationally in the Graduate Medical Admission Examination for Australia; Dean of Medicine Scholarship, University of Sydney; Commonwealth Learning Scholarship, Australian Government; Medical Foundation Research Scholarship, University of Sydney; Bercovici Medal, University of Sydney; Churchill Fellowship to Harvard Medical School, Sir Winston Churchill Trust Australia; PhD Scholarship, National Health and Medical Research Council of Australia; PhD Scholarship, National Heart Foundation of Australia; Prize for highest ranked clinician researcher, Royal Australasian College of Physicians and the National Health and Medical Research Council of Australia; Commonwealth Endeavour Fellowship to Harvard University, Massachusetts; Institute of Technology & the Brigham and Women's Hospital; Cardiometabolic Research Prize, Cardiac Society of Australia and New Zealand; Inducted Fellow, European Society of Cardiology

C. Contributions to Science

CI LAL research interest spans clinical, basic sciences and translational research into cardiovascular disease, specifically heart failure and coronary artery disease, both of which are leading causes of morbidity and mortality in Australia. With respect to basic and translational research, these centre upon big data analysis of human heart samples to unlock potential mechanisms of heart failure through 'merge omics' (multi nodal and network analysis of unbiased transcriptomic, metabolomic and proteomic data). CI LAL collaborates with other Australian groups to establish links between basic molecular targets and clinical endpoints. CI LAL's clinical research examines major public health problems, linking epidemiology to clinical outcomes with the view to change international clinical guidelines. Total peer-reviewed funding to date \$11 million (\$5.5 million as a Chief Investigator).

Cardiovascular molecular biology

Mengbo Li, Benjamin L. Parker, Evangeline Pearson, Benjamin Hunter, Jacob Cao, Yen Chin Koay, Oneka Guneratne, David E. James, Jean Yang, **Sean Lal**^{*}, John O'Sullivan^{*} (*co-senior authors). Core functional nodes and sex-specific pathways in human ischaemic and dilated cardiomyopathy. (2020) *Nature Communications* (11, 2843). Impact factor 17. This is the most extensive big data analysis of human heart failure to unlock potential mechanisms of heart failure through 'merge omics' (multi nodal and network analysis of unbiased metabolomic and proteomic mass spec).

Jacob Cao, Yen Chin Koay, Lake-Ee Quek, Benjamin Parker, **Sean Lal***, **John O'Sullivan*** (*co-senior authors). Myocardial Metabolic Perturbations in Advanced Ischemic and Non-Ischemic Dilated Human Heart Failure. (2019) *European Journal of Heart Failure*. 21(8), 1042-1045. Impact factor 17. This was the first study to analyse human cardiac tissue metabolomics in heart failure, demonstrating several changes in energy/substrate utilization in the failing heart.

Clinical Cardiology

Sean Lal, Irina Kotchetikova, Jacob Cao, Daniel Jackson, Rachael Cordina and David S Celermajer. Heart failure admissions and poor subsequent outcomes in adults with congenital heart disease. (2017) *European Journal of Heart Failure* Apr; 20(4): 812-815. Impact factor 17. This was the first study to demonstrate the high mortality rate (as early as 3 months) post first admission with heart failure in this vulnerable cohort.

Nelson Wang, Jordan Fulcher, Nishan Abeysuriya, Michele McGrady, Ian Wilcox, David Celermajer, **Sean Lal**. Tricuspid Regurgitation is associated with increased mortality independent of pulmonary pressures and right heart failure. (2019) *European Heart Journal* 40, 476-484. Impact factor 39. This article was editorialised in the *European Heart Journal*. This study was guideline changing and editorialized twice by this journal, the number one in its field, whereby tricuspid regurgitation was shown to be an independent predictor of mortality regardless of etiology.

Nelson Wang, Jordan Fulcher, Nishan Abeysuriya, Laura Park, Shejil Kumar, Gian Luca Di Tanna, Ian Wilcox, Anthony Keech, Anthony Rodgers, **Sean Lal**. More intensive LDL-C lowering reduces major vascular events beyond current recommendations: systematic review and meta-analysis of 327,037 patients. *The Lancet Diabetes and Endocrinology* 8 (1): 36-49 Impact factor 39. This article was editorialised in *The Lancet Diabetes and Endocrinology*. This study has been editorialized and now with an invited editorial to highlight the need for early intervention to lower LDL in younger at risk populations, in addition to the need to lower LDL in older higher risk populations beyond the current lipid guidelines.

Responsibilities impacting on track record

From 2007 until 2015, I worked **full-time** as a clinician. Since 2014, when I received my FRACP (Cardiology) I have maintained a clinical workload as a heart failure specialist at RPA that includes inpatient and outpatients care, on-call roster including for acute heart failure and mechanical circulatory support, cardiac MRI (level III international accreditation) and teaching medical students and junior doctors.

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: Kabanov, Alexander V

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

POSITION TITLE: Mescal S. Ferguson Distinguished Professor

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
M.V. Lomonosov Moscow State University	MS	06/1984	Chemistry
M.V. Lomonosov Moscow State University	PHD	01/1987	Chemical kinetics & catalysis
M.V. Lomonosov Moscow State University	DSC	01/1990	Chemistry, Biochemistry

A. Personal Statement

My main research interests and expertise are in polymeric drug delivery systems for small molecules, nucleic acids, and proteins for treatment of cancer and CNS diseases. I have contributed work on block copolymer micelles, polyplexes, nanogels, macrophage-based drug and gene delivery carriers, and exosomes that have been now widely spread in the nanomedicine and drug delivery field. My efforts have contributed to translation, clinical evaluation, and approval of polymeric micelle-based delivery systems. I co-discovered the ultrahigh-capacity poly(2-oxazoline) micelles and most recently led the program of evaluation of this technology for delivery of agents modifying tumor microenvironment to treat triple-negative breast cancer. I have conducted extensive research on delivery of RNA and DNA including vaccines for cancer and infectious diseases. I am committed and vested in translational research to develop novel therapies. I have trained over 70 graduate students and postdoctoral scientists half of whom are women and underrepresented minorities. Thirteen of my past trainees hold faculty appointments. I am currently the PI of the NIH T32 Carolina Cancer Nanotechnology Training Program. Prior to that I was PI of NIH Center of Biomedical Research Excellence (CoBRE) grant (P20) "Nebraska Center of Nanomedicine" at the University of Nebraska Medical Center (UNMC), Omaha, NE. CoBRE are multidisciplinary centers that augment and strengthen institutional biomedical research capacity by developing biomedical faculty research capability, mentoring junior faculty, and enhancing research infrastructure to carry out a multidisciplinary, collaborative program. Therefore, I have ample experience in leading complex, cross-disciplinary projects with a principal research and training components.

1. Lim C, Ramsey JD, Hwang D, Teixeira SCM, Poon C-D, Strauss JD, Rosen EP, Sokolsky-Papkov M, Kabanov AV. Drug-dependent morphological transitions in spherical and worm-like polymeric micelles define stability and pharmacological performance of micellar drugs. *Small* 2021, e2103552.
2. Vinod N, Hwang D, Azam SH, Van Swearingen AED, Wayne E, Fussell SC, Sokolsky-Papkov M, Pecot CV, **Kabanov AV**. High Capacity poly(2-oxazoline) formulation of TLR 7/8 agonist extends survival in a chemo-insensitive, metastatic model of Lung Adenocarcinoma. *Sci Adv.* 2020; 6:eaba5542.
3. Wan X, Min Y, Bludau H, Keith A, Sheiko SS, Jordan R, Wang AZ, Sokolsky-Papkov M, **Kabanov AV**. Drug combination synergy in worm-like polymeric micelles improves treatment outcome for small cell and non-small cell lung cancer. *ACS Nano.* 2018, 12(3):2426-2439. PMCID: PMC6331221.
4. Wan X, Beaudoin JJ, Vinod N, Min Y, Makita N, Bludau H, Jordan R, Wang A, Sokolsky M, **Kabanov AV**. Co-delivery of paclitaxel and cisplatin in poly(2-oxazoline) polymeric micelles: Implications for drug loading, release, pharmacokinetics and outcome of ovarian and breast cancer treatments. *Biomaterials.* 2019, 192:1-14. PMCID: PMC6331221.

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

R01 CA264488-03	KABANOV, ALEXANDER V (PI)	08/01/21-07/31/25
Towards translation of Nanoformulated Paclitaxel-Platinum Combination		
This proposal is to obtain pre-clinical data for translation of a novel nanotechnology-based immunotherapeutic drug to treat triple negative breast cancer that is currently associated with poor prognosis. Role: PI		
T32 CA196589-09	KABANOV, ALEXANDER V (PI)	07/01/15-06/30/25
Carolina Cancer Nanotechnology Training Program (C-CNTP)		
This program is to train postdoctoral research fellows who will successfully pursue careers in academia, industry, and government agencies using nanotechnology to diagnose and treat cancer. Role: PI		
1R21NS135362-01 , National Institute of Neurological Disorders and Stroke	KABANOV, ALEXANDER V (PI)	09/20/23 – 08/31/25
Naturally Targeted Exosomal TLR7/8 Agonist for Immunotherapy of Medulloblastoma		
The aims are to determine 1) if exosomes improve the tumor distribution of resiquimod (exo-Res) and 2) if exo-Res enhances the anti-tumor efficacy of resiquimod in medulloblastoma-bearing mice. Role: PI		
R01 CA184088 National Cancer Institute	KABANOV, ALEXANDER V (PI)	01/01/15-12/31/21
Liposomal Doxorubicin and Pluronic Combination for Cancer Therapy		
We propose a simple strategy to promote the release of doxorubicin from the liposomal particles directly within the tumor matrix, while sensitizing this tumor using Pluronic copolymers. Role: PI		
1R01NS102412 National Institutes of Health	BATRAKOVA, ELENA (PI)	03/1/18 – 11/30/22
Cell-based Platform for Gene Delivery to the Brain		
We explore how PBM interact with brain cells and facilitate horizontal gene transfer upon neurodegeneration, potentially opening other cell-based gene delivery systems to treat Parkinson's disease. Role: Co-I		
R01NS112019 National Institutes of Health	BATRAKOVA, ELENA (PI)	09/01/19 - 06/30/24
Extracellular Vesicles for CNS Delivery of Therapeutic Enzymes to Treat Lysosomal Storage Disorders		
We explore the extracellular vesicles, released by macrophages, as a platform for efficient and targeted brain delivery of therapeutic lysosomal enzymes to treat Lysosomal Storage Diseases (LSDs). Role: Co-I		

B. Positions, Scientific Appointments, and Honors

Positions and Employment

2015 -	Director, T32 Carolina Cancer Nanotechnology Training Center, UNC-Chapel Hill, NC
2012 -	Mescal S. Ferguson Distinguished Professor, UNC Eshelman School of Pharmacy, NC
2012 -	Co-Director / Director, Carolina Institute for Nanomedicine, UNC-Chapel Hill, NC
2012 -	Member, UNC Lineberger Comprehensive Cancer Center, UNC-Chapel Hill, NC
2012 -	Director, Center for Nanotechnology in Drug Delivery, UNC-Chapel Hill, NC
2008 - 2012	Founding Director, NIH CoBRE Nebraska Center for Nanomedicine, UNMC, Omaha, NE
2004 - 2012	Founding Director, Center for Drug Delivery and Nanomedicine, UNMC, Omaha, NE
2004 - 2012	Parke-Davis Endowed Chair in Pharmaceutics, College of Pharmacy, UNMC, Omaha, NE
2002 - 2022	Professor (adjunct/visiting/secondary), Founding Director, Laboratory of Chemical Design of Bionanomaterials (2011 - 2022), MSU, Moscow, Russia
2001 - 2012	Professor of Pharmaceutical Sciences, Eppley Institute for Cancer Research, Genetics, Cell Biology and Anatomy, Pharmacology and Experimental Neuroscience, UNMC, Omaha, NE
1995 - 2012	Member, UNMC Eppley Cancer Center, UNMC, Omaha, NE
1994 - 2001	Associate Professor, College of Pharmacy, UNMC, Omaha, NE
1988 - 1993	Head, Laboratory and Division of Biopolymers, Institute of Applied Molecular Biology, National Research Center of Molecular Diagnostics and Therapy, Moscow, Russia
1987 - 1997	Research Fellow (Junior, Common, Senior, & Leading), Department of Chemical Enzymology, M.V. Lomonosov Moscow State University (MSU), Moscow, Russia

Other Experience and Professional Memberships

2018 -	President (2018-2020) and CEO, Russian American Science Association, Corp. (Boston, MA)
2018 - 2022	Member, Grants Council of Russian Federation (resigned in March 2022)
2003 - 2008	Member / Chair (2006-08), NIH BMBI study section
2003 -	Founder, Nanomedicine and Drug Delivery Symposium series (nanodds.org)
1995 -	Co-founder/founder and/or director, Supratek Pharma, Inc.; InnovaForm Technologies, LLC; Neuronano Pharma, Inc.; SoftKemo Pharma; BendaRx; Ostrea Bio, DelAQUA

Honors

2022	Founders award, Controlled Release Society
2021	Fellow, American Association for Advancement of Science
2019	Corresponding member (elected), Russian Academy of Sciences
2018	Fellow, Controlled Release Society
2018	Life Sciences award (Outstanding research from a university), Triangle Business Journal
2017	George Gamow award, Russian American Science Association
2017	Fellow, US National Academy of Inventors
2014	Fellow, American Institute for Medical and Biological Engineering
2014, 2018	Highly Cited (Pharmacology & Toxicology), Thompson Reuters / Clarivate Analytics
2013	Member (elected), Academia Europaea (The Academy of Europe)
2009	Scientist Laureate, UNMC
2007	Outstanding Research & Creative Activity (ORCA) award, University of Nebraska
1995	CAREER award, National Science Foundation
1988	Lenin Komsomol Prize, USSR

C. Contributions to Science

1. In 1989 we were first to use polymeric micelles as a nanoparticle delivery platform for small drug molecules and then developed the first-in-man polymeric micelle drug to treat cancer. Our work was followed by investigation of polymeric micelles by numerous groups across the globe and marketing approval of polymeric micelle drugs. More recently we developed poly(2-oxazoline) micelles with ultra-high drug loading allowing to 1) greatly enhance the solubility and stability of drugs and drug candidates (by a factor of up to 100,000), 2) improve their efficacy and safety, and 3) widen the therapeutic window. Our patents were licensed to or purchased by several companies to complete the final development of the anticancer therapeutics.
 - a. Wan X, Beaudoin JJ, Vinod N, Min Y, Makita N, Bludau H, Jordan R, Wang A, Sokolsky M, **Kabanov AV**. Co-delivery of paclitaxel and cisplatin in poly(2-oxazoline) polymeric micelles: Implications for drug loading, release, pharmacokinetics and outcome of ovarian and breast cancer treatments. *Biomaterials*. 2018 Oct 31;192:1-14. PubMed PMID: 30415101.
 - b. Luxenhofer R, Schulz A, Roques C, Li S, Bronich TK, Batrakova EV, Jordan R, **Kabanov AV**. Doubly amphiphilic poly(2-oxazoline)s as high-capacity delivery systems for hydrophobic drugs. *Biomaterials*. 2010 Jun;31(18):4972-9. PubMed PMID: 20346493; PubMed Central PMCID: PMC2884201.
 - c. **Kabanov AV**, Nazarova IR, Astafieva IV, Batrakova EV, Alakhov VY, Yaroslavov AA, Kabanov VA. Micelle formation and solubilization of fluorescent probes in poly(oxyethylene-b-oxypropylene-b-oxyethylene) solutions. *Macromolecules*. 1995 March; 28(7):2303-2314.
 - d. **Kabanov AV**, Chekhonin VP, Alakhov VYu, Batrakova EV, Lebedev AS, Melik-Nubarov NS, Arzhakov SA, Levashov AV, Morozov GV, Severin ES. The neuroleptic activity of haloperidol increases after its solubilization in surfactant micelles. Micelles as microcontainers for drug targeting. *FEBS Lett.* 1989 Dec 4;258(2):343-5. PubMed PMID: 2599097.
2. We discovered pharmacological effects of Pluronic block copolymers: 1) Sensitization of multidrug resistant and cancer stem cells to overcome and prevent drug resistance in cancer; 2) Increased permeability of drugs in intestinal and blood-brain barriers due to inhibition of drug efflux transporters to increase delivery of drugs across these barriers, and 3) Enhanced and prolonged expression of naked DNA in skeletal muscle to increase gene transfer. In each case established the molecular mechanisms and structural requirements for

the copolymers. Our findings advanced the use of block copolymers in pharmaceutical formulations by academia and industry.

- a. Batrakova EV, Li S, Vinogradov SV, Alakhov VY, Miller DW, Kabanov AV. Mechanism of pluronic effect on P-glycoprotein efflux system in blood-brain barrier: contributions of energy depletion and membrane fluidization. *J Pharmacol Exp Ther.* 2001 Nov;299(2):483-93. PubMed PMID: 11602658.
 - b. Lemieux P, Guérin N, Paradis G, Proulx R, Chistyakova L, **Kabanov A**, Alakhov V. A combination of poloxamers increases gene expression of plasmid DNA in skeletal muscle. *Gene Ther.* 2000 Jun;7(11):986-91. PubMed PMID: 10849559.
 - c. Batrakova EV, Han HY, Miller DW, **Kabanov AV**. Effects of pluronic P85 unimers and micelles on drug permeability in polarized BBMEC and Caco-2 cells. *Pharm Res.* 1998 Oct;15(10):1525-32. PubMed PMID: 9794493.
 - d. Alakhov VYu, Moskaleva EYu, Batrakova EV, **Kabanov AV**. Hypersensitization of multidrug resistant human ovarian carcinoma cells by pluronic P85 block copolymer. *Bioconjug Chem.* 1996 Mar-Apr;7(2):209-16. PubMed PMID: 8983343.
3. In late 80-ies and early 90-ies we were first to publish on the design of polyion complexes of DNA with synthetic polycations for transfection of cells that became prototypes of current polyplexes for cell transfection. Today many polycationic transfection reagents descending from this work are marketed and used in cell biology, and biotechnology. To enable administration of polyplexes to live organisms we invented the use of cationic block copolymers to produce core-shell polyplex nanoparticles with polyion complex core and inert non-ionic shell for delivery of nucleic acids. Today many academic and industrial scientists commonly use this technology for nucleic acids delivery.
- a. Roy S, Zhang K, Roth T, Vinogradov S, Kao RS, **Kabanov A**. Reduction of fibronectin expression by intravitreal administration of antisense oligonucleotides. *Nat Biotechnol.* 1999 May;17(5):476-9. PubMed PMID: 10331808.
 - b. **Kabanov AV**, Vinogradov SV, Suzdaltseva YG, Alakhov VYu. Water-soluble block polycations as carriers for oligonucleotide delivery. *Bioconjug Chem.* 1995 Nov-Dec;6(6):639-43. PubMed PMID: 8608176.
 - c. **Kabanov AV**, Kabanov VA. DNA complexes with polycations for the delivery of genetic material into cells. *Bioconjug Chem.* 1995 Jan-Feb;6(1):7-20. PubMed PMID: 7711106.
 - d. Kabanov AV, Kiselev VI, Chikindas ML, Astaf'eva IV, Glukhov AI. [Increase in the transforming activity of plasmid DNA by means of its inclusion in the interpolyelectrolytic complex with a carbon-chain cation]. *Dokl Akad Nauk SSSR.* 1989 May-Jun;306(1):226-9. PubMed PMID: 2526724.
4. In mid 90-ies we described formation of polyion complex micelles through ionic complexation of block polyelectrolytes. We invented cross-linked polyelectrolyte nanogels and polymeric micelles with cross-linked polyion cores. Due to their softness, stability, and lack of defined "surface" these materials have shown promise for targeted drug delivery to tumors. Recent work used core-shell polyplex nanoparticles as a platform approach for delivery of therapeutic proteins and demonstrated the proof-of-concept in animal models for treatment of brain injury, hypertension, spinal cord injury, CWA poisoning and other applications. The technology holds promise for protein-based biopharmaceuticals since it provides a method of packaging proteins, keeping them stable for long time and releasing without any loss of activity.
- a. Jiang Y, Fay JM, Poon CD, Vinod N, Zhao Y, Bullock K, Qin S, Manickam DS, Yi X, Banks WA, **Kabanov AV**. Nanoformulation of Brain-Derived Neurotrophic Factor with Target Receptor-Triggered-Release in the Central Nervous System. *Adv Funct Mater.* 2018 Feb 7;28(6)PubMed PMID: 29785179; PubMed Central PMCID: PMC5958903.
 - b. Efremenko EN, Lyagin IV, Klyachko NL, Bronich T, Zavyalova NV, Jiang Y, **Kabanov AV**. A simple and highly effective catalytic nanozyme scavenger for organophosphorus neurotoxins. *J Control Release.* 2017 Feb 10;247:175-181. PubMed PMID: 28043864.
 - c. Bronich TK, Keifer PA, Shlyakhtenko LS, **Kabanov AV**. Polymer micelle with cross-linked ionic core. *J Am Chem Soc.* 2005 Jun 15;127(23):8236-7. PubMed PMID: 15941228.
 - d. Kabanov AV, Bronich TK, **Kabanov VA**, Yu K, Eisenberg A. Spontaneous formation of vesicles from complexes of block ionomers and surfactants. *J. Am. Chem. Soc.* 1998; 120:9941-9942.

5. We proposed to use macrophages loaded with polyion complexes as carriers for transfer of therapeutic proteins and genes to sites of inflammation in the brain. Discovered the phenomenon of horizontal transfer of gene and siRNA from transfected macrophages to new host cells, allowing use of macrophages as disease site-specific non-viral gene delivery vectors. Demonstrated that exosomes can carry protein therapeutics to the inflamed brain. These findings were applied for treatment of Parkinson's disease, lysosomal storage diseases and cancer in animal models.
 - a. Yuan D, Zhao Y, Banks WA, Bullock KM, Haney M, Batrakova E, **Kabanov AV**. Macrophage exosomes as natural nanocarriers for protein delivery to inflamed brain. *Biomaterials*. 2017 Oct;142:1-12. PubMed PMID: 28715655; PubMed Central PMCID: PMC5603188.
 - b. Mahajan V, Gaymalov Z, Alakhova D, Gupta R, Zucker IH, **Kabanov AV**. Horizontal gene transfer from macrophages to ischemic muscles upon delivery of naked DNA with Pluronic block copolymers. *Biomaterials*. 2016 Jan;75:58-70. PubMed PMID: 26480472; PubMed Central PMCID: PMC4644506.
 - c. Haney MJ, Zhao Y, Harrison EB, Mahajan V, Ahmed S, He Z, Suresh P, Hingtgen SD, Klyachko NL, Mosley RL, Gendelman HE, **Kabanov AV**, Batrakova EV. Specific transfection of inflamed brain by macrophages: a new therapeutic strategy for neurodegenerative diseases. *PLoS One*. 2013;8(4):e61852. PubMed PMID: 23620794; PubMed Central PMCID: PMC3631190.
 - d. Batrakova EV, Li S, Reynolds AD, Mosley RL, Bronich TK, **Kabanov AV**, Gendelman HE. A macrophage-nanozyme delivery system for Parkinson's disease. *Bioconjug Chem*. 2007 Sep-Oct;18(5):1498-506. PubMed PMID: 17760417; PubMed Central PMCID: PMC2677172.

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: Dawn Bowles

ERA COMMONS USER NAME (credential, e.g., agency login): bowle009

POSITION TITLE: Assistant Professor

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 Louisiana at Lafayette LA	BS	06/1990	Microbiology
Louisiana State University Medical Center, Shreveport, LA	PhD	07/1998	Microbiology and Immunology
Louisiana State University Medical Center, Shreveport, LA	Post-doc	08/1999	Virology
University of North Carolina, Chapel Hill, NC	Post-doc	10/2024	Gene Therapy

A. Personal Statement

I am currently the co-director of the Duke Human Heart Repository and have considerable practical knowledge in the collection, storage, and usage of human samples (blood, urine, myocardial tissues) to address basic and translational research problems in human heart failure and disease. I will provide human cardiovascular samples needed for this proposal. Although I perform basic and translational cardiovascular research using this rare and valuable resource, I am also committed to sharing excess materials through collaborations with other investigators. Much of my independent research which has focused on basic, translational, and clinical research on causes and treatments of cardiac injury, disease, and failure. I have assessed a variety of therapeutics including pharmacological, gene, cell and mechanical therapy candidates to ameliorate cardiac dysfunction, disease, and failures utilizing an array of animal models (both large and small) and human bio specimens. I have continued an active translational research effort examining different forms of therapies as an avenue to alter myocardial function and treat heart failure. Much of this effort has been done collaboratively with Dr. Carmelo A. Milano. Combined recent efforts have focused on the development of clinically applicable methods to achieve efficient gene delivery to the adult heart. I am also an expert on the development, manufacture, purification, and administration of the various viral vectors. I have over 18 years of experience with adeno-associated virus (AAV) vectors and have developed scores of such vectors. As a post-doc in the laboratory of Dr. R. Jude Samulski, a renowned expert in the field of gene therapy, my research focused on AAV biology with the emphasis on AAV vector development. I currently direct the construction of numerous viral vectors and implementation of these vectors in both cell culture systems and in vivo models on several funded projects.

Ongoing and recently completed projects that I would like to highlight include:NASA NNH20ZDA001N **Bowles** (PI) 12/01/2021 – 11/30/2024

A Multi-omics and Multi-Species Examination of Combined Environmental Stressors of Space Exploration The key central objective of this proposal is to obtain a multi omics and physiological understanding of the impact of space radiation and weightlessness individually and together on the cardiovascular system using two rodent models: rat neonatal cardiomyocytes (RNNC) and C57BL/6 mice.

1R01-HL152723-01A1 Fujise (PI)
Fortilin, CTNNA3, and the Heart

06/2021 – 01/2026

This proposal will use human heart samples diagnosed with ischemic and nonischemic cardiomyopathy and non-failing tissues in order to assess the effect of fortilin and CTNNA on the human heart and the potential to become part of a future treatment option for heart failure.

R21-AI175164-01 Turek (PI) 03/6/2023 -02/28/2025 Transplantation of Cryopreserved Thymus

This proposal aims to determine the ability of cryopreserved cultured thymus tissue implantation (cCTTI) to re-establish thymus function and naive T cell numbers in thymectomized and conditioned recipient pigs.

Solid Biosciences **Bowles** (PI) 3/3/2023 - 3/2/2024

Gene Therapy for Cardiac Transplantation

Specific Aim 1. Assessment of immune suppressive function using immunogenic tumor models. Specific Aim 2. To test the hypothesis that PD-L1 truncated (PD-L1T) or PD-L1 secreted (PD-L1S) overexpression in a murine heart transplant model will promote graft survival and increase the time to rejection.

1U01-AI170064-01 Barbas (PI) 07/19/2022 – 04/30/2027

Genetic engineering of kidney allografts by ex vivo perfusion delivery of adeno-associated viral vectors For this proposal, we have assembled a team of investigators with expertise in ex vivo organ perfusion, the use of adeno-associated viral (AAV) vectors for gene therapy, and kidney transplantation.

Regeneron **Bowles** (PI) 01/3/2023 – 01/02/2025

Transcriptional profiling of human myocardial tissues to identify etiology-specific gene expression signature in the failing heart

The goals of this research project are to ensure tissue quality of the Duke Human Heart Repository is adequate, logistics are aligned, and data output are generally as expected based on published historical human heart failure tissue sample data.

BRASH 1901 NASA **Bowles** (PI) 1/1/19-9/30/21

Gene Therapy Countermeasure for Detrimental Effects of Space Radiation

The overall objective of this proposal is to perform proof of concept studies in the development of a gene therapy to improve an astronaut's resilience against space radiation.

National Aeronautics and Space Administration NNX16AK20G **Bowles** (PI) 5/12/16-11/30/22 Proteomic signatures of space radiation induced cardiovascular degeneration

To evaluate the consequences of low dose, chronic space radiation, or mixed field space radiation on the dynamics of the cardiac proteome and to understand how the radiation induced changes relate to cardiovascular function.

NASA 80JSC019N1-OMNIUS2 **Bowles** (PI) 1/1/21-10/13/22

Radiation Resistance Conferred by Small Proline Rich Proteins (SPRRs)

Project Goal and Specific Aims: We will molecularly explore the overexpression of several small proline rich proteins (SPRRs) using viral vector mediated approaches.

The following citations are examples of studies performed collaboratively with other investigators studying biomaterials from the Duke Human Heart Repository that demonstrate the robustness of the Duke Human Heart Repository:

1. Beak JY, Kang HS, Huang W, Myers PH, **Bowles DE**, Jetten AM, Jensen BC. The nuclear receptor ROR α protects against angiotensin II-induced cardiac hypertrophy and heart failure. *Am J Physiol Heart Circ Physiol.* 2019 Jan 1;316(1):H186-H200. Epub 2018 Nov 2. PMID: 30387679; PMCID: PMC6383360.
2. Hayashi H, Hess DT, Zhang R, Sugi K, Gao H, Tan BL, **Bowles DE**, Milano CA, Jain MK, Koch WJ, Stamler JS. S-Nitrosylation of β -Arrestins Biases Receptor Signaling and Confers Ligand Independence. *Mol Cell.* 2018 May 3;70(3):473-487.e6. PMID: 29727618; PMCID: PMC5940012.
3. Wang T, O'Brien EC, Rogers JG, Jacoby DL, Chen ME, Testani JM, **Bowles DE**, Milano CA, Felker GM, Patel CB, Bonde PN, Ahmad T. Plasma Levels of MicroRNA-155 Are Upregulated with Long-Term Left

- Ventricular Assist Device Support. *ASAIO J.* 2017 Sep/Oct;63(5):536-541. PMID: 28319523; PMCID: PMC5585122.
4. Jensen BC, Bultman SJ, Holley D, Tang W, de Ridder G, Pizzo S, **Bowles D**, Willis MS. Upregulation of autophagy genes and the unfolded protein response in human heart failure. *Int J Clin Exp Med.* 2017;10(1):1051-1058. Epub 2017 Jan 30. PMID: 28794819; PMCID: PMC5546743.

B. Positions, Scientific Appointments, and Honors

2019-	Founder Caripae, LLC
2012 -	Co-Director, Duke Human Heart Repository, Duke University Medical Center, Durham, NC
2006 -	Assistant Professor, Duke University Medical Center, Durham, NC
2005 - 2006	Assistant Research Professor, Duke University Medical Center, Durham, NC
2004 - 2005	Instructor Temporary, Duke University Medical Center, Durham, NC

Other Experience and Professional Membership

2015 -	Member, International Society for Heart and Lung Transplantation
2014 -	Adhoc reviewer, PLOSone
2013 -	Member, International Society for Biological and Environmental Repositories
2012 -	Ad hoc reviewer, Virology
2010 - 2011	Member, Society for Clinical and Translational Science
2010 -	Ad hoc reviewer, Journal of Molecular and Cellular Cardiology
2002 - 2014	Member, American Society of Gene and Cell Therapy
1995 -	Full Member, American Society of Virology
1990 -	Member, The Honor Society of Phi Kappa Phi

C. Contributions to Science

1. Molecular Virology and Gene Regulation. My research experience began as a graduate student in the laboratory of Dr. Dennis J. O'Callaghan whose research focused on the use of equine herpesvirus type 1 (EHV1) as a model system to study the regulation of herpesvirus gene expression and herpesvirus defective interfering particles. Specifically, my dissertation research centered on the characterization of the EICP0 protein of equine herpesvirus type 1 (EHV1). When I entered Dr. O'Callaghan's laboratory essentially nothing was known about this protein. It was speculated that this protein was functioning as a regulator of viral gene expression based on sequence alignments with other viral regulatory proteins. It should be emphasized that no molecular reagents were available for this protein at that time and that my work generated important reagents for the study of this protein, defined the kinetics of EICP0 expression during an infection, determined that this protein was an important and potent regulator of EHV-1 gene expression, and defined the domains of this protein important for its regulatory function.

- a. **Bowles DE**, Holden VR, Zhao Y, O'Callaghan DJ. The ICP0 protein of equine herpesvirus 1 is an early protein that independently transactivates expression of all classes of viral promoters. *J Virol.* 1997 Jul;71(7):4904-14. PMID: 9188552; PMCID: PMC191720.
- b. Kim SK, **Bowles DE**, O'Callaghan DJ. The gamma2 late glycoprotein K promoter of equine herpesvirus 1 is differentially regulated by the IE and EICP0 proteins. *Virology.* 1999 Apr 10;256(2):173-9. PMID: 10191181 PMCID: N/A.
- c. **Bowles DE**, Kim SK, O'Callaghan DJ. Characterization of the trans-activation properties of equine herpesvirus 1 EICP0 protein. *J Virol.* 2000 Feb;74(3):1200-8. PMID: 10627530; PMCID: PMC111454.

2. Adeno-associated Viral Vector Molecular Biology. As a post-doc in the laboratory of R. Jude Samulski, a renowned expert in the field of gene therapy, my research focused on AAV biology with the emphasis on AAV vector development. At the time, AAV serotype 2 was the only serotype utilized for clinical trials despite its limited transduction efficiency in various target organs such as skeletal and cardiac muscle. Using molecular modeling of the three-dimensional crystal structure of the AAV2 capsid as a guide, the AAV2.5 chimeric capsid, composed of amino acids from both AAV1 and AAV2 was generated via site directed mutagenesis. AAV2.5 exhibited

properties of both parental capsids such that efficient skeletal muscle transduction was inherited from AAV1, while heparin binding properties were acquired from AAV2. Interestingly, the AAV2.5 chimera had reduced antigenic cross reactivity to either parental capsid suggesting the possibility of avoiding preexisting neutralizing antibodies against AAV2 prevalent in the human population. The AAV2.5 mini-dystrophin vector was utilized in a phase 1 clinical trial for Duchenne Muscular Dystrophy, the first example of a clinical trial utilizing a custom designed AAV vector. The results demonstrated that these new types of vectors were safe and well tolerated in patients. It laid the foundation for the next generation of customized AAV vectors tailored for individual clinical application. I continued my work studying the AAV capsid in relation to tissue transduction. In subsequent studies, I evaluated the influence of these five amino acids, engineered individually and in groups in the context of both the AAV2 and AAV3b capsids, on the transduction of murine skeletal and cardiac muscle to map the amino acids responsible as the beginning effort to understand the mechanism responsible for the enhanced skeletal muscle transduction of AAV2.5.

- a. **Bowles DE**, McPhee SW, Li C, Gray SJ, Samulski JJ, Camp AS, Li J, Wang B, Monahan PE, Rabinowitz JE, Grieger JC, Govindasamy L, Agbandje-McKenna M, Xiao X, Samulski RJ. Phase 1 gene therapy for Duchenne muscular dystrophy using a translational optimized AAV vector. *Mol Ther*. 2012 Feb;20(2):443-55. PMID: 22068425; PMCID: PMC3277234
- b. Li C, Diprimio N, **Bowles DE**, Hirsch ML, Monahan PE, Asokan A, Rabinowitz J, Agbandje-McKenna M, Samulski RJ. Single amino acid modification of adeno-associated virus capsid changes transduction and humoral immune profiles. *J Virol*. 2012 Aug 86(15):7752-9. PubMed PMID: 22593151; PubMed Central PMCID: PMC3421647.
- c. Piacentino V 3rd, Milano CA, Bolanos M, Schroder J, Messina E, Cockrell AS, Jones E, Krol A, Bursac N, Mao L, Devi GR, Samulski RJ, **Bowles DE**. X-linked inhibitor of apoptosis protein-mediated attenuation of apoptosis, using a novel cardiac-enhanced adeno-associated viral vector. *Hum Gene Ther*. 2012 Jun;23(6):635-46. PMID: 22339372; PMCID: PMC3392616.
- d. Messina EL, Nienaber J, Daneshmand M, Villamizar N, Samulski J, Milano C, **Bowles DE**. Adeno-associated viral vectors based on serotype 3b use components of the fibroblast growth factor receptor signaling complex for efficient transduction. *Hum Gene Ther*. 2012 Oct;23(10):1031-42. PMID: 22680698; PMCID: PMC3472518.

3. Translational Studies using gene and other therapies as an avenue to alter myocardial function and treatment of heart failure in small and large animal models of cardiovascular disease. I have continued an active translational research effort examining different forms of therapies (including gene therapy) as an avenue to alter myocardial function and treat heart failure. Much of this effort has been done collaboratively with Dr. Carmelo A. Milano. Combined recent efforts have focused on the development of clinically applicable methods to achieve efficient gene delivery to the adult heart. The most promising experiments have focused on gene delivery to the porcine heart using an ex vivo perfusion approach. With regards to possible gene therapy, this type of perfusion storage allows for intracoronary delivery of high concentrations of viral vectors with continuous recirculation under metabolically favorable conditions. We have been able to evaluate the utility of ex-vivo warm blood perfusion as a method of viral vector delivery to the heart prior to experimental transplantation in the porcine. Our major findings are unprecedented transgene expression achieved in all regions of the allograft including the coronary vasculature. Despite global expression in the allograft, no transgene expression was evident in any of the recipient's other organs. Finally, duration of perfusion (with the viral vector) is in line with current clinical transplants and would not require any additional time relative to the current clinical practice.

- a. Bishawi M, Roan JN, Milano CA, Daneshmand MA, Schroder JN, Chiang Y, Lee FH, Brown ZD, Nevo A, Watson MJ, Rowell T, Paul S, Lezberg P, Walczak R, **Bowles DE**. A normothermic ex vivo organ perfusion delivery method for cardiac transplantation gene therapy. *Sci Rep*. 2019 May 29;9(1):8029: 31142753; PMCID: PMC6541710.
- b. Lima B, Lam GK, Xie L, Diesen DL, Villamizar N, Nienaber J, Messina E, **Bowles D**, Kontos CD, Hare JM, Stamler JS, Rockman HA. Endogenous S-nitrosothiols protect against myocardial injury. *Proc Natl Acad Sci U S A*. 2009 Apr 14; 106(15):6297-302. PMID: 19325130; PMCID: PMC2669330.
- c. Villamizar NR, Crow JH, Piacentino V 3rd, DiBernardo LR, Daneshmand MA, Bowles DE, Groh MA, Milano CA. Reproducibility of left atrial ablation with high-intensity focused ultrasound energy in a calf model. *J Thorac Cardiovasc Surg*. 2010 Dec; 140(6):1381-7.e1. PMID: 20934725; PMCID: PMC4165600.

- d. Schechter MA, Southerland KW, Feger BJ, Linder D Jr, Ali AA, Njoroge L, Milano CA, **Bowles DE**. An isolated working heart system for large animal models. *J Vis Exp.* 2014 Jun 11; PMID: 24962492; PMCID: PMC4189428.

4. Establishment of the Duke Human Heart Repository. One of the most profound contributions a patient can provide to the scientific community is the donation of human tissue for research purposes. However, without committed individuals in place to acquire and store these biospecimens, these valuable samples would be discarded, precluding important discoveries that may be obtained from their study. For the full potential of these donated bio specimens to be realized, these specimens should be annotated with patient, molecular, and clinical information. In addition, the biological specimens and associated data cannot languish in freezers and databases, but must reside in the hands of investigators who can best drive the science. I have devoted a considerable amount of my professional effort developing the Duke Human Heart Repository (DHHR) which acquires, processes, stores, annotates and disseminates these cardiac bio specimens to the research community. There are approximately 40,000 samples from over 1300 patients currently in the DHHR inventory. This robust biorepository has enabled over 50 investigators to conduct well-powered studies to answer key questions regarding human cardiac physiology and disease and contributes to the advancement of science and development of improved treatment for patients with heart disease and heart failure.

- a. Schechter MA, Hsieh MK, Njoroge LW, Thompson JW, Soderblom EJ, Feger BJ, Troupes CD, Hershberger KA, Ilkayeva OR, Nagel WL, Landinez GP, Shah KM, Burns VA, Santacruz L, Hirschey MD, Foster MW, Milano CA, Moseley MA, Piacentino V 3rd, **Bowles DE**. Phosphoproteomic profiling of human myocardial tissues distinguishes ischemic from non-ischemic end stage heart failure. *PLoS One.* 2014;9(8):e104157. PMID: 25117565; PMCID: PMC4130503.
- b. Lorenzana-Carrillo MA, Gopal K, Byrne NJ, Tejay S, Saleme B, Das SK, Zhang Y, Haromy A, Eaton F, Mendiola Pla M, **Bowles DE**, Dyck JRB, Ussher JR, Michelakis ED, Sutendra G. TRIM35-mediated degradation of nuclear PKM2 destabilizes GATA4/6 and induces P53 in cardiomyocytes to promote heart failure. *Sci Transl Med.* 2022 Nov 2;14(669):eabm3565. Epub 2022 Nov 2. PMID: 36322626.
- c. Chakraborty A, Peterson NG, King JS, Gross RT, Mendiola Pla M, Thennavan A, Zhou KC, DeLuca S, Bursac N, **Bowles DE**, Wolf MJ, Fox DT. Conserved chamber-specific polyploidy maintains heart function in *Drosophila*. *Development.* (2023).
- d. Mohammed M, Ogunlade B, Elgazzaz M, Berdasco M, Lakkappa N, Ghita I, Guidry JJ, Sriramula S, Xu J, Restivo L, Mendiola Pla MA, **Bowles DE**, Beyer AM, Yue X, Lazartigues E. Nedd4-2 up-regulation is associated with ACE2 ubiquitination in hypertension. *Cardiovascular Research.* (2023).

5. Insights into the influence of space travel stress on cardiovascular system. One of the most exciting goals of in the 21st century is human space exploration and proposed travel to Mars. With exploration comes risk. NASA is particularly concerned about the stressors of space exploration on cardiovascular function. I have received NASA funding on multiple research projects on this topic.

- a. Bryan J. Feger, J. Will Thompson, Laura G. Dubois, Reddy P. Kommaddi, Matthew W. Foster, Rajashree Mishra, Sudha K. Shenoy, Yared H. Kidane, M. Arthur Moseley, Lisa A. Scott Carnell, and **Dawn E. Bowles**. Microgravity induces proteomic changes involved in endoplasmic reticulum stress and mitochondrial protection" *Scientific Reports* 6, Article number 34091 (2016). PMID: 27670941; PMCID: PMC5037457.
- b. Bishawi M, Lee FH, Abraham DM, Glass C, Blocker SJ, Cox DJ, Brown ZD, Rockman HA, Mao L, Slaba TC, Dewhirst MW, Truskey GA, **Bowles DE**. Late onset cardiovascular dysfunction in adult mice resulting from galactic cosmic ray exposure. *iScience.* 2022 Mar 16;25(4):104086. PMID: 35378858; PMCID: PMC8976132.
- c. Davis CM, Allen AR, **Bowles DE**. Consequences of space radiation on the brain and cardiovascular system. *J Environ Sci Health C Toxicol Carcinog.* 2021;39(2):180-218.1891825. PMID: 33902387.
- d. Brown ZD, Bishawi M, Roan JN, Lee F, Nevo A, Watson M, **Bowles DE**. The use of an inexpensive processing aid device (the Mouse PAD) to facilitate rodent tissue banking. *Biotechniques.* 2020 Jul;69(1):364-368. Epub 2020 May 18. PMID: 32418443.

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: Edward T.H. Yeh

eRA COMMONS USER NAME (credential, e.g., agency login): EYEH01

POSITION TITLE: Professor and Chairman, Department of Internal Medicine

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 California, Berkeley	BA	06/1976	Biochemistry
University of California, Davis	MD	06/1980	Medicine
Harvard University	Post-Doc	06/1986	Immunology

A. Personal Statement

In 2000, I founded the Department of Cardiology at the University of Texas MD Anderson Cancer Center. My laboratory made the seminal discovery that Topoisomerase 2b (Top2b) is the molecular basis of anthracycline-induced cardiotoxicity (*Nature Medicine* 18:1639-42, 2012). I used a genetic approach to demonstrate that Top2b-deletion in the adult cardiomyocytes prevents acute and chronic doxorubicin-induced cardiotoxicity. This discovery changed the age-old paradigm that anthracycline toxicity is due to ROS generation alone and showed that Top2b can be targeted by dexrazoxane to prevent doxorubicin-induced cardiotoxicity. I am a leader in cardio-oncology and have written several major reviews (*Circulation* 109:3122, 2004, *JACC* 53:2231, 2009, 70:2536, 2017, 70:2552, 2017) and edited a textbook (Cancer and the heart, two editions) in this emerging field. Dr. Pestell has revealed his recent studies showing that CCR5i are dual function compounds in that they augment the ability of doxorubicin to treat cancer while protecting the heart from doxorubicin-induced damage. I am pleased to collaborate with Dr. Pestell as a consultant on his SBIR Phase II grant submission entitled "Improving Outcomes in Cancer Treatment-Related Cardiotoxicity".

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

R01 HL126916

YEH (PI)

4/1/2015-9/31/2020

Doxorubicin-induced Cardiotoxicity: the Role of Topoisomerase 2b

RO1 HL151993

YEH (co-I)

6/15/2020-5/31/2025

Prevention of heart failure-induced by doxorubicin with early administration of dexrazoxane

Citations:

- a) Zhang S, Liu X, Bawa-Khalfe T, Lu LS, Lyu YL, Liu LF, **Yeh ET.** (2012) Identification of the molecular basis of doxorubicin-induced cardiotoxicity. *Nat Med.* Nov; 18(11):1639-42. PubMed PMID: 23104132.

- b. **Yeh ETH**, Chang H. Oncocardiology: (2016) Past, Present, and the Future. *JAMA Cardiology*, December, 1:1066-1072, PubMed PMID: 27541948. PMCID: PMC5788289
- c. Chang HM, Moudgil R, Scarabelli T, Okwuosa T, **Yeh ET** (2017) Cardiovascular complications of cancer therapy. Part 1, *Journal of American College of Cardiology*, 70:2536-2551. PubMed PMID: 29145954 PMCID: PMC5825187
- d. Chang HM, Okwuosa T, Scarabelli T, Moudgil R, Yeh ET (2017) Cardiovascular complications of cancer therapy. Part 2, *Journal of American College of Cardiology*, 70:2552-2565. PubMed PMID: 29145955 PMCID: PMC5825188

B. Positions and Honors

Positions and Employment

- 2020- Professor and Chairman, Department of Internal Medicine, University of Arkansas for Medical Sciences, Little Rock, AR
- 2016 - 2020 Director, Center for Precision Medicine, University of Missouri, Columbia, MO
- 2016 - 2019 Professor and Chairman, Department of Medicine, University of Missouri, Columbia, MO
- 2000 - 2016 Professor and Chairman, Dept. of Cardiology, MD Anderson Cancer Center, Houston, TX
- 1998 - 2000 Professor of Medicine, University of Texas Houston Medical School, Houston, TX
- 1992 - 1998 Associate Professor of Medicine, University of Texas Houston Medical School, Houston, TX
- 1987 - 1992 Assistant Professor of Medicine, Harvard Medical School, Boston, MA

Other Experience and Professional Memberships

- 2017 - present Section Editor, *Journal of American College of Cardiology*
- 2017 - 2020 Member and Chair, NIH, Cardiac Contractility, Hypertrophy, and Failure study section
- 2009 - 2014 Editorial Board Member, *Journal of Biological Chemistry*
- 2004 - 2016 Editorial Board Member, *Circulation*
- 1995 - 2004 Associate Editor, *Circulation*

Honors

- 2019 Elected Member, Association of University Cardiologists
- 2016 Elected Fellow, American Association for the Advancement of Science
- 2008 McNair Scholar, Texas Heart Institute and the McNair Foundation
- 2006 Elected Academician, Academia Sinica
- 2005 Outstanding Advocate Award, American Heart Association-Houston,
- 2001 Distinguished Alumnus Award, University of California-Davis Medical School
- 2000 Elected Member, Association of American Physicians
- 1993 Elected Member, American Society of Clinical Investigation
- 1992 Established Investigator, American Heart Association
- 1987 Investigator Award, Arthritis Foundation

C. Contributions to Science

- 1. My earliest contribution to science was the elucidation of the biosynthetic pathway of the mammalian glycosylphosphatidylinositol (GPI) anchor. Using a panel of GPI anchor mutants, I defined the intermediates in the mammalian GPI anchor biosynthetic pathway. My work contributed to the elucidation of the molecular defect in paroxysmal nocturnal hemoglobinuria.
 - a. DeGasperi R, Thomas LJ, Sugiyama E, Chang HM, Beck PJ, Orlean P, Albright C, Waneck G, Sambrook JF, Warren CD., **Yeh ET** (1990) Correction of a defect in mammalian GPI anchor biosynthesis by a transfected yeast gene. *Science*. Nov 16; 250(4983):988-91. PubMed PMID: 1978413.
 - b. Sugiyama E, DeGasperi R, Urakaze M, Chang HM, Thomas LJ, Hyman R, Warren CD, **Yeh ET** (1991) Identification of defects in glycosylphosphatidylinositol anchor biosynthesis in the Thy-1 expression mutants. *J Biol Chem*. Jul 5; 266(19):12119-22. PubMed PMID: 1829456.
 - c. Urakaze M, Kamitani T, DeGasperi R, Sugiyama E, Chang HM, Warren CD, **Yeh ET** (1992) Identification of a missing link in glycosylphosphatidylinositol anchor biosynthesis in mammalian cells. *J Biol Chem*. Apr 5; 267(10):6459-62. PubMed PMID: 1313004.

- d. **Yeh ET**, Rosse WF. (1994) Paroxysmal nocturnal hemoglobinuria and the glycosylphosphatidylinositol anchor. *J Clin Invest*. Jun; 93(6):2305-10. PubMed PMID: 8200963.
2. In 1994, my laboratory cloned a ubiquitin-like protein called Sentrin, which was eventually renamed SUMO (Small ubiquitin-like modifiers). I hold the patent for the Sentrin (SUMO) gene and its application to protect against cell death signaling. (U.S. Patent No. 7,179,650; February 20, 2007). I identified three SUMOs (SUMO-1, SUMO-2, SUMO-3), the SUMO E1 activating enzymes, and E2 ligase (Ubc9).
- Okura T, Gong L, Kamitani T, Wada T, Okura I, Wei CF, Chang HM, **Yeh ET**. (1996) Protection against Fas/APO-1- and tumor necrosis factor-mediated cell death by a novel protein, sentrin. *J Immunol*. Nov 15; 157(10):4277-81. PubMed PMID: 8906799.
 - Kamitani T, Nguyen HP, **Yeh ET**. (1997) Preferential modification of nuclear proteins by a novel ubiquitin-like molecule. *J Biol Chem*. May 30; 272(22):14001-4. PubMed PMID: 9162015.
 - Gong L, Kamitani T, Fujise K, Caskey LS, **Yeh ET**. (1997) Preferential interaction of sentrin with a ubiquitin-conjugating enzyme, Ubc9. *J Biol Chem*. Nov 7; 272(45):28198-201. PubMed PMID: 9353268.
 - Kamitani T, Kito K, Nguyen HP, Fukuda-Kamitani T, **Yeh ET**. (1998) Characterization of a second member of the sentrin family of ubiquitin-like proteins. *J Biol Chem*. May 1; 273(18):11349-53. PubMed PMID: 9556629.
3. I cloned the first mammalian Sentrin/SUMO-specific protease (SENP) and proposed the nomenclature and classification for this important class of enzymes that regulates SUMO-modified proteins. Furthermore, I showed that SENPs play critical roles in hypoxia signaling, embryonic development, and homologous recombination. I also hold patents for SENP1 and the use of SENP1 as a cancer biomarker and target for cancer therapy. (U.S. Patent No. 6,596,527; July 22, 2003 and No. 7,579,152; August 25, 2009).
- Gong L, Millas S, Maul GG, **Yeh ET**. (2000) Differential regulation of sentrinized proteins by a novel sentrin-specific protease. *J Biol Chem*. Feb 4; 275(5):3355-9. PubMed PMID: 10652325.
 - Cheng J, Kang X, Zhang S, **Yeh ET**. (2007) SUMO-specific protease 1 is essential for stabilization of HIF1alpha during hypoxia. *Cell*. Nov 2; 131(3):584-95. PubMed PMID: 17981124; PubMed Central PMCID: PMC2128732.
 - Kang X, Qi Y, Zuo Y, Wang Q, Zou Y, Schwartz RJ, Cheng J, **Yeh ET**. (2010) SUMO-specific protease 2 is essential for suppression of polycomb group protein-mediated gene silencing during embryonic development. *Mol Cell*. Apr 23; 38(2):191-201. PubMed PMID: 20417598; PubMed Central PMCID: PMC2879644.
 - Dou H, Huang C, Singh M, Carpenter PB, **Yeh ET**. (2010) Regulation of DNA repair through deSUMOylation and SUMOylation of replication protein A complex. *Mol Cell*. Aug 13; 39(3):333-45. PubMed PMID: 20705237; PubMed Central PMCID: PMC2928994.
4. My laboratory was the first to show that NEDD8, a ubiquitin-like protein, can conjugate to other substrates. I identified the activating and conjugating enzymes for NEDD8 and showed the NEDD8 modification plays a critical role in cancer pathogenesis.
- Kamitani T, Kito K, Nguyen HP, **Yeh ET**. (1997) Characterization of NEDD8, a developmentally downregulated ubiquitin-like protein. *J Biol Chem*. Nov 7; 272(45):28557-62. PubMed PMID: 9353319.
 - Gong L, **Yeh ET**. (1999) Identification of the activating and conjugating enzymes of the NEDD8 conjugation pathway. *J Biol Chem*. Apr 23; 274(17):12036-42. PubMed PMID: 10207026.
 - Gong L, Kamitani T, Millas S, **Yeh ET**. (2000) Identification of a novel isopeptidase with dual specificity for ubiquitin- and NEDD8-conjugated proteins. *J Biol Chem*. May 12; 275(19):14212-6. PubMed PMID: 10799498.
 - Gao F, Cheng J, Shi T, **Yeh ET**. (2006) Neddylation of a breast cancer-associated protein recruits a class III histone deacetylase that represses NFkappaB-dependent transcription. *Nat Cell Biol*. 2006 Oct; 8(10):1171-7. PubMed PMID: 16998474.

5. I showed that Topoisomerase 2b (Top2b) is the molecular basis of anthracycline-induced cardiotoxicity. This discovery changed the 40-year-old paradigm that anthracycline toxicity is due to ROS generation alone, leading to establishment of the era of personalized cardio-protection for patients undergoing chemotherapy and development of several novel strategies: 1) synthesizing Top2 inhibitors that are specific for Top2a to avoid cardiotoxicity, 2) using Top2b to predict patient's susceptibility to anthracycline before treatment, 3) using Top2b inhibitor to prevent anthracycline-induced cardiotoxicity.
 - a. Zhang S, Liu X, Bawa-Khalfe T, Lu LS, Lyu YL, Liu LF, **Yeh ET**. (2012) Identification of the molecular basis of doxorubicin-induced cardiotoxicity. *Nat Med*. Nov; 18(11):1639-42. PubMed PMID: 23104132.
 - b. **Yeh ET**, Chang H. *Oncocardiology*: (2016) Past, Present, and the Future. *JAMA Cardiology*, December, 1:1066-1072, PubMed PMID: 27541948. PMCID: PMC5788289
 - c. Chang HM, Moudgil R, Scarabelli T, Okwuosa T, **Yeh ET** (2017) Cardiovascular complications of cancer therapy. Part 1, *Journal of American College of Cardiology*, 70:2536-2551. PubMed PMID: 29145954 PMCID: PMC5825187
 - d. Chang HM, Okwuosa T, Scarabelli T, Moudgil R, **Yeh ET** (2017) Cardiovascular complications of cancer therapy. Part 2, *Journal of American College of Cardiology*, 70:2552-2565. PubMed PMID: 29145955 PMCID: PMC5825188

Complete List of Published Work in My Bibliography

<http://www.ncbi.nlm.nih.gov/myncbi/edward.yeh.1/bibliography/40495023/public/?sort=date&direction=ascending>

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: Javid J. Moslehi

ERA COMMONS USER NAME (credential, e.g., agency login): jmoslehi1

POSITION TITLE: Associate Professor

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
Johns Hopkins University	BA	06/1996	Biology
University of Connecticut School of Medicine	MD	06/2001	Medicine

A. Personal Statement

I am a clinical cardiologist, cardio-oncologist and a myocyte biologist who is interested in cardiovascular complications associated with novel targeted cancer therapies and the lessons that one can learn from such complications about basic cardiovascular biology. I serve on the advisory board of Light Seed and am well qualified to provide advice on the grant entitled: "*Improving Outcomes in Cancer Treatment-Related Cardiotoxicity*" My independent basic and translational research laboratory focuses on signal transduction in the myocardium and vasculature. I also direct the cardio-oncology program at Vanderbilt. This program includes a clinical service focused on the cardiovascular health of cancer patients and cancer survivors as well as a translational research component.

Our group has been interested in the intersection of cardiovascular and immune systems. This new interest came about after we defined new clinical syndromes of cancer immunotherapy-induced myocarditis, pericarditis, and other cardiovascular toxicities. In collaboration with oncology colleagues, we have defined new clinical syndromes of immune checkpoint inhibitor (ICI)-associated myocarditis (Johnson et al, *NEJM*, 2016; Moslehi et al, *Lancet*, 2018) and other ICI-associated cardiovascular toxicities, including pericarditis and vasculitis (Salem et al, *Lancet Oncology*, 2018). I have utilized my expertise as a myocyte and mouse biologist to generate 2 separate pre-clinical models of ICI-associated myocarditis, which we will have utilized to dissect mechanisms of ICI-associated toxicities. These mouse models have already helped us develop better diagnostic and therapeutic strategies for our patients (Bonaca et al. *Circulation*, 2019; Salem et al. *NEJM*, 2019; We et al, *Cancer Discovery*, 2020). My interest in this space has recently expanded to other inflammatory cardiomyopathies, including giant cell myocarditis, acute cellular rejection (ACR) following cardiac transplantation, and other forms of myocarditis. I have been a co-author on several recent major American Heart Association (AHA) as well as European Society of Cardiology (ESC) statements on this new field of "Cardio-Immunology" (Kociol et al, *Circulation*, 2020; de Boer RA et al, *European Journal of Heart Failure*, 2020; Ammirati et al, *Circulation Heart Failure*, 2020).

I have a long history of mentorship, both in the clinic and in the laboratory. I also believe that cardio-oncology is an excellent career path for physician-scientists. When I moved to Vanderbilt, I established a Graduate Medical Education (GME) approved fellowship training the next generation of physician-scientists in the field of cardio-oncology. This is the first such program in the country. I am currently setting up a similar training program at UCSF. Indeed, over the last several years (going back to prior to GME approval), I have trained 11 fellows in cardio-oncology. Of the 9 that have completed this training, 5 have moved on to academic jobs and are currently Assistant or Associate Professors of medicine at University of Massachusetts, Harvard Medical School (Brigham and Women's Hospital), Virginia Commonwealth University, University of Groningen and Sorbonne University (or in training). A total of 24 trainees have participated in work in my laboratory over the last decade, includin g

undergraduate and graduate students and post-doctoral fellows. About 40% of these have been women. 6 have been underrepresented minorities.

Research I'd like to highlight:

R01 HL156021 (MPI: Moslehi, Balko)

05/01/2021 - 04/30/2026

Immunologic and Antigenic Drivers of Immune Checkpoint Inhibitor-Associated Myocarditis

To seek to define the T cells responsible for the etiology and pathogenesis of ICI-myocarditis and to demonstrate that specific T cell populations are both necessary and sufficient to drive pathogenesis (Aim 1). To seek to define the antigen targets of ICI-myocarditis in mice and in patients (Aim 2). Role: Principal Investigator

R01 HL155990 (MPI: Moslehi, Johnson)

05/01/2021 -04/30/2026

NIH

Long-Term Cardiovascular Sequelae of Cancer Immunotherapies

To test the hypothesis that cardiac ischemia potentiates increased immune infiltration and inflammation in pharmacologic and genetic mouse models of ICI-myocarditis (Aim 1). To assess the long-term impact of ICI on cardiovascular disease utilizing a large retrospective cohort of long-term survivors treated with ICI, as well as a prospective cohort (Aim 2). Role: Principal Investigator

R01 HL141466 (PI: Moslehi)

08/01/2019 - 05/31/2024

NIH

Novel mechanisms and predictors of VEGF receptor inhibitor-associated hypertension

To investigate the mechanism by which VEGF signaling regulates ET-1 transcription and expression. (2) To determine the impact of endothelin receptor antagonism on VEGFR-TKI hypertension. (3) Develop a novel precision risk predictor for VEGFR-TKI-related hypertension. Role: Principal Investigator

R01 CA227481 (PI: Balko/Johnson)

04/01/2019 - 03/31/2024

NIH

Patient and tumor-specific biomarkers and mechanisms that predict irAEs resulting from checkpoint inhibition To identify pre-existing indicators of subclinical autoimmunity that are present in patients with immunotherapy that develop irAEs and/or clinical response. (2) To evaluate cell-mediated mechanisms of autoimmunity that arise in response to immune checkpoint blockade in patients experiencing irAEs. (3) To determine whether common TCRs are expanded in tumor and sites of irAEs. Role: Co-Investigator

R01 HL123968 (PI: Wu)

04/01/2018 - 03/31/2022

NIH

Modeling susceptibility to chemotherapy-induced cardiotoxicity using human iPSC To generate iPSC lines from cancer patient with doxorubicin.

To perform a combination of ATAC-seq, CHIP-seq, and mass spectrometry to identify genes regulated by topoisomerase II-beta. Role: Co-Investigator

R01 HL141851(PI: Wu)

08/01/2018- 06/30/2022

NIH

Modeling tyrosine kinase inhibitor-induced vascular dysfunction using human iPSC

To generate iPSC lines from cancer patient with TKI-induced vascular toxicities versus those with no toxicity. To perform a combination of kinase sequencing, ATAC-seq, CHIP-seq to identify genes regulated by VEGF inhibition in patients treated with VEGFR-TKI. (3) To develop a risk predictor for TKI-induced vascular toxicity using iPSC Role: Co-Investigator

B. Positions, Scientific Appointments, and Honors

Positions and Employment

2021 - Present	Associate Professor in Residence, UCSF (Starting October 2021)
2021 - Present	Chief, Section of Cardio-Oncology, Immunology, and Metabolism, UCSF
2021 - Present	William Grossman Distinguished Professor in Cardiology, UCSF
2019 - 2021	Associate Professor of Medicine, Vanderbilt School of Medicine
2019 - 2021	Co-Director, Vanderbilt Program for Optimizing Immuno-Oncology Therapy (VPOINT)
2014 - 2021	Director, Cardio-Oncology Program, Vanderbilt School of Medicine
2014 - 2019	Assistant Professor of Medicine, Vanderbilt School of Medicine

2009 - 2014	Instructor, Harvard Medical School
2009 - 2014	Associate Physician, Department of Medicine, Brigham and Women's Hospital
2009 - 2014	Associate Physician, Dana-Farber Cancer Institute
2009 - 2014	Co-Director, Cardio-Oncology Program, Brigham and Women's Hospital/DanaFarber Cancer Institute
2007 - 2009	Research Fellow, Dana-Farber Cancer Institute, Boston, MA
2006 - 2007	Fellow in Vascular Medicine, Brigham and Women's Hospital, Boston, MA
2004 - 2006	Fellow in Cardiology, Brigham and Women's Hospital, Boston, MA
2001 - 2004	Resident in Internal Medicine, Johns Hopkins Hospital, Baltimore, MD
1998 - 1999	Stanley J. Sarnoff Cardiovascular Research Fellow: Harvard Medical School

Honors

- 2020 American Society of Clinical Investigation (ASCI)
2016 Stanley J. Sarnoff Alumni Achievement Award 2020
2010 Lerner Research Award, Brigham and Women's Hospital, Harvard Medical School
2009 Heart Failure Society of America Research Fellowship Award
2009 Thomas W. Smith Fellowship Award in Heart Failure Research, Brigham and Women's Hospital, Division of Cardiology
2009 Watkins Discovery Award Program in Cardiovascular Medicine, Brigham and Women's Hospital, Harvard Medical School
2006 Harvard Medical School Resident Teaching Award
1998 Stanley J. Sarnoff Fellowship in Cardiovascular Research
1996 Azrael Fellowship for excellence in journalism: Johns Hopkins University
1994 Fortuna Iseman Klotz Memorial Scholarship: Johns Hopkins University

C. Contribution to Science (from > 210 peer-reviewed publications)

1. Cardiovascular Toxicities Associated with Cancer Immunotherapies: I have collaborated with oncology colleagues to define new clinical syndromes of cardiovascular toxicity associated with cancer immunotherapies, especially immune checkpoint inhibitors (ICI). One clinical syndrome (ICI-associated myocarditis) often presents with concurrent, early progressive and refractory cardiac electrical instability, and fulminant myocarditis with a robust presence of T-cell and macrophage infiltrates. We have started studies exploring the mechanisms of this new clinical syndrome, why certain cancer patients are especially at risk and the implications of this syndrome for other cardiac diseases, including other types of myocarditis and cardiac transplant rejection. My laboratory has established several mouse models of these new clinical syndromes which are allowing us to develop preventive and treatment strategies for these new clinical syndromes.

- a) Bonaca MP, Olenchock BA, Salem JE, Wiviott, SD, Ederhy S, Cohen, A, Stewart, G, Choueiri TK, Di Carli M, Allenbach YA, Kumbhani DJ, Heinzerling L, Amiri-Kordestani L, Lyon AR, Thavendiranathan P, Padera R, Lichtman A, Liu PP, Johnson DB, **Moslehi J.** Myocarditis in the Setting of Cancer Therapeutics: Proposed Definitions for Emerging Clinical Syndromes in Cardio-Oncology. *Circulation*. 2019. 140(2): 80-91.
- b) Salem JE, Manouchehri A, Moey M, Lebrun-Vignes B, Bastarache L, Pariente A, Gobert A, Spano JP, Balko JM, Bonaca MP, Roden DM, Johnson DB, **Moslehi JJ.** Spectrum of Cardiovascular Toxicities Associated with Immune Checkpoint Inhibitors. *Lancet Oncology*. 2018. 19(12):1579- 1589. PMID: 30442497
- c) **Moslehi J**, Salem JE, Sosman JA, Lebrun-Vignes B, Johnson DB. Rapid Increase in Reporting of Fatal Immune Checkpoint Inhibitor associated Myocarditis. *Lancet*. 2018. 391(10124):933. PMID: 29536852
- d) Johnson DB, Balko JM, Compton ML, Chalkias S, Gorham J, Xu Y, Hicks M, Puzano I, Alexander MR, Bloomer TL, Becker J, Slosky DA, Phillips EJ, Pilkinton MA, Craig-Owens L, Kola N, Plautz G, Reshef DS, Deutsch JS, Deering RP, Olenchock BA, Lichtman AH, Roden DM, Seidman CE, Koralnik IJ, Seidman JG, Hoffman RD, Taube JM, Diaz Jr LA, Anders, RA, Sosman JA, **Moslehi JJ.** Fulminant Myocarditis with Combination Immune Checkpoint Blockade. *N Engl J Med*. 2016. 375:1749-1755. PMID: 27806233

2. Cardio-Oncology: I have established myself as an expert in cardio-oncology. I founded the cardio-oncology program at the Brigham and Women's Hospital/Dana-Farber Cancer Institute in 2009 before being recruited to direct the cardio-oncology program at Vanderbilt in 2014. In both settings, I have created clinical and research programs involving multiple clinicians, researchers and fellows. Clinically, I have established preventive and treatment cardiovascular protective measures for specific groups of cancer patients and am a thought leader in this growing field. My research program includes a basic research program where we use cell-based and animal-based models to dissect the mechanism of toxicities from novel cancer therapies. These models have shed light into the novel regulatory signaling pathways in the cardiovascular system. I work closely with cardiologists and oncologists locally, nationally and internationally. I have also started several collaborations with industry and regulatory bodies (including the US FDA) in cardio-oncology. In 2019, I was named the first chair of the American Heart Association (AHA) cardio-oncology subcommittee. At Vanderbilt, I have established a Graduate Medical Education (GME) approved fellowship training the next generation of physician-scientists in the field of cardio-oncology.

- a) **Moslehi J.** Cardiovascular Toxic Effects of Targeted Cancer Therapies. *N Engl J Med.* 2016. 1475-1467. PMID: 27732808
- b) Sarosiek KA, Fraser C, Muthalagu N, Bhola PD, Chang W, McBrayer SK, Cantlon A, Fisch S, Golomb-Mello G, Ryan JA, Deng J, Jian B, Corbett C, Goldenberg M, Madsen JR, Liao R, Walsh D, Sedivy J, Murphy DJ, Carrasco DR, Robinson S, **Moslehi J.**, Letai A. Developmental Regulation of Mitochondrial Apoptosis by c-Myc Governs Age- and Tissue-Specific Sensitivity to Cancer Therapeutics. *Cancer Cell.* 2017. 31(1):142-156. PMID: 28017613
- c) Bellinger AM, Arteaga CL, Force T, Humphreys BD, Demetri GD, Druker BJ, **Moslehi J.** Cardio-Oncology: how new targeted cancer therapies and precision medicine can inform cardiovascular discovery. *Circulation.* 2015. 132(23):2248-58. PMID: 26644247.
- d) Groake JD, Cheng S, **Moslehi J.** Cancer-drug Discovery and Cardiovascular Surveillance. *N Engl J Med.* 2013;369(19):1779-81. PMID: 24180496

3. Immune-Related Toxicities Associated with Immune Checkpoint Inhibitors (ICI): ICI have revolutionized cancer care and are now (in 9/19) approved for 16 different cancers. However, immune-related adverse events affecting multiple organs occur in unpredictable fashion and may cause severe morbidity and even death. My laboratory has developed several mouse models where we recapitulate ICI-associated toxicities and are utilizing these models to better elucidate mechanisms of toxicity but also formulate treatment strategies. In addition, we hope these models provide insights into interaction of the immune system with specific organs (e.g., the heart). Clinically, along with my colleague, Dr. Douglas Johnson, I co-direct the Vanderbilt Program for Optimizing Immuno-Oncology Therapy (V-POINT), a clinical group that brings a multi-disciplinary approach to the care of patients with ICI-toxicities. We have already utilized this program to better define the nature of the toxicities but also identify the patients at risk of toxicities.

- a) Wei SC, Meijers WC, Axelrod ML, Anang NAS, Screever EM, Wescott EC, Johnson DB, Whitley E, Lehmann L, Courand PY, Mancuso JJ, Himmel LE, Lebrun-Vignes B, Wleklinski MJ, Knollmann BC, Srinivasan J, Li Y, Atolagbe OT, Rao X, Zhao Y, Wang J, Ehrlich LIR, Sharma P, Salem JE, Balko JM, **Moslehi JJ***, Allison JP*. A Genetic Mouse Model Recapitulates Immune Checkpoint Inhibitor-Associated Myocarditis and Supports a Mechanism-Based Therapeutic Intervention. *Cancer Discovery.* 2021;CD-20-0856. PMID: 33257470 (*Co-Senior/Co-Corresponding)
- b) Johnson DB, McDonnell WJ, Gonzalez-Ericsson PI, Al-Rohil RN, Mobley BC, Salem JE, Wang DY, Sanchez V, Wang Y, Chastain CA, Barker K, Liang Y, Warren S, Beechem JM, Menzies AM, Tio M, Long GV, Cohen JV, Guidon AC, O'Hare M, Chandra S, Chowdhary A, Lebrun-Vignes B, Goldinger SM, Rushing EJ, Buchbinder EI, Mallal SA, Shi C, Xu Y, **Moslehi JJ**, Sanders ME, Sosman JA, Balko JM. A Case Report of Clonal EBV-like Memory CD4+ T Cell Activation in Fatal Checkpoint Inhibitor- induced Encephalitis. *Nat Med.* 2019 [Epub ahead of print] PMID: 31332390
- c) Salem JE, Allenbach Y, Vozy A, Brechot N, Johnson DB, **Moslehi JJ**, Kerneis M. Abatacept for Severe Immune Checkpoint Inhibitor-Associated Myocarditis. *N Engl J Med.* 2019;380(24):2377- 2379. PMID: 31189043

- d) Wang DY, Salem JE, Cohen JV, Chandra S, Menzer C, Ye F, Zhao S, Das S, Beckermann KE, Ha L, Rathmell WK, Ancell KK, Balko JM, Bowman C, Davis EJ, Chism DD, Horn L, Long GV, Carlino MS, Lebrun-Vignes B, Eroglu Z, Hassel JC, Menzies AM, Sosman JA, Sullivan RJ, **Moslehi JJ**, Johnson DB. Fatal Toxic Effects Associated with Immune Checkpoint Inhibitors: A Systematic Review and Meta-Analysis. *JAMA Oncol.* 2018. PMID: 30242316

4. Cardiovascular toxicities associated with Tyrosine Kinase Inhibitors (TKI): A focus of my research over the last five years, has been defining the cardiovascular complications that arise as a result of TKI. This includes both the cardiac and vascular complications associated with these drugs. Using mice, we have shown that a mechanism of the cardiomyopathy associated with VEGFR-TKI is cardiac microvascular dysfunction, induction of myocardial hypoxia and stabilization and activation of hypoxia-inducible factor (see section 4 below). Our work has helped define how patients are treated for clinically when they have cardiac and vascular effects of TKI. At Vanderbilt, we have devised algorithms for cardiovascular care of patients treated with TKI, which emphasize monitoring and prevention strategies to mitigate cardiovascular effects of TKI.

- a) Sharma A, Burridge PW, McKeithan WL, Serrano R, Shukla P, Sayed N, Churko JM, Kitani T, Wu H, Holmström A, Matsa E, Zhang Y, Kumar A, Fan AC, Del Álamo JC, Wu SM, **Moslehi JJ**, Mercola M, Wu JC. High-throughput screening of tyrosine kinase inhibitor cardiotoxicity with human induced pluripotent stem cells. *Sci Transl Med.* 2017. 9(377). PMID: 28202772
- b) Li W, Croce K, Steensma DP, McDermott DF, Ben-Yehuda O, **Moslehi J.** Vascular And Metabolic Implications Of Novel Targeted Cancer Therapies. *J Am Coll Card.* 2015 Sep 8. PMID: 26337996
- c) **Moslehi J**, Deininger M. Tyrosine Kinase Inhibitor Associated Cardiovascular Toxicity in Chronic Myeloid Leukemia. *J Clin Oncol.* 2015;33(35):4210-8. PMID: 26371140
- d) Uraizee I, Cheng S, And **Moslehi J.** Reversible Cardiomyopathy Associated With Sunitinib and Sorafenib. *N Engl J Med.* 2011 Oct 27. PMID: 22030001

5. Myocardial Hypoxic Signaling: The master transcription factor hypoxia-inducible factor (HIF) has emerged as a central mechanism by which tissues sense and respond to hypoxia. HIF regulation is mostly post-transcriptional. The Prolyl Hydroxylase Domain-Containing Protein (PHD; also called EglN) prolyl hydroxylases translate changes in oxygen bioavailability into changes in the abundance of HIF. Under low oxygen conditions, such as during ischemia, PHD function is impaired and HIF becomes active. In work that I started as a post-doctoral fellow, I showed a critical role for the EglN/PHD/HIF pathway in the cardiovascular system in biologically relevant models such as ischemic protection and ischemic cardiomyopathy.

- a) Olenchock BA*, **Moslehi J***, Baik A, Davidson S, Williams J, Gibson W, Pierce K, Miller C, Hanse E, Kelekar A, Sullivan L, Wagers A, Clish C, Vander Heiden MG, Kaelin WG Jr. Inhibition of the EglN1 oxygen sensor and rerouting of a-ketoglutarate are sufficient for remote ischemic protection. *Cell.* 2016. 164(5):884- 95. PMID: 26919427 (*co-first authors)
- b) b. Akbay EA, **Moslehi J**, Christensen CL, Saha S, Tchaicha JH, Ramkissoon SH, Stewart K, Carretero J, Kikuchi E, Zhang H, Cohoon TJ, Murray S, Liu W, Uno K, Fisch S, Jones K, Gurumurthy S, Gliser C, Choe S, Keenan M, Son J, Stanley I, Losman JA, Padera R, Bronson RT, Asara JM, Abdel-Wahab O, Amrein PC, Fathi AT, Danial N, Kimmelman AC, Kung AL, Ligon KL, Yen KE, Kaelin Jr WG, Bardeesy N, Wong K-K. D-2- hydroxyglutarate produced by mutant IDH2 causes cardiomyopathy and neurodegeneration in mice. *Genes Dev.* 2014. 28(5):479-90. PMID: 24589777
- c) **Moslehi J***, Minamishima YA*, Shi J, Neuberg D, Charytan DM, Padera RF, Signoretti S, Liao R, Kaelin WG Jr. Loss of hypoxia-inducible factor prolyl hydroxylase activity in cardiomyocytes phenocopies ischemic cardiomyopathy. *Circulation.* 2010. 122(10):1004-16. PMID: 20733101 (*co-first authors)
- d) Minamishima YA, **Moslehi J**, Bardeesy N, Cullen D, Bronson RT, Kaelin WG Jr. Somatic Inactivation of the PHD2 Prolyl Hydroxylase Causes Polycythemia and Congestive Heart Failure. *Blood.* 2008;111(6):3236-44. PMID: 18096761

Complete List of Published Work in MyBibliography:

<https://www.ncbi.nlm.nih.gov/myncbi/browse/collection/40735802/?sort=date&direction=ascending>

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: Altieri, Dario C

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

POSITION TITLE: Director, Cancer Center

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 Milan, Milan, Italy	M.D.	10/1982	Medicine
University of Milan, Milan, Italy	Postgrad Specialty	10/1985	Hematology
The Scripps Research Institute, La Jolla, CA	Postdoctoral	07/1989	Immunology
Yale University, New Haven, CT	M.A. (Hon)	10/1999	Arts and Science

A. Personal Statement

Our laboratory studies the role of mitochondria in cancer. We pursue the overarching hypothesis that multiple mitochondrial functions in bioenergetics, buffering of reactive oxidative species (ROS), inter-organelle communication with the ER and retrograde gene expression are invariably reprogrammed in malignancy and exploited to support tumor cell proliferation, evasion from multiple forms of cell death and heightened tumor cell motility and invasion. We use a multidisciplinary portfolio of biochemical, cellular, and molecular approaches *in vitro*, xenograft and genetic mouse models of localized and metastatic disease, *in vivo*, and analysis of clinically annotated primary patient samples to elucidate how mitochondrial reprogramming affects tumor maintenance, progression, and treatment response. Although mechanistic in nature, our research goals have clear translational and disease-relevant implications. Our work has demonstrated that therapeutic targeting of mitochondrial reprogramming in cancer is feasible, and may uniquely disable multiple mechanisms of disease progression, including metastatic competence across the spectrum of genetically heterogeneous tumors (*ClinicalTrials.gov NCT04827810*). Over the past three decades, our laboratory has consistently provided a strong and committed mentoring environment for the education and training of dozens of cancer scientists, with several former trainees successfully progressing in independent careers as senior investigators at leading universities in the US and abroad.

Ongoing projects that I would like to highlight include:

R35 CA220446 (Outstanding Investigator Award from the National Institutes of Health/National Cancer Institute)
Title: Tumor Plasticity

Role: PI

09/01/17-08/31/24

The proposed studies will dissect the cellular and molecular requirements of tumor plasticity as a novel hallmark of cancer, credential its relevance in xenograft and genetic mouse models of localized and metastatic disease, and exploit emerging vulnerabilities of these pathways for innovative cancer drug discovery.

B. Positions, Scientific Appointments, and Honors

Positions and Scientific Appointments

2016 – 2019	Editorial Board Member, Scientific Reports
2015 – Present	Editorial Board Member, The Journal of Clinical Investigation
2015 – Present	President and Chief Executive Officer, The Wistar Institute, Philadelphia, PA
2013 – 2016	Senior Editor, Molecular Cancer Research
2010 – Present	Robert and Penny Fox Distinguished Professor, The Wistar Institute, Philadelphia, PA
2010 – Present	Director, The Wistar Institute Cancer Center, Philadelphia, PA
2010 – 2015 PA	Executive Vice President and Chief Scientific Officer, The Wistar Institute, Philadelphia, PA
2004 – 2009	Editorial Board Member, Cancer Research
2003 – 2005	Editorial Board Member, Journal of Biological Chemistry
2002 – 2010 University of Massachusetts Medical School, Worcester, MA	Eleanor Eustis Farrington Professor and Founding Chair. Department of Cancer Biology, University of Massachusetts Medical School, Worcester, MA
2002 – 2009 Worcester, MA	Director, UMass Memorial Cancer Center, University of Massachusetts Medical School, Worcester, MA
2001 – 2004	Editorial Board Member, Cell Death and Differentiation
1999 – 2002 Yale University School of Medicine, New Haven, CT	Professor with Tenure. Department of Pathology, Boyer Center for Molecular Medicine, Yale University School of Medicine, New Haven, CT
1998 – 2003	Charter Member and Chair, NIH Pathology A Study Section
1994 – 1999 University School of Medicine, New Haven, CT	Associate Professor. Department of Pathology, Boyer Center for Molecular Medicine, Yale University School of Medicine, New Haven, CT
1989 – 1994 Research Institute, La Jolla, CA	Assistant Member. Departments of Immunology and Vascular Biology, The Scripps Research Institute, La Jolla, CA

Honors

2023	Citations: 65,074; h-index: 112; i10-index: 252
2017 – Present	NCI Outstanding Investigator Award (OIA) 1 R35 CA220446-01
2004 – 2014	NIH MERIT Award 2 R01 HL54131
1997 – Present	Member, American Society of Clinical Investigation
1994 – 1999	American Heart Association Established Investigator

C. Contributions to Science

1. **Multifunctional *survivin* signaling in cancer.** Our laboratory is credited with the discovery and characterization of the *survivin* gene. With over 9,100 citations currently in PubMed, *survivin* is recognized as a fundamental *cancer* gene, a pleiotropic molecular hub for multiple pathways of cell survival, mitosis, adaptation to stress and metabolic reprogramming, as well as a validated therapeutic target in the clinic. Our contributions in this field have run the gamut from the discovery of *survivin* to the characterization of its unique role at the interface between cell death and mitosis in cancer, to clinical validation as a therapeutic target and predictive/prognostic disease biomarker

- a. Ambrosini G, Adida C, **Altieri DC.** (1997). A novel anti-apoptosis gene, *survivin*, expressed in cancer and lymphoma. *Nat Med*, 3:917-921. PMID:9256286.
- b. Li F, Ambrosini G, Chu EY, Plescia J, Tognin S, Marchisio PC, **Altieri DC.** (1998). Control of apoptosis and mitotic spindle checkpoint by survivin. *Nature*, 396:580-584. PMID:985999
- c. Smith SD, Wheeler MA, Plescia J, Colberg JW, Weiss RM, **Altieri DC.** (2000). Urine detection of survivin and diagnosis of bladder cancer. *JAMA*, 285:324-328. PMID:11176843.
- d. Mehrotra S, Languino LR, Raskett CM, Mercurio AM, Dohi T, **Altieri DC.** (2010). IAP regulation of metastasis. *Cancer Cell*, 17:53-64. PMCID: PMC2818597.

2. **Mitochondrial proteostasis in tumor adaptation.** Over the past decade, our work uncovered a novel role of protein folding quality control in mitochondria as a key driver of tumor progression. These studies elucidated mechanisms of protein homeostasis, or proteostasis maintained by Heat Shock Protein-90 (Hsp90) chaperones as well as AAA+ proteases in mitochondria, characterized their role in adaptive regulation of

apoptosis, metabolic reprogramming and retrograde gene expression, and identified novel mechanisms of tumor adaptation to microenvironment stress stimuli, including hypoxia or exposure to molecular therapy.

- a. Kang BH, Plescia J, Dohi T, Rosa J, Doxsey SJ, **Altieri DC**. (2007). Regulation of tumor cell mitochondrial homeostasis by an organelle-specific Hsp90 chaperone network. *Cell*, 131:257-270. PMID:17956738.
 - b. Chae YC, Caino MC, Lisanti S, Ghosh JC, Dohi T, Danial NN, Villanueva J, Ferrero S, Vaira V, Santambrogio L, Bosari S, Languino LR, Herlyn M, **Altieri DC**. (2012). Control of tumor bioenergetics and survival stress signaling by mitochondrial Hsp90s. *Cancer Cell*, 22:331-344. PMCID: PMC3615709.
 - c. Chae YC, Angelin A, Lisanti S, Kossenkov AA, Speicher KD, Wang H, Powers JF, Tischler AS, Pacak K, Fliedner S, Michalek RD, Karoly ED, Wallace DC, Languino LR, Speicher DW, **Altieri DC**. (2013). Landscape of the mitochondrial Hsp90 metabolome in tumors. *Nat Commun*, 4:2139. PMCID: PMC3732457.
 - d. Seo JH, Rivadeneira DB, Caino MC, Chae YC, Speicher DW, Tang HY, Vaira V, Bosari S, Palleschi A, Rampini P, Kossenkov AV, Languino LR, **Altieri DC**. (2016). The Mitochondrial Unfoldase-Peptidase Complex ClpXP Controls Bioenergetics Stress and Metastasis. *PLoS Biol*, 14(7):e1002507. PMCID: PMC4936714.
3. **Mitochondria and metastasis.** How tumors that switch to an inefficient glycolytic metabolism, i.e. the Warburg effect manage to accomplish highly energy-demanding tasks of cell motility and invasion has long remained elusive. Our work demonstrated that mitochondrial oxidative phosphorylation is required to fuel membrane dynamics of cell motility, resulting in increased tumor chemotaxis, invasion, and metastasis. Mechanistically, we showed that this pathway involves the redistribution of energetically active mitochondria to the peripheral cytoskeleton of tumor cells, where they provide a concentrated, *spatiotemporal* energy source to power membrane lamellipodia dynamics, turnover of focal adhesion complexes, and sustained phosphorylation of cell motility kinases.
- a. Caino MC, Chae YC, Vaira V, Ferrero S, Nosotti M, Martin NM, Weeraratna AT, O'Connell M, Jernigan D, Fatatis A, Languino LR, Bosari S, **Altieri DC**. (2013). Metabolic stress regulates cytoskeletal dynamics and metastasis of cancer cells. *J Clin Invest*, 123:2907-2920. PMCID: PMC3998961.
 - b. Caino MC, Ghosh JC, Chae YC, Vaira V, Rivadeneira DB, Faversani A, Rampini P, Kossenkov AV, Aird KM, Zhang R, Webster MR, Weerarathna AT, Bosari S, Languino LR, **Altieri DC**. (2015). PI3K therapy reprograms mitochondrial trafficking to fuel tumor cell invasion. *Proc Natl Acad Sci USA*, 112:8638-43. PMCID: PMC4507184.
 - c. Caino MC, Seo JH, Aguinaldo A, Wait E, Bryant KG, Kossenkov AV, Hayden JE, Vaira V, Morotti A, Ferrero S, Bosari S, Gabrilovich DI, Languino LR, Cohen AR, **Altieri DC**. (2016). A neuronal network of mitochondrial dynamics regulates metastasis. *Nat Commun*, 7:13730. PMCID: PMC5187409.
 - d. Agarwal E, Altman BJ, Ho Seo J, Bertolini I, Ghosh JC, Kaur A, Kossenkov AV, Languino LR, Gabrilovich DI, Speicher DW, Dang CV, **Altieri DC**. (2019). Myc regulation of a mitochondrial trafficking network mediates tumor cell invasion and metastasis. *Mol Cell Biol*, 39(14). pii: e00109-19. PMCID: PMC6597883.
4. **Mitochondrial control of tumor plasticity.** Recent findings from our group have demonstrated that mitochondrial reprogramming is a universal cancer trait that imparts unique plasticity to a full spectrum of tumor responses, from early-stage malignant transformation to full blown tumor growth, to regulation of *go-or-grow* decisions, the dynamic and reversible switch between cell proliferation and cell migration states. We showed that mitochondrial control of tumor plasticity involves panoply of signaling pathways, including generation of ROS, exosome-dependent intercellular communication, and stabilization of HIF1 resulting in a transcriptionally-active, pseudo-hypoxic state.

- a. Chae YC, Vaira V, Caino MC, Tang HY, Seo JH, Kossenkov AV, Ottobrini L, Martelli C, Lucignani G, Bertolini I, Locatelli M, Bryant KG, Ghosh JC, Lisanti S, Ku B, Bosari S, Languino LR, Speicher DW, **Altieri DC**. (2016). Mitochondrial Akt regulation of hypoxic tumor reprogramming. *Cancer Cell*, 30:257-272. PMCID: PMC5131882.
- b. Li J, Agarwal E, Bertolini I, Seo JH, Caino MC, Ghosh JC, Kossenkov AV, Liu Q, Tang H-Y, Goldman AR, Languino LR, Speicher DW, **Altieri DC**. (2020). The mitophagy effector FUNDC1 controls mitochondrial

- reprogramming and cellular plasticity in cancer. *Science Signal*, 13(642):eaaz8240. PMCID: PMC7484983.
- c. Bertolini I, Ghosh JC, Kossenkov AV, Mulugu S, Krishn SR, Vaira V, Qin J, Plow EF, Languino LR, **Altieri DC**. (2020). Small extracellular vesicle regulation of mitochondrial dynamics reprograms a hypoxic tumor microenvironment. *Dev Cell*, S1534-5807(20)30588-8. PMCID: PMC7606608.
 - d. Agarwal E, Goldman AR, Tang HY, Kossenkov AV, Ghosh JC, Languino LR, Vaira V, Speicher DW, **Altieri DC** (2021) A cancer ubiquitome landscape identifies metabolic reprogramming as target of Parkin tumor suppression. *Science Adv*, 7(35):eabg7287. PMCID: PMC8386929.

5. **Novel cancer drug discovery approaches.** Our laboratory has pioneered the concept of targeting mitochondrial reprogramming for novel cancer therapeutics. We use a combination of mitochondrial-targeted peptidomimetic antagonists and small molecule ATPase inhibitors to disrupt mitochondrial Hsp90-directed protein folding, inhibit oxidative bioenergetics and abolish MFF cytoprotection at the mitochondrial outer membrane. In preclinical studies, these first-in-class mitochondrial-targeted agents (*Shepherdin*, *Gamitrinib*, *MFF 8-11*) were well-tolerated, demonstrated a unique “mitochondriotoxic” mechanism of action, and delivered potent, cytotoxic anticancer activity alone or in combination in localized and disseminated tumor models, *in vivo*. A publicly funded, first-in-human phase I clinical trial of mitochondrial-targeted Hsp90 inhibitor, *Gamitrinib* in patients with advanced cancer is currently ongoing (*ClinicalTrials.gov* NCT04827810).

- a. Plescia J, Salz W, Xia F, Pennati M, Zaffaroni N, Daidone MG, Meli M, Dohi T, Fortugno P, Nefedova Y, Gabrilovich D, Colombo M, **Altieri DC**. (2005). Rational design of Shepherdin, a novel anticancer agent. *Cancer Cell*, 7:457-468. PMID:15894266.
- b. Kang BH, Plescia J, Song HY, Meli M, Colombo G, Beebe K, Scroggins B, Neckers L, **Altieri DC**. (2009). Combinatorial drug design targeting multiple cancer signaling networks controlled by mitochondrial Hsp90. *J Clin Invest*, 119:454-464. PMCID: PMC2648691.
- c. Siegelin MD, Dohi T, Raskett CM, Orlowsky GM, Powers CM, Gilbert CA, Ross AH, Plescia J, **Altieri DC**. (2011). Exploiting the mitochondrial unfolded protein response for cancer therapy in mice and human cells. *J Clin Invest*, 121:1349-1360. PMCID: PMC3069780
- d. Seo JH, Chae YC, Kossenkov AV, Lee YG, Tang HY, Agarwal E, Gabrilovich DI, Languino LR, Speicher DW, Shastrula PK, Storaci AM, Ferrero S, Gaudioso G, Caroli M, Tosi D, Giroda M, Vaira V, Rebecca VW, Herlyn M, Xiao M, Fingerman D, Martorella A, Skordalakes E, **Altieri DC**. (2020). MFF regulation of mitochondrial cell death is a therapeutic target in cancer. *Cancer Res*, 79(24):6215-6226. PMCID: PMC6911621.

Complete List of Published Work in MyBibliography:
<https://pubmed.ncbi.nlm.nih.gov/?term=altieri%20dc&sort=date>

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

UEI*: EKE1RJKQSGL9

Budget Type*: Project Subaward/Consortium

Enter name of Organization: LIGHTSEED, INC.

Start Date*: 12-01-2024

End Date*: 11-30-2025

Budget Period: 1

A. Senior/Key Person												
Prefix	First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base	Calendar	Academic	Summer	Requested	Fringe	Funds Requested (\$)*
						Salary (\$)	Months	Months	Months	Salary (\$)*	Benefits (\$)*	
1.	Xuanmao		Jiao		PD/PI	221,900.00	2.64			48,818.00	11,716.00	60,534.00
2.	Zhiping		Li		Co-Investigator	84,700.00	6.12			43,197.00	10,367.00	53,564.00

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

Additional Senior Key Persons:	File Name:	Total Senior/Key Person	114,098.00
--------------------------------	------------	-------------------------	------------

B. Other Personnel														
Number of Personnel*	Project Role*	Calendar Months				Academic	Months	Summer	Months	Requested	Salary (\$)*	Fringe	Benefits*	Funds Requested (\$)*
		1	Post Doctoral Associates	6.0						29,293.00	7,030.00	36,323.00		
Graduate Students														
Undergraduate Students														
Secretarial/Clerical														
1	Total Number Other Personnel									Total Other Personnel	36,323.00			
										Total Salary, Wages and Fringe Benefits (A+B)	150,421.00			

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

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

UEI*: EKE1RJKQSGL9

Budget Type*: Project Subaward/Consortium

Organization: LIGHTSEED, INC.

Start Date*: 12-01-2024

End Date*: 11-30-2025

Budget Period: 1

C. Equipment Description		Funds Requested (\$)*
List items and dollar amount for each item exceeding \$5,000		
Equipment Item		
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*: EKE1RJKQSGL9

Budget Type*: Project Subaward/Consortium

Organization: LIGHTSEED, INC.

Start Date*: 12-01-2024

End Date*: 11-30-2025

Budget Period: 1

F. Other Direct Costs		Funds Requested (\$)*
1. Materials and Supplies		100,000.00
2. Publication Costs		
3. Consultant Services		
4. ADP/Computer Services		
5. Subawards/Consortium/Contractual Costs		492,466.00
6. Equipment or Facility Rental/User Fees		
7. Alterations and Renovations		
8. Hull and Associates		25,000.00
	Total Other Direct Costs	617,466.00

G. Direct Costs		Funds Requested (\$)*
	Total Direct Costs (A thru F)	767,887.00

H. Indirect Costs			
Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base (\$)	Funds Requested (\$)*
1 . MTDC	40.0	300,421.00	120,168.00
		Total Indirect Costs	120,168.00
Cognizant Federal Agency (Agency Name, POC Name, and POC Phone Number)			

I. Total Direct and Indirect Costs		Funds Requested (\$)*
	Total Direct and Indirect Institutional Costs (G + H)	888,055.00

J. Fee		Funds Requested (\$)*
		62,164.00

K. Total Costs and Fee		Funds Requested (\$)*
		950,219.00

L. Budget Justification*	File Name: BudJust_20240329.pdf
--------------------------	---------------------------------

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

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

UEI*: EKE1RJKQSGL9

Budget Type*: Project Subaward/Consortium

Enter name of Organization: LIGHTSEED, INC.

Start Date*: 12-01-2025

End Date*: 11-30-2026

Budget Period: 2

A. Senior/Key Person

Prefix	First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base	Calendar	Academic	Summer	Requested	Fringe	Funds Requested (\$)*
						Salary (\$)	Months	Months	Months	Salary (\$)*	Benefits (\$)*	
1.	Xuanmao		Jiao		PD/PI	221,900.00	2.64			48,818.00	11,716.00	60,534.00
2.	Zhiping		Li		Co-Investigator	87,238.00	6.12			44,491.00	10,678.00	55,169.00

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

Additional Senior Key Persons: File Name: Total Senior/Key Person **115,703.00**

B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary (\$)*	Fringe Benefits*	Funds Requested (\$)*
1	Post Doctoral Associates		6.0		30,171.00	7,241.00	37,412.00
	Graduate Students						
	Undergraduate Students						
	Secretarial/Clerical						
1	Total Number Other Personnel				Total Other Personnel		37,412.00
					Total Salary, Wages and Fringe Benefits (A+B)		153,115.00

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

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

UEI*: EKE1RJKQSGL9

Budget Type*: Project Subaward/Consortium

Organization: LIGHTSEED, INC.

Start Date*: 12-01-2025

End Date*: 11-30-2026

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*: EKE1RJKQSGL9

Budget Type*: Project Subaward/Consortium

Organization: LIGHTSEED, INC.

Start Date*: 12-01-2025

End Date*: 11-30-2026

Budget Period: 2

F. Other Direct Costs		Funds Requested (\$)*
1. Materials and Supplies		80,000.00
2. Publication Costs		
3. Consultant Services		
4. ADP/Computer Services		
5. Subawards/Consortium/Contractual Costs		506,939.00
6. Equipment or Facility Rental/User Fees		
7. Alterations and Renovations		
8. Hull and Associates		25,000.00
9. Charles River		100,000.00
Total Other Direct Costs		711,939.00

G. Direct Costs		Funds Requested (\$)*
Total Direct Costs (A thru F)		865,054.00

H. Indirect Costs		Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base (\$)	Funds Requested (\$)*
1 . MTDC			40.0	358,116.00	143,246.00
Total Indirect Costs					143,246.00
Cognizant Federal Agency (Agency Name, POC Name, and POC Phone Number)					

I. Total Direct and Indirect Costs		Funds Requested (\$)*
Total Direct and Indirect Institutional Costs (G + H)		1,008,300.00

J. Fee		Funds Requested (\$)*
		70,581.00

K. Total Costs and Fee		Funds Requested (\$)*
		1,078,881.00

L. Budget Justification*		File Name: BudJust_20240329.pdf
RESEARCH & RELATED Budget {F-K} (Funds Requested)		

BUDGET JUSTIFICATION

Upon careful review of the budget required to complete the Aims as outlined, and further review of the eligible waiver topics, LightSeed Therapeutics respectfully request the following budget, as outlined. This document justifies the budget and provides a basis for inclusion for the waiver topics. The NIH published the guidance entitled “National Institutes of Health SBA-Approved SBIR/STTR Topics for Awards over Statutory Budget Limitations Approved 5/26/2023. Within the NHLBI, on page 24, section D: “Therapeutics (drugs, devices, gene therapy, or other biologics) development of heart, lung, blood, and sleep-related diseases and disorders” is listed as an eligible topic. Accordingly, we request a hard cap budget waiver for this effort.

Personnel (Y1: \$150,421; Y2: \$153,116)

A. Senior/Key Personnel

Xuanmao Jiao, M.D., Ph.D. (Y1 – Y2: 22% effort, 2.64 cal. mo.). Dr. Jiao is an Associate Professor (Research) at the Baruch S. Blumberg Institute, who works in the Pestell Laboratory, at the Lankenau Institute for Medical Research (LIMR). Dr. Jiao is an expert in primary culture of murine mammary epithelial cells and 3D matrigel culture and imaging for tumors. His prior work includes studies of i) stem cells and their culture. ii). identification of the utility of a compound from the LOPAC 1280 library to reduce Dox-induced iPSC toxicity iii). substantial experience using Luc2 stable metastatic breast cancer cell lines to follow growth in real time in mice. He has extensive experience with *in vivo* imaging using the mutant luc reporter C57mg breast cancer stable lines. Dr. Jiao works full time at the bench and will conduct the studies in Aim 1 and 2. Dr. Jiao will contribute 22% effort to this project.

Zhiping Li PhD (Y1-Y2: 51% effort, 6.12 cal. mo.). Dr. Li is an Associate Professor, at the Baruch S. Blumberg Institute who is also involved directly with this project at the level of bench research. Dr Li has worked with Dr Pestell for more than 20 years and whose technical expertise is attested by the high-quality publications shown in her biosketch, Dr. Li has a broad published background in the mechanisms of cell death and G protein coupled receptor signaling. She has experience with tumor response to Doxorubicin and will conduct tumor analysis described in the presence of the new cardio protectants. Her efforts will remain directed to tumor responses and the immunological response described in Aim. 2. The studies outlined are highly labor intensive and we are keenly aware of the critical importance of Dr. Li's involvement in this project. Dr. Li will contribute 3 calendar months to this project.

B. Other Personnel.

PostDoctoral Fellow (TBA) (100% effort, 6 cal. mo.). A postdoctoral fellow with experience in animal husbandry and mouse models of cancer will be recruited for the studies. This individual will be involved in the tissue culture-based analysis of breast cancer cell lines and measurements and analysis of the breast tumors that develop in the mice.

The fringe benefits for employees at LightSeed Therapeutics are 24% of the salary requested.

C. Equipment. No funds requested.

D. Travel. No funds requested.

E. Participant/Trainee Support Costs. Not applicable.

F. Other Direct Costs (Y1: \$767,887; Y2: \$865,055)

1. Materials and Supplies (Y1: \$100,000; Y2: \$80,000)

Funds will be utilized to purchase lab supplies, drugs, animals, etc.: \$25,000 are requested to purchase sufficient supplies to adequately conduct this study.

1. Maraviroc will be obtained from Selleck chemicals \$1270/gram.

2. PEGylated liposome (a) Doxorubicin Liposomes (PEGylated) (Doxil) will be obtained from Encapsula NanoSciences (catalogue number SKU# DOX-1000) 5-ml \$800.00. (Alternative vendors if supply becomes compromised include FormuMax Scientific (catalogue number NC1488576\$335.00 for 2ml or Creative Biostructure Kit-068K \$1150 2ml or Creative Biolabs , \$2680 5ml).
3. Non-PEGylated liposome (Myocet) Sopherion Therapeutics 2mg/ml 910\$/ml
4. Polymeric micelle (PM) SP1049C will be prepared and provided to us as a collaboration with Professor Alexander Kabanov's laboratory (please see his letter of support) and is included in the Budget Justification section for organizational convenience and clarity to the Reviewers.
5. Polymeric nanoparticle (LivaTag) will be obtained from Valerio Therapeutics (letter of support indicates provision by Valerio Therapeutics and is included in the Budget Justification section for organizational convenience and clarity to the Reviewers).
6. Additional chemicals, price and source. Nitisinone,(Sigma Aldrich ID:SML0269) 10mg. Price \$67.80 4-amino-1,8-naphthalimide (Sigma Aldrich ID:A0966), 20mg Price \$169, NSC405020 (Sigma Aldrich ID: SML0518), 5mg Price \$123, CyPPA (Sigma Aldrich ID: C5493), 5mg Price \$149, Metoclopramide hydrochloride (Sigma Aldrich ID: M0763), 10G Price \$69.60, Pitstop 2 (Sigma Aldrich ID: SML1169), 5mg Price \$115, HA155(Sigma Aldrich ID: SML0914),5mg Price \$122, JW74 (Sigma Aldrich ID: SML0227), 5mg Price \$120, BIX (Sigma Aldrich ID: SML1073), 5mg Price \$118 O-(Carboxymethyl)hydroxylamine hemihydrochloride (Sigma Aldrich ID: C13408) 1G Price \$99.60
7. \$55,000 is requested for purchase and maintenance (acquisition of animals, housing, cage cleaning, food, breeding, and other related expenses). All mice are housed in a special, germ-free environment in the Lankenau Institute for Medical Research (LIMR) Barrier Facilities.

2. Publication Costs. No funds requested.

3. Consultant Services (Y1: \$25,000 Y2: \$25,000).

Consultants Regulatory (Y1 and 2: \$25,000). Advice on the regulatory pathway necessary to ensure the appropriate work is conducted in the proposal for the IND of the clinical trial that will be conducted in the futures phases of these studies.

Consultants GLP safety and toxicology (Y2: \$100,000). Charles River will serve as the consultants for GLP safety and toxicology for the combination of maraviroc and the different reformulated DOX.

4. ADP/Computer Services. No funds requested.

5. Subaward/Consortium/Contractual (Y1: \$492,466; Y2: \$506,939)

Baruch S. Blumberg Institute (Y1: \$354,365; Y2: \$366,034)

See attached justification and detailed budget.

Lankenau Institute for Medical Research (Y1: \$138,101; Y2: \$140,905)

See attached justification and detailed budget.

6. Equipment or Facility Rental/User Fees. No funds requested.

7. Alterations and Renovations. No funds requested.

H. Indirect Costs. LightSeed Therapeutics, Inc. does not yet have an established indirect cost rate with a federal agency. Based upon a projection of the facilities and administration activities and costs projected for the next year, the company has applied the allowable indirect cost rate of 40%.

J. Fee.

A 7 percent profit or fee is being requested to provide support for company growth and to cover costs which are deemed unallowable through SBIR (e.g., legal expenses). This fee contributes to the growth and development of LightSeed Therapeutic, Inc by allowing expansion resources and personnel development. The fee is consistent with the normal profit margin provided for research and development work.

RESEARCH & RELATED BUDGET - Cumulative Budget

	Totals (\$)
Section A, Senior/Key Person	229,801.00
Section B, Other Personnel	73,735.00
Total Number Other Personnel	2
Total Salary, Wages and Fringe Benefits (A+B)	303,536.00
Section C, Equipment	0.00
Section D, Travel	0.00
1. Domestic	0.00
2. Foreign	0.00
Section E, Participant/Trainee Support Costs	0.00
1. Tuition/Fees/Health Insurance	0.00
2. Stipends	0.00
3. Travel	0.00
4. Subsistence	0.00
5. Other	0.00
6. Number of Participants/Trainees	0
Section F, Other Direct Costs	1,329,405.00
1. Materials and Supplies	180,000.00
2. Publication Costs	0.00
3. Consultant Services	0.00
4. ADP/Computer Services	0.00
5. Subawards/Consortium/Contractual Costs	999,405.00
6. Equipment or Facility Rental/User Fees	0.00
7. Alterations and Renovations	0.00
8. Other 1	50,000.00
9. Other 2	100,000.00
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)	1,632,941.00
Section H, Indirect Costs	263,414.00

Section I, Total Direct and Indirect Costs (G + H)	1,896,355.00
Section J, Fee	132,745.00
Section K, Total Costs and Fee (I + J)	2,029,100.00

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

UEI*: NAYCKSB7F68

Budget Type*: Project Subaward/Consortium

Enter name of Organization: Baruch S. Blumberg Institute

Start Date*: 12-01-2024

End Date*: 11-30-2025

Budget Period: 1

A. Senior/Key Person												
	Prefix First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base	Calendar	Academic	Summer	Requested	Fringe	Funds Requested (\$)*
						Salary (\$)	Months	Months	Months	Salary (\$)*	Benefits (\$)*	
1.	Richard	G.	Pestell		PD/PI		1.2			22,190.00	5,325.00	27,515.00
2.	Taimoor		Khan		Post-Doctoral Fellow		6.0			28,440.00	6,826.00	35,266.00
3.	Danni		Li		Post-Doctoral Fellow		6.0			28,440.00	6,826.00	35,266.00

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

Additional Senior Key Persons:	File Name:	Total Senior/Key Person	98,047.00
--------------------------------	------------	-------------------------	-----------

B. Other Personnel												
Number of Personnel*	Project Role*	Calendar Months			Academic	Months	Summer	Months	Requested Salary (\$)*	Fringe	Benefits*	Funds Requested (\$)*
		1	2	3	4	5	6	7	8	9	10	11
1	Post Doctoral Associates								58,586.00		14,060.00	72,646.00
	Graduate Students											
	Undergraduate Students											
	Secretarial/Clerical											
1	Total Number Other Personnel									Total Other Personnel		72,646.00
										Total Salary, Wages and Fringe Benefits (A+B)		170,693.00

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

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

UEI*: NAYCKSJB7F68

Budget Type*: Project Subaward/Consortium

Organization: Baruch S. Blumberg Institute

Start Date*: 12-01-2024

End Date*: 11-30-2025

Budget Period: 1

C. Equipment Description

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

Equipment Item

Funds Requested (\$)*

Total funds requested for all equipment listed in the attached file

Total Equipment 0.00

Additional Equipment: File Name:

D. Travel

Funds Requested (\$)*

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

2. Foreign Travel Costs

Total Travel Cost 0.00

E. Participant/Trainee Support Costs

Funds Requested (\$)*

1. Tuition/Fees/Health Insurance

2. Stipends

3. Travel

4. Subsistence

5. Other:

Number of Participants/Trainees

Total Participant Trainee Support Costs

0.00

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

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

UEI*: NAYCKSJB7F68

Budget Type*: Project Subaward/Consortium

Organization: Baruch S. Blumberg Institute

Start Date*: 12-01-2024

End Date*: 11-30-2025

Budget Period: 1

F. Other Direct Costs		Funds Requested (\$)*
1. Materials and Supplies		28,785.00
2. Publication Costs		
3. Consultant Services		
4. ADP/Computer Services		
5. Subawards/Consortium/Contractual Costs		
6. Equipment or Facility Rental/User Fees		22,000.00
7. Alterations and Renovations		
8. Data Management and Sharing Costs		0.00
	Total Other Direct Costs	50,785.00

G. Direct Costs		Funds Requested (\$)*
	Total Direct Costs (A thru F)	221,478.00

H. Indirect Costs			
Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base (\$)	Funds Requested (\$)*
1 . MTDC	60.0	221,478.00	132,887.00
			Total Indirect Costs
			132,887.00
Cognizant Federal Agency	DHHS, Ernest Kinneer (214)767-3261		
(Agency Name, POC Name, and POC Phone Number)			

I. Total Direct and Indirect Costs		Funds Requested (\$)*
Total Direct and Indirect Institutional Costs (G + H)		354,365.00

J. Fee		Funds Requested (\$)*

K. Total Costs and Fee		Funds Requested (\$)*
		354,365.00

L. Budget Justification*	File Name: BSBI_Budget_Justification.pdf
--------------------------	--

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

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

UEI*: NAYCKSB7F68

Budget Type*: Project Subaward/Consortium

Enter name of Organization: Baruch S. Blumberg Institute

Start Date*: 12-01-2025

End Date*: 11-30-2026

Budget Period: 2

A. Senior/Key Person												
	Prefix First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base	Calendar	Academic	Summer	Requested	Fringe	Funds Requested (\$)*
						Salary (\$)	Months	Months	Months	Salary (\$)*	Benefits (\$)*	
1.	Richard	G.	Pestell		PD/PI		1.2			22,190.00	5,326.00	27,516.00
2.	Taimoor		Khan		Post-Doctoral Fellow		6.0			29,293.00	7,030.00	36,323.00
3.	Danni		Li		Post-Doctoral Fellow		6.0			29,293.00	7,030.00	36,323.00
Total Funds Requested for all Senior Key Persons in the attached file										Total Senior/Key Person		100,162.00
Additional Senior Key Persons: File Name:												

B. Other Personnel											
Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary (\$)*	Fringe Benefits*	Funds Requested (\$)*				
1	Post Doctoral Associates				60,342.00	14,482.00	74,824.00				
	Graduate Students										
	Undergraduate Students										
	Secretarial/Clerical										
1	Total Number Other Personnel					Total Other Personnel	74,824.00				
Total Salary, Wages and Fringe Benefits (A+B)											174,986.00

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

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

UEI*: NAYCKSJB7F68

Budget Type*: Project Subaward/Consortium

Organization: Baruch S. Blumberg Institute

Start Date*: 12-01-2025

End Date*: 11-30-2026

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

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

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

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

RESEARCH & RELATED BUDGET - SECTIONS F-K, Budget Period 2

UEI*: NAYCKSJB7F68

Budget Type*: Project Subaward/Consortium

Organization: Baruch S. Blumberg Institute

Start Date*: 12-01-2025

End Date*: 11-30-2026

Budget Period: 2

F. Other Direct Costs		Funds Requested (\$)*
1. Materials and Supplies		28,785.00
2. Publication Costs		
3. Consultant Services		
4. ADP/Computer Services		
5. Subawards/Consortium/Contractual Costs		
6. Equipment or Facility Rental/User Fees		25,000.00
7. Alterations and Renovations		
8. Data Management and Sharing Costs		0.00
Total Other Direct Costs		53,785.00

G. Direct Costs		Funds Requested (\$)*
Total Direct Costs (A thru F)		228,771.00

H. Indirect Costs			
Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base (\$)	Funds Requested (\$)*
1 . MTDC	60.0	228,771.00	137,263.00
Total Indirect Costs			137,263.00
Cognizant Federal Agency			DHHS, Ernest Kinneer (214)767-3261
(Agency Name, POC Name, and POC Phone Number)			

I. Total Direct and Indirect Costs		Funds Requested (\$)*
Total Direct and Indirect Institutional Costs (G + H)		366,034.00

J. Fee		Funds Requested (\$)*

K. Total Costs and Fee		Funds Requested (\$)*
		366,034.00

L. Budget Justification*	File Name: BSBI_Budget_Justification.pdf
--------------------------	--

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

BUDGET JUSTIFICATION Baruch S. Blumberg Institute

PERSONNEL

Richard G. Pestell, M.D., Ph.D. (10% effort) (PI of BSBI subcontract)

Dr. Pestell received his M.D. and Ph.D. from the University of Melbourne. Dr. Pestell's clinical training was in Oncology and Endocrinology. He is ranked in the world by Google Scholar, cell-cycle control (#1) and breast cancer (#11) for >30 years. Prior to his current position he was Executive Vice President of Thomas Jefferson University and Director of the Sidney Kimmel Cancer Center and Chairman of the Department of Cancer Biology, Thomas Jefferson University (2005-2015). He was responsible for the oversite of ~120 clinical trials/yr from 2002-2015. Dr. Pestell has been the founder of six Biotechnology companies based on his intellectual property. He has more than 650 publications, more than 120 in the last 5 years, predominantly in the area of cell-cycle, breast cancer and mouse models of cancer. He is the inventor of the technology being tested in the current application. Dr. Pestell has been a lecturer in inducible transgenics at the NCI-supported Annual Mouse Developmental Genetics Course at AECOM, at the Jackson Labs meetings on new transgenic developments, and has published 30 papers using transgenic mice and 5 using knockout mice including the genesis of mammary gland-targeted ponasterone-inducible transgenics. Dr. Pestell will oversee the design of all experiments and will assist in writing all manuscripts. Dr. Pestell will contribute 10% total effort to this project (1.2 calendar months) (as recommended by the Reviewer). 10% effort will be provided as CEO of LightSeed and will be contributed by the BSBI subcontract in which he will coordinate and evaluate the research.

Taimoor Khan, PhD, (50% effort). (Post-Doctoral Fellow). Dr. Khan completed his PhD at Shanghai Jiao Tong University, Shanghai, China on a highly competitive Chinese Government Fellowship for International PhD students and a Post-Doctoral Fellowship at UCSF. He has published in the area of cell cycle control and breast tumor growth relevant to the current proposal. He will be directly involved in breast tumor implantation studies measuring the impact of CCR5 inhibitors on the impact of alternate reformulations of doxorubicin induced tumor growth and cardiotoxicity. Under the guidance of Dr. Ashton, he will participate in the echocardiographic analysis of the mice in this study.

Danni Li. M.D, PhD, (50% effort). (Post-Doctoral Fellow). Dr. Li completed her M.D. and PhD degrees at the University of Tongji, Shanghai, China. She is familiar with assessing animal models of cancer in response to therapies and analyzing cardiac tissue and cell lines for cell survival as proposed herein. She will be involved in the labor-intensive studies measuring the impact of CCR5 inhibitors on the impact of alternate reformulations of doxorubicin induced tumor growth and cardiotoxicity. She will also provide specimens as a collaborative bridge with our CRO for the GLP safety and toxicology of the combination of maraviroc and DOX in Aim 3.

Post-Doctoral Fellow (TBA) (100% effort). A postdoctoral fellow with experience in animal husbandry and mouse models of cardiotoxicity and or cardiac cell death will be recruited for the studies. This individual will be involved in the tissue culture based analysis of cardiac cell lines and measurements and analysis of the cardiotoxicity that develop in the mice.

SUPPLIES:

\$28,785 is requested to purchase sufficient supplies to adequately conduct this study.

FACILITY USER FEES

\$22,000 includes the facility licenses to use all facilities at BSBI, LIMR and or Wistar for the proposed research as described in the facility section. Year 2 will increase to \$25,000 as it is anticipated that more statistical analysis will be required in year 2, as substantial data will be generated during year 1.

ADDITIONAL NOTES

1. The indirect cost rate at Baruch S. Blumberg Institute is 60%.
2. The fringe benefit rates at Baruch S. Blumberg Institute are 24% for faculty and staff.

RESEARCH & RELATED BUDGET - Cumulative Budget

	Totals (\$)
Section A, Senior/Key Person	198,209.00
Section B, Other Personnel	147,470.00
Total Number Other Personnel	2
Total Salary, Wages and Fringe Benefits (A+B)	345,679.00
Section C, Equipment	0.00
Section D, Travel	0.00
1. Domestic	0.00
2. Foreign	0.00
Section E, Participant/Trainee Support Costs	0.00
1. Tuition/Fees/Health Insurance	0.00
2. Stipends	0.00
3. Travel	0.00
4. Subsistence	0.00
5. Other	0.00
6. Number of Participants/Trainees	0
Section F, Other Direct Costs	104,570.00
1. Materials and Supplies	57,570.00
2. Publication Costs	0.00
3. Consultant Services	0.00
4. ADP/Computer Services	0.00
5. Subawards/Consortium/Contractual Costs	0.00
6. Equipment or Facility Rental/User Fees	47,000.00
7. Alterations and Renovations	0.00
8. Other 1	0.00
9. Other 2	0.00
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)	450,249.00
Section H, Indirect Costs	270,150.00

Section I, Total Direct and Indirect Costs (G + H)	720,399.00
Section J, Fee	0.00
Section K, Total Costs and Fee (I + J)	720,399.00

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

UEI*: 1232175626A1

Budget Type*: Project Subaward/Consortium

Enter name of Organization: Lenkenau Institute for Medican Research

Start Date*: 12-01-2024

End Date*: 11-30-2025

Budget Period: 1

A. Senior/Key Person

Prefix	First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base	Calendar	Academic	Summer	Requested	Fringe	Funds Requested (\$)*
						Salary (\$)	Months	Months	Months	Salary (\$)*	Benefits (\$)*	
1.	Anthony		Ashton		Subaward PI	158,557.00	1.5			19,820.00	6,075.00	25,895.00

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

Additional Senior Key Persons: File Name: Total Senior/Key Person **25,895.00**

B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary (\$)*	Fringe Benefits*	Funds Requested (\$)*
1	Post Doctoral Associates		4.68		22,815.00	6,993.00	29,808.00
	Graduate Students						
	Undergraduate Students						
	Secretarial/Clerical						
1	Total Number Other Personnel					Total Other Personnel	29,808.00
						Total Salary, Wages and Fringe Benefits (A+B)	55,703.00

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

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

UEI*: 1232175626A1

Budget Type*: Project Subaward/Consortium

Organization: Lenkenau Institute for Medican Research

Start Date*: 12-01-2024

End Date*: 11-30-2025

Budget Period: 1

C. Equipment Description		Funds Requested (\$)*
List items and dollar amount for each item exceeding \$5,000		
Equipment Item		
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*: 1232175626A1

Budget Type*: Project Subaward/Consortium

Organization: Lenkenau Institute for Medican Research

Start Date*: 12-01-2024

End Date*: 11-30-2025

Budget Period: 1

F. Other Direct Costs		Funds Requested (\$)*
1. Materials and Supplies		26,500.00
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	26,500.00

G. Direct Costs		Funds Requested (\$)*
	Total Direct Costs (A thru F)	82,203.00

H. Indirect Costs		Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base (\$)	Funds Requested (\$)*
1 .	MTDC		68.0	82,203.00	55,898.00
Cognizant Federal Agency					55,898.00
(Agency Name, POC Name, and POC Phone Number)					

I. Total Direct and Indirect Costs		Funds Requested (\$)*
	Total Direct and Indirect Institutional Costs (G + H)	138,101.00

J. Fee		Funds Requested (\$)*

K. Total Costs and Fee		Funds Requested (\$)*
		138,101.00

L. Budget Justification*		File Name: LIMR_BudJust.pdf

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

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

UEI*: 1232175626A1

Budget Type*: Project Subaward/Consortium

Enter name of Organization: Lenkenau Institute for Medican Research

Start Date*: 12-01-2024

End Date*: 11-30-2025

Budget Period: 2

A. Senior/Key Person

Prefix	First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base	Calendar	Academic	Summer	Requested	Fringe	Funds Requested (\$)*
						Salary (\$)	Months	Months	Months	Salary (\$)*	Benefits (\$)*	
1.	Anthony		Ashton		Subaward PI	163,314.00	1.5			20,414.00	6,257.00	26,671.00

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

Additional Senior Key Persons: File Name: Total Senior/Key Person **26,671.00**

B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary (\$)*	Fringe Benefits*	Funds Requested (\$)*
1	Post Doctoral Associates	4.68			23,499.00	7,202.00	30,701.00
	Graduate Students						
	Undergraduate Students						
	Secretarial/Clerical						
1	Total Number Other Personnel					Total Other Personnel	30,701.00
					Total Salary, Wages and Fringe Benefits (A+B)		57,372.00

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

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

UEI*: 1232175626A1

Budget Type*: Project Subaward/Consortium

Organization: Lenkenau Institute for Medican Research

Start Date*: 12-01-2024

End Date*: 11-30-2025

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*: 1232175626A1

Budget Type*: Project Subaward/Consortium

Organization: Lenkenau Institute for Medican Research

Start Date*: 12-01-2024

End Date*: 11-30-2025

Budget Period: 2

F. Other Direct Costs		Funds Requested (\$)*
1. Materials and Supplies		26,500.00
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	26,500.00

G. Direct Costs		Funds Requested (\$)*
	Total Direct Costs (A thru F)	83,872.00

H. Indirect Costs		Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base (\$)	Funds Requested (\$)*
1 .	MTDC		68.0	83,872.00	57,033.00
Cognizant Federal Agency					57,033.00
(Agency Name, POC Name, and POC Phone Number)					

I. Total Direct and Indirect Costs		Funds Requested (\$)*
	Total Direct and Indirect Institutional Costs (G + H)	140,905.00

J. Fee		Funds Requested (\$)*

K. Total Costs and Fee		Funds Requested (\$)*
		140,905.00

L. Budget Justification*		File Name: LIMR_BudJust.pdf

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

Lankenau Institute for Medical Research (LIMR)

Budget Justification

A. Key/Senior Staff

Anthony Ashton, Ph.D. (Co- Investigator, 1.50 Calendar Months; 12.50% effort). Dr. Ashton (Professor, Division of Cardiovascular Medicine, Lankenau Institute of Medical Research) is involved directly with project management and bench research for this proposal. Dr. Ashton has a broad published background in mechanisms of cell death, angiogenesis, immunology, and G protein coupled receptor signaling. He has experience with echocardiography and in vivo imaging of tumors and will assess the cardiac and tumor response to Doxorubicin in the presence of the new cardio protectants. His efforts will remain directed to optimizing the timing, dose and form of DOX that works optimally with CCR5i to inhibit tumor growth/metastasis and salvage myocardial health *in vivo* and *in vitro* (Aim 1) and in the performance and interpretation of the *in vivo* breast cancer models in Aim 2. The studies outlined are highly labor intensive and we are keenly aware of the critical importance of Dr. Ashton's involvement in this project. Salary support for his effort is requested for both years of the proposal.

Other Personnel

TBA (Post-Doctoral Fellow 4.68 Calendar Months; 39% effort). A Post-Doctoral Fellow will be hired to measure the impact of CCR5 inhibitors on the impact of alternate reformulations of doxorubicin induced cardiotoxicity. Under the guidance of Dr. Ashton, she/he will participate in the echocardiographic analysis of the mice and animal care in this study, isolation of primary myocytes and assessment of cardiotoxicity *in vitro*. Salary Support for their efforts are requested.

Fringe Benefits: are requested at 30.65% of the salary.

A 3% incremental increase is added to the requested personnel costs in year 2.

SUPPLIES:

\$26,500 is requested to purchase sufficient supplies to adequately conduct this study.

F & A: The Lankenau Institute for Medical Research negotiated Facilities and Administrative rate of 68% on the Modified Total Direct Costs.

RESEARCH & RELATED BUDGET - Cumulative Budget

	Totals (\$)
Section A, Senior/Key Person	52,566.00
Section B, Other Personnel	60,509.00
Total Number Other Personnel	2
Total Salary, Wages and Fringe Benefits (A+B)	113,075.00
Section C, Equipment	0.00
Section D, Travel	0.00
1. Domestic	0.00
2. Foreign	0.00
Section E, Participant/Trainee Support Costs	0.00
1. Tuition/Fees/Health Insurance	0.00
2. Stipends	0.00
3. Travel	0.00
4. Subsistence	0.00
5. Other	0.00
6. Number of Participants/Trainees	0
Section F, Other Direct Costs	53,000.00
1. Materials and Supplies	53,000.00
2. Publication Costs	0.00
3. Consultant Services	0.00
4. ADP/Computer Services	0.00
5. Subawards/Consortium/Contractual Costs	0.00
6. Equipment or Facility Rental/User Fees	0.00
7. Alterations and Renovations	0.00
8. Other 1	0.00
9. Other 2	0.00
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)	166,075.00
Section H, Indirect Costs	112,931.00

Section I, Total Direct and Indirect Costs (G + H)	279,006.00
Section J, Fee	0.00
Section K, Total Costs and Fee (I + J)	279,006.00

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	579,102	670,758	0	0	0	1,249,860

SBIR/STTR Information

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

 DOE HHS USDA Other:

SBC Control ID:*

001657833

Program Type (select only one)*

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

Application Type (select only one)*

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

Phase I Letter of Intent Number:

* Agency Topic/Subtopic:

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

1a. Do you certify that at the time of award your organization will meet the eligibility criteria for a small business as defined in the funding opportunity announcement?* Yes No

1b. Anticipated Number of personnel to be employed at your organization at the time of award.* 2

1c. Is your small business majority owned by venture capital operating companies, hedge funds, or private equity firms?* Yes No1d. Is your small business a Faculty or Student-Owned entity?* Yes No2. Does this application include subcontracts with Federal laboratories or any other Federal Government agencies?* Yes No

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

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

If no, provide an explanation in an attached file. Explanation:*

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

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

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

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

Attach File:*

CP_Final_Lightseed_20240328.pdf

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.*

COMMERCIALIZATION PLAN

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

A.1. Project Overview—Doxorubicin (DOX) is one of the most widely used chemotherapeutic agent for cancer in the world, but it remains a serious cause of morbidity and mortality in children and adults due to dose-dependent cardiomyopathy.¹ LightSeed has identified the anti-retroviral drug maraviroc, which also inhibits cysteine-cysteine chemokine receptor 5 (CCR5, a.k.a. CD195), to elicit a dual function by limiting DOX-induced cardio toxicity while enhancing DOX-induced cancer cell killing.

DOX is widely used to treat lymphomas, myelomas, sarcoma, breast, ovarian, gastric, lung, thyroid, and pediatric cancers.² Anthracyclines are administered for the treatment of childhood cancers (50 to 60%), breast cancer patients (30%), elderly lymphoma patients (up to 70%), and other cancers). DOX toxicity is therefore relevant to a broad number of cancers. With cancer survivors estimated at 19 million in the USA by 2025, Dox-induced cardiotoxicity is considered to be part of the “cardio-oncology epidemic”. DOX-induced cardio toxicity is the leading cause of discontinuation of treatment and can lead to cardiovascular-associated death.³ DOX remains a serious cause of morbidity and mortality in children and adults by inducing a dose-dependent cardiomyopathy, which is often lethal or requires heart transplantation.¹ About 10% of patients treated with DOX experience cardio toxicity within the first year, increasing to 25% over 5 years,⁴ with the risk of congestive heart failure (CHF) increasing with the cumulative dose of DOX. Dexrazoxane is the only cardio protectant currently approved by the FDA, with the specific indication to treat women with metastatic breast cancer who have received a cumulative dose of 300 mg/m² of DOX. Dexrazoxane can cause myelotoxicity⁵ and, less likely, secondary malignancies.⁶ Alternative strategies to minimize DOX-induced cardio toxicity include antioxidants, which were ineffective,^{7,8} and new DOX liposomal reformulations currently under development, including Myocet,⁹ which causes mucositis and hand-foot syndrome.¹⁰ Thus, there is a clear unmet need for the development of novel cardio protectants to reduce or eliminate DOX-induced cardio toxicity.

LightSeed aims to improve the therapeutic potential of anthracyclines by providing both cardio protection and enhanced cancer cell killing. Partially funded by a Phase I award (1R43HL164131-01A1), we have identified several small molecules that 1) prevented DOX-induced toxicity in cardiac cells, and 2) enhanced cancer cell killing by DOX, including the CCR5 inhibitors maraviroc and vicriviroc. CCR5 is overexpressed in pediatric and adult cancers, including approximately 50% of breast cancer patients,¹¹ where it induces cancer stemness, cell survival, and DNA repair. Maraviroc achieved cardiac protection in a mouse model of chronic DOX cardio toxicity, in which DOX treatment was shown to enhance expression of CCR5. We have also identified through high throughput screening using a human cardiomyocyte model of DOX toxicity, an additional 10 compounds that provided cardioprotection, are regarded as safe in humans, and are not currently either published or patented as cardioprotectants. This Phase II renewal will develop the CCR5i maraviroc and/or vicriviroc as cardio protectants and further asses the additional 10 cardio protectants we identified in the Phase 1 study:

A.2. Technical Objectives

The goal of the proposed study is to develop novel cardio protectants with “dual function”, including the CCR5i maraviroc and/or vicriviroc, to prevent DOX-induced cardiotoxicity. This Phase II proposal will complete the following Specific Aims:

Aim 1. Determine the impact of CCR5 inhibitors on DOX-induced cardiotoxicity. We will test the combination of maraviroc and vicriviroc with several DOX reformulations currently under development, including **PEGylated liposomes** (Doxil), **non-pegylated liposomes** (Myocet), **polymeric micelles** (PM) (SP1049C), and **polymeric nanoparticles** (PNP) (LivaTag), in a murine model of DOX-induced cardiotoxicity, and test the optimal sequence of administration. We will then assess the cardio protectant activity of ten additional small molecules with “dual function” identified in our Phase I proposal in combination with the optimal DOX reformulation, which will select the most effective DOX/cardio protectant combination and sequence of administration to advance to Aims 2 and 3. **Milestones:** Determine the IC₅₀ for cardio protection in canine myocytes and human cardiomyocytes; identify the optimal DOX reformulation that provides maximal cardio protection provided by the CCR5i to the alternate DOX reformulation and sequence of administration that provides optimal cardio protection by CCR5i. These studies will identify the best cardio protectant for subsequent combination studies.

Aim 2. Assess CCR5 inhibitors in a syngeneic TNBC model. Based on the sequencing of administration established in aim 1, we will assess the functional synergy between DOX reformulation and cardio protectant in C57BL/6 mice implanted with orthotopic PY8119 tumors. Tumor-bearing mice (n=10/group) will be treated with

the optimal DOX reformulation / cardio protectant combination identified in Aim 1; mice will be treated with vehicle or drugs alone as controls. Tumor volume (caliper) and body weight will be measured 3 times per week; daily clinical assessments will be performed as early signs of toxicity. Overall survival will be recorded for each treatment group. At endpoint, animals will be sacrificed; blood will be collected for analytical chemistry. Tumors and major organs will be collected for histopathological evaluation. Cardiac function will be monitored under anesthesia by electrocardiogram and echocardiography. Milestones: Demonstrate that the combination inhibits tumor growth by ≥60% compared to vehicle, more efficiently than either drug alone, with no cardiotoxicity.

Aim 3. GLP safety and toxicology of the combination of DOX and CCR5i. We will work with Charles River Labs to perform a preliminary safety/toxicity study of reformulated DOX and cardio protectant in Sprague Dawley rats. Milestones: ≤10% body weight loss; no histopathological liabilities; no cardiotoxicity.

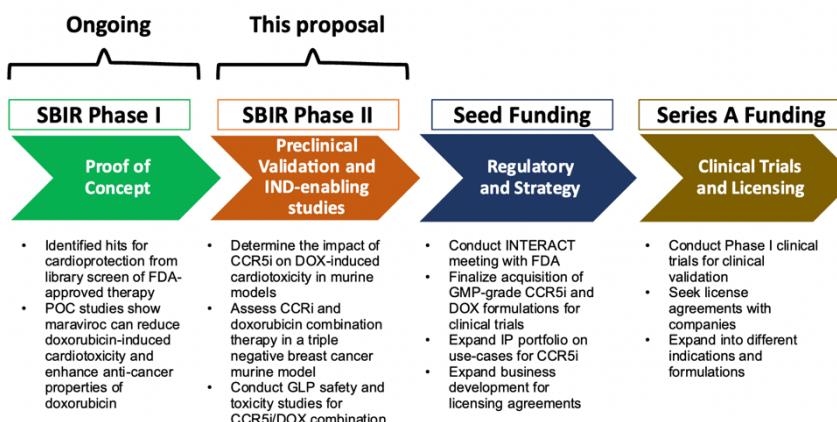
A.3. Gap / Medical Need to be Addressed—Doxorubicin, while extremely potent in fighting cancer, and therefore one of the most widely used chemotherapeutic agents in the world, can cause long-term cardiotoxicity that either 1) prevent physicians from prescribing doxorubicin to at risk patients or 2) reduce a patient's quality of life after surviving cancer. Developing a drug that can reduce cardiotoxicity can therefore both increase cancer survivors and also improve quality of life after cancer. LightSeed is committed to developing therapies that will benefit these stakeholders:

- **Physicians:** The availability of more cardioprotective drugs when prescribing doxorubicin can greatly enhance a physician's ability to treat cancers. To prevent cardiotoxicity, physicians can only limit the cumulative dose, use alternative formulations of DOX, or alter administration schedules of DOX.¹² Furthermore, doctors cannot prescribe doxorubicin to patients with significant comorbidities and cardiovascular risks.¹³ The only drug currently available to prevent cardiotoxicity is dexrazoxane, which is only partially cardioprotective with significant side effects. Adding new cardioprotective drugs to a physician's arsenal will enable administration of higher doses of doxorubicin to patients who were previously at risk and to patients with cardiac risk factors.¹⁴
- **Patients:** While doxorubicin can save a cancer patient, it can also significantly decrease a patient's lifespan after cancer treatment. Up to 34% of patients receiving DOX experience nonreversible cardiotoxicity.^{15,16} Furthermore, the second leading cause of morbidity and mortality in breast cancer survivors is cardiovascular disease.^{15,16} Development of cardioprotective drugs that can decrease the risk of cardiovascular disease will allow patients to have higher quality of life and longer lifespans. In the pediatric population

A.4. Position of this SBIR Project in the Product Development and Regulatory Pathway—This SBIR Phase II project will allow LightSeed to further develop our lead CCR5 inhibitor candidates, maraviroc and vicriviroc, as a repurposed drug for cardioprotection after chemotherapy (patent #US20230035491A1 submitted) (**Figure 1**). This project will be a continuation of our SBIR Phase I Project (1R43HL164131-01A1), where we identified several FDA-approved compounds that can be repurposed to lower cardiotoxicity of doxorubicin (DOX) chemotherapy. From this screen, our lead candidates, maraviroc and vicriviroc, demonstrated the ability to both 1) provide cardiac protection in a mouse model of chronic DOX-induced cardiotoxicity and 2) enhance the anti-cancer effects of DOX. This SBIR Phase II project will further evaluate maraviroc and vicriviroc as a drug to reduce cardiotoxicity with multiple formulations of DOX and in a breast cancer model. This Phase II project will serve as IND-enabling studies for determining GLP safety and toxicity of the combination of DOX and our CCR5 inhibitors maraviroc or vicriviroc. According to our regulatory advisors and FDA's guidance ICH M3(R2), combination toxicity studies on advanced cancers are generally not warranted unless there is a specific cause

for concern under clinically relevant conditions, especially for investigator-initiated INDs.¹⁷ Therefore, LightSeed predicts that additional preclinical data will not be necessary, though this will be confirmed when LightSeed pursue an INTERACT meeting with the FDA. Completion of these studies will allow LightSeed to pursue Phase I clinical trials for studying CCR5 inhibitors for reducing DOX-induced cardiotoxicity.

Figure 1: Timeline of this SBIR project in the commercial pathway.



B. Company

B.1. Corporate Structure and Mission—LightSeed is a biotechnology company whose mission is to improve the quality of life of cancer patients by developing approaches to reduce serious side effects from chemotherapy and radiation. Founded in 2006 as a precision gene therapy company developing methods for light-activated gene therapy to reduce complications, LightSeed more recently identified CCR5 as a target receptor that is activated in patients who required heart transplants for heart failure after chemotherapy for cancer. LightSeed is therefore developing CCR5 inhibitors (CCR5i) that can confer cardioprotective properties after chemotherapy treatment. Our lead candidate, maraviroc, is a small molecule that has shown cardiac protection in preclinical models of chronic cardiovascular toxicity after doxorubicin treatment while also enhancing the anti-cancer properties of doxorubicin. Maraviroc is currently approved for treating HIV infection (Selzentry).¹⁸ Our other lead candidate, vicriviroc, has been deemed safe but failed Phase III clinical trials for HIV.¹⁹ LightSeed seeks breast cancer as the first target indication for preventing chemotherapy-induced cardiomyopathies. Additional cancer indications will be pursued upon successful demonstration of CCR5i for reducing doxorubicin-induced cytotoxicity.

B.2. Management Team — LightSeed is led by a management team that has previously developed 3 biotechnology companies with one successful exit as well as experts in oncology and cardiovascular disease. Furthermore, the advisory board is supplemented by scientific and clinical leaders in cardiotoxicity, oncology, pathology, and cell death.

Founder & CEO – Dr. Richard Pestell, MD, PhD, MBA: Dr. Pestell is a world-renown clinician scientist specializing in oncology with over 700 peer-reviewed publications and more than 95,000 citations. Previously, Dr. Pestell founded the biotechnology companies EcoGenome, StromaGenesis, and ProstaGene, which was sold in 2018. In addition, Dr. Pestell has been the director of two NCI Cancer Centers: Lombardi Comprehensive Cancer Center at Georgetown University and the Sidney Kimmel Cancer Center at Thomas Jefferson University. Most recently, Dr. Pestell was the Executive Vice President at Thomas Jefferson University, where he helped oversee an annual budget of \$5.6 billion, eleven hospitals, 30,000 employees, and 160 undergraduate and graduate programs. Dr. Pestell received his MD and PhD from the University of Melbourne, his MBA from NYU Stern School of Business, and completed his clinical fellowship at Massachusetts General Hospital. He is an expert in breast and prostate cancer, cancer stem cells, and cell-cycle research in which he is ranked #1 by Google Scholar. He has been working on cardio protectants and cardiac death for several decades,²⁰⁻²⁸ with his first publication studying another chelating agent Desferrioxamine to reverse heart failure.^{28,29}

Chief Science Officer – Dr. Anthony Ashton, PhD: Dr. Ashton is a leading expert in cardiovascular research, including mechanisms of cardiac death and mechanisms governing cardiac progenitor cell function. He is also the Scientific Director in Perinatal Research at the Kolling Institute of Medical Research as well as a Professor at the Lankenau Institute for Medical Research. Dr. Ashton received his PhD from the University of New South Wales. Then, he spent 12 years conducting cardiovascular research at the Albert Einstein College of Medicine. At LightSeed, Dr. Ashton provides expertise in cardiotoxicity and pediatric cardiovascular disease.

B.3. Company Advisors

Dr. Rick Kitsis, MD. – Head of Clinical Advisory Board: Dr. Kitsis is a Professor and the Director of the Wilf Family Cardiovascular Research Institute at Albert Einstein College of Medicine. Dr. Kitsis is a leading expert in mechanisms governing cardiac death. Previously, Dr. Kitsis was the Chief of Cardiology at Albert Einstein College of Medicine, where he also researched cardiovascular medicine. Dr. Kitsis received his MD at the University of California, San Francisco, and completed his cardiology fellowship at Montefiore Medical Center. At LightSeed, Dr. Kitsis will advise on the clinical affairs for developing cardiotoxicity inhibitors.

Dr. Javid Moslehi, MD. –Clinical Advisory Board: Dr. Moslehi is a cardiologist specializing in cardiovascular health of cancer patients. Currently, Dr. Moslehi is the chief of the University of California, San Francisco's cardio-oncology and immunology section and Professor in Cardiology. His research focuses on the cardiovascular system in cancer patients and survivors, as well as inflammatory heart conditions like myocarditis. Dr. Moslehi completed his MD at the University of Connecticut School of Medicine and at Brigham and Women's Hospital he completed fellowship in cardiology and postdoctoral research fellowship in oncology. Dr. Moslehi will advise LightSeed on clinical affairs for developing cardiotoxicity drugs specifically for cancer patients.

Dr. Chris Albanese, PhD. – Scientific Advisory Board: Dr. Albanese is a Professor in the Departments of Oncology and Pathology at Georgetown University, as well as a member of the Molecular Oncology program. He is also the Executive Director of the Center for Translational Imaging and the Director of the Urogenital Program in the Center for Cellular Reprogramming at Georgetown Medical Center. He received his PhD in Biology at Cardiff University. An expert in the molecular and genetic causes of cancer and developing preclinical models of diseases, Dr. Albanese will advise LightSeed on preclinical studies and disease models.

Dr. Martin Schnermann, PhD. – Scientific Advisory Board: Dr Schnermann is a Senior Investigator at the National Cancer Institute's Center for Cancer Research. He is an expert in using organic chemistry for cancer drug discovery as well as developing optical approaches for drug delivery. Dr. Schenermann received his PhD in Chemistry at the Scripps Institute and a postdoctoral fellowship at the University of California, Irvine. Dr. Schnermann provides expertise in medicinal chemistry of small molecules and drug delivery at LightSeed.

Dr. Stanley Fricke, PhD. – Scientific Advisory Board: Dr. Fricke is a Professor in Radiology and Director of Medical Physics Program at Georgetown University. He is also the CEO of HyperMC2, a consulting firm for medical physics equipment. Dr. Fricke received his PhD in Physics from the Università degli Studi di Torino. He is also a Physicist at Children's National Health System. Dr. Fricke brings expertise in medical imaging to LightSeed.

Goodwin Proctor – Legal and Intellectual Property Counsel: Goodwin Proctor is a top legal firm with experience with full-service intellectual property practice and legal counsel in the life sciences. They will assist LightSeed with IP protection and consult in all legal matters.

Eisner Advisory Group – Accounting Counsel: Eisner Advisory Group is a leading accounting firm that LightSeed is working with for accounting and financial matters.

Hull and Associates – Regulatory Consultants: Hull and Associates brings decades of experience with regulatory affairs and compliance in the pharmaceutical industry.

B.4. History of Funding—To date, LightSeed has received approximately \$10 million in nondilutive funding from the NIH, the Kimmel Cancer Center, and the Pennsylvania Department of Health to develop its pipeline for developing therapies to reduce side effects in cancer patients after chemotherapy and radiation, including both cardiotoxicity inhibitors and inducible gene therapy drugs. Furthermore, LightSeed received a SBIR Phase I grant (1R43HL164131-01A1) to screen and conduct proof-of-concept studies for repurposing FDA-approved drugs to reduce chemotherapy-induced cardiotoxicity, where we identified maraviroc as our lead candidate.

B.5. Plan to Develop from a Small Technology R&D Business to a Successful Commercial Entity—LightSeed is developing a portfolio of therapeutic assets for reducing side effects of cancer patients. This includes both our efforts in repurposing FDA-approved drugs for lowering cardiotoxicity after chemotherapy and inducible gene therapy modalities. Our lead products, CCR5i maraviroc and virciviroc, have shown the ability to lower cardiotoxicity in DOX models as well as enhance DOX-mediated cancer cell death. We are now pursuing derisking studies to further validate maraviroc and virciviroc. We will be requesting an INTERACT meeting with the FDA to understand the requirements for IND-enabling studies to move towards clinical trials. Our first target indication for our CCR5i's is for doxorubicin treatment in breast cancer patients, but positive clinical trial results will allow us to expand into other cancer indications that are treated by doxorubicin and other chemotherapies that increase cardiotoxicity. Success of maraviroc and/or virciviroc in Phase I clinical trials will allow LightSeed to license our therapy to pursue licensing opportunities. Furthermore, we have discovered additional compounds with potential for reducing cardiotoxicity after chemotherapy and will continue to research those compounds. These efforts will build out a multi-dimensional portfolio of assets that will make LightSeed attractive to pharmaceutical companies for acquisition. Our current predicted timeline for commercialization of our CCR5i is shown in **Figure 2**.

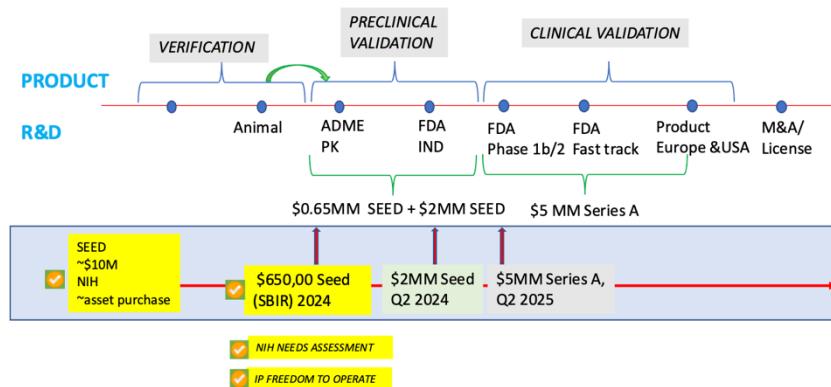


Figure 2: Timeline of CCR5i commercialization strategy.

Currently, we have a research team at Baruch S. Blumberg Institute overseeing our R&D efforts, with expertise in high-throughput screening, compound validation, synthetic chemistry, structure determination, and *in vivo* animal models. LightSeed also has access to facilities at the Lankenau Institute for Medical Research and the

Wistar Institute. Upon successful completion of derisking studies for maraviroc and virciviroc, LightSeed will seek to expand its management team, including recruitment of a Chief Operating Officer and Chief Financial Officer. Expansion will also allow LightSeed to expand financing efforts in raising capital for conducting IND-enabling

studies and clinical trials.

C. Market, Customer, and Competition

C.1. Market Size—The overall cardiovascular drug market was worth \$81 billion globally in 2022, growing at a CAGR of 1.3% and expected to be worth \$92 billion by 2032.³⁰ One of the market trends shaping the cardiovascular drug market is the focus on cardio-oncology. Cardio-oncology will grow faster than other cardiovascular drug focus within the next 10 years, despite capturing a small percentage of the overall cardiovascular market today. As many cancer treatments have cardiotoxicity-induced side effects, the demand for drugs to reduce cardiotoxicity is growing.

A key driver of the cardio-oncology market is the growth of the doxorubicin market. The global doxorubicin market was valued at \$1.1 billion dollars in 2022 with a 5.8% CAGR.³¹ Doxorubicin is prescribed for a variety of cancers, including breast cancer, ovarian cancer, leukemia, and more (Figure 3).³² The drivers of high growth include an aging population, rise in incidence of certain cancers, and increase in approvals for doxorubicin generics. Furthermore, development of various formulations of doxorubicin to enhance delivery and reduce toxicity, such as liposomes, micelles, or polymers, will further drive growth.³³ An increase in doxorubicin treatments, however, also increases the number of patients suffering from severe side effects. The incidence of doxorubicin-induced acute cardiotoxicity is approximately 11%, while doxorubicin cardiomyopathy incidence can be up to 36% when the dose exceeds 600 mg/m².³⁴ This risk increases even further when doxorubicin is administered as a combination therapy or used with radiation. **Cancer patients therefore need a treatment option to reduce cardiotoxicity after doxorubicin treatment.**

Breast cancer dominates the market for doxorubicin treatment and is therefore LightSeed's first target indication for maraviroc. The global doxorubicin market for breast cancer in 2022 was approximately \$374 million with a CAGR of 5.6%.³¹ More than 300,000 new cases of breast cancer were diagnosed in the US in 2023, and 1 out of 8 women in the US will be diagnosed with breast cancer in her lifetime.³⁵ Improvements in therapies for breast cancer, including doxorubicin (DOX) and its various formulations, have resulted in greater than 90% survival rate after 5 years. DOX can be given as an adjuvant or neoadjuvant to breast cancer surgery, as single agent treatments for metastatic and invasive breast cancers, or as part of a combination therapy.¹³ However, doxorubicin can induce nonreversible cardiotoxicity in patients, with reports of cardiotoxicity after chemotherapy as high as 34%.^{15,16} Breast cancer survivors have an increased risk of cardiovascular diseases, which is the second leading cause of morbidity and mortality.^{15,16} These chemotherapy drugs, while effective in treating breast cancer, will still ultimately lead to shorter lifespans for patients. Therefore, the need for a cardioprotective drug to prevent cardiovascular disease after doxorubicin treatment is necessary for breast cancer patients.

Customer

Physicians like oncologists are in need of more cardioprotective drugs to prescribe to patients who are undergoing doxorubicin and other chemotherapy treatments. Currently, cardioprotective strategies available to physicians prescribing doxorubicin include limiting the cumulative dose, altering administration or continuous infusion, using alternative formulations, or administering currently approved cardioprotective drugs (See Competition section for more information).¹² Beyond just reducing the risk of cardiovascular disease in survivors, physicians can limit prescribing doxorubicin to patients with significant comorbidities and cardiovascular risks.¹³ Cardioprotective drugs could permit physicians to administer higher doses doxorubicin to patients who were previously at risk, prescribe doxorubicin to patients with cardiac risk factors, and also decrease the risks of future cardiovascular morbidities.¹⁴ Therefore, discovering more cardioprotective drugs will allow physicians greater options to treat breast cancer patients at risk of cardiotoxicity.

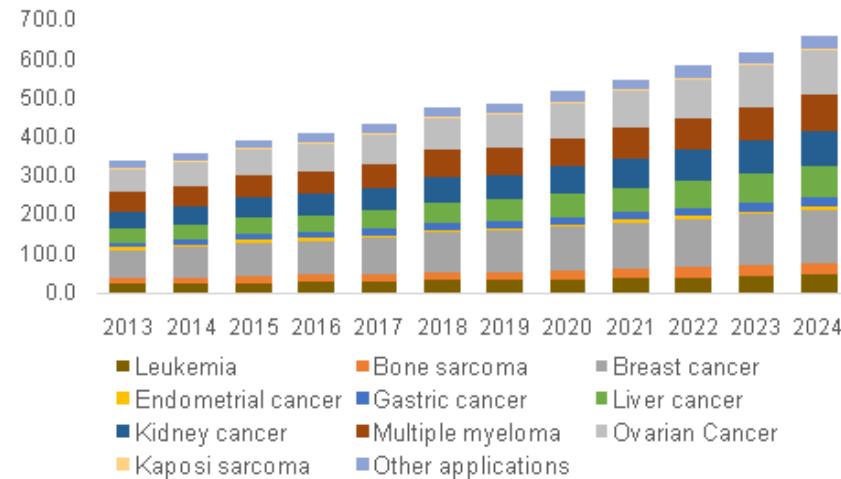


Figure 3: North American doxorubicin market by cancer indication in USD millions. Reproduced from Grand View Research

C.2. Market Entry Challenges—This SBIR project is designed to address the primary barrier to market entry: demonstrating that CCR5i maraviroc and/or vicriviroc can reduce chemotherapy-induced cardiotoxicity in cancer patients. To derisk maraviroc and vicriviroc, we will pursue preclinical testing with murine models of doxorubicin-induced cardiotoxicity as well as cancer models for combination therapy of doxorubicin and maraviroc/ vicriviroc with comparison made to an incumbent technology Dexrazoxane. Furthermore, we will perform GLP safety and toxicity studies of doxorubicin and maraviroc/vicriviroc combination therapy for IND-enabling studies. Success of this project will allow LightSeed to schedule an INTERACT meeting with the FDA to progress towards clinical trials for maraviroc and/or vicriviroc in reducing doxorubicin-induced cardiotoxicity in breast cancer patients. Success of Phase I clinical trials will allow LightSeed to start seeking licensing agreements.

Future market entry challenges center around adoption and marketing of maraviroc as a treatment to reduce cardiotoxicity after chemotherapy treatment. Upon demonstrating clinical success of maraviroc and/or vicriviroc, we will push to include these CCR5i in the National Comprehensive Cancer Network guidelines for treating breast cancer.³⁶ In addition, we will pursue presenting our results at conferences such as the American Association for Cancer Research, American Society for Clinical Oncology, Global Cardio Oncology Summit, and more. We will interview key opinion leaders with our regulatory consultants Hull and Associates to best understand the needs of clinicians.

C.3. Competition—The only FDA-approved drug for preventing doxorubicin-induced cardiotoxicity currently is Dexrazoxane. Dexrazoxane can decrease the risk of doxorubicin-induced cardiotoxicity, allow patients to receive higher doses of DOX, and be used in conjunction with

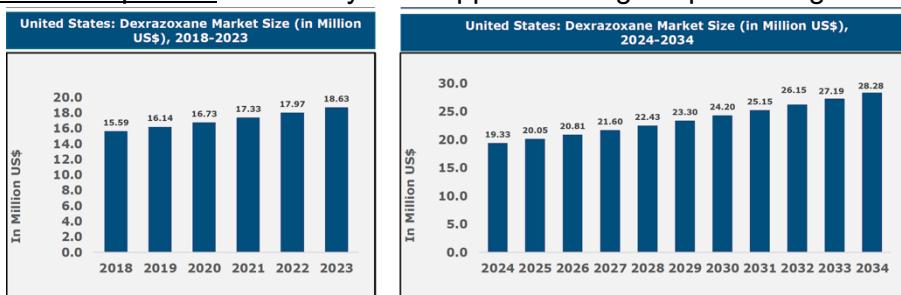


Figure 4. The USA Dexrazoxane market size current (left hand panel) and forecast (right hand panel).

doxorubicin in patients with cardiac risk factors.¹⁴ Despite these advantageous, dexrazoxane induces myelotoxicity, which has similar side effects to doxorubicin, making it difficult to distinguish which drug is inducing side effects.³⁷ Dexrazoxane has been shown to be incompletely cardioprotective; it does not fully suppress doxorubicin cardiotoxicity because it only acts on some of the cardiotoxic mechanisms.³⁸ Dexrazoxane has a narrow approval for metastatic breast cancer patients receiving 300 mg/m² of doxorubicin.³⁹ Narrow approval has led to a small market size for dexrazoxane, shown in Figure 4. Uridine triacetate (Vistogard) has been approved to treat cardiotoxicity after fluoropyrimidine chemotherapy and is being explored for doxorubicin-induced cardiotoxicity.

Other drugs tested for doxorubicin-induced cardiac dysfunction include beta-blockers, angiotensin inhibitors and receptor blockers, mineralocorticoid receptor antagonists, sodium-glucose transport protein 2 inhibitors, aldosterone antagonists, and statins, but have yet to yield much clinical success (Table 1).^{38,40} In addition, several companies and research groups are looking at developing newer drugs with potentially new mechanisms of action to combat chemotherapy-induced cardiotoxicity. Montelukast decreases doxorubicin-induced cardiotoxicity by inhibiting reactive oxygen species activation of NF- κ B,⁴¹ while sulforaphane decreases ROS through Nrf2 activation.⁴²

NovoMedix is developing dual mTOR inhibitor/AMPK agonists including NM043, NM922 and NM1155, which showed anti-apoptotic effects in cardiac myocyte cell culture and attenuated fibrosis in an aortic constriction model.^{43,44} Auransa has discovered a drug through their AI platform that can potentially mitigate doxorubicin-induced cardiotoxicity.⁴⁵ LightSeed is developing CCR5 inhibitors to protect against doxorubicin-induced cardiotoxicity, which is an unexplored mechanism of

Table 1. Potential Competing Technologies

Potential Competitor ⁴³	Mechanism of Action ⁴³	Shortcomings of the drug ⁴³
Dexrazoxane ⁴³	Iron chelation ⁴³	Incomplete cardio protection. Induces myelotoxicity, making it difficult to distinguish whether side effects are from doxorubicin or dexrazoxane. ⁴³
Beta blockers ⁴³	Treatment of heart failure (no effect on Dox cardio toxicity) ⁴³	Mixed results in clinical trials, no significant cardio protection ⁴³
Angiotensin inhibitors and receptor blockers ⁴³	Treatment of heart failure (no effect on Dox cardio toxicity) ⁴³	No improvement in left ventricular contraction ⁴³
Adiponectin agonist peptide (ADP355) ⁴³	Reduced ROS, enhanced antioxidant levels ⁴³	Modest effect in animal models ²⁵
Statins (Atorvastatin, NCT02943590) ⁴³	Activation of AMPK ⁴³	Benefits and risks still unclear ⁴³
Montelukast (NCT05959889) and Sulforaphane (NCT03934905) ⁴³	Leukotriene receptor antagonist (montelukast) or Nrf2 activation (sulforaphane) ⁴³	Not yet determined ⁴³
Empagliflozin (NCT06103279) ⁴³	Unknown (Sodium-glucose co-transporter-2 inhibitor) ²⁶	Not yet determined, Clinical trials to begin end of 2023 ⁴³
Trimetazidine ⁴³ (EudraCT2016-002270-12) ⁴³	Metabolic inhibition of fatty acid oxidation ⁴³	Not yet determined ⁴³
NM043, NM922 & NM1155 (NovoMedix) ⁴³	Activation of AMPK and mTOR inhibitor ⁴³	Benefits and risks still unclear ⁴³
AU-018 (Auransa) ⁴³	Unknown ⁴³	Unknown ⁴³

action compared to traditional drugs for reducing DOX-induced cardiotoxicity.

Another method to reduce cardiotoxicity of doxorubicin has been to develop **alternative formulations** and derivatives. For example, liposomal doxorubicin, both PEGylated and non-PEGylated, decreases cardiotoxicity and other side effects over conventional DOX.³³ The current USA market size

of \$470 mm with expected 2034 market of \$890 MM. Additional efforts to develop doxorubicin formulations, including polymer nanoparticles, micelles, hydrogels, and more, are currently being investigated in clinical trials and are displayed in **Table 2**.³³ The market is currently unknown. Other alternative formulations include creating safer derivatives and analogs of doxorubicin or prodrug versions of doxorubicin.⁴⁶ These alternative formulations and derivatives, however, reduce but do not fully eliminate cardiotoxicity. **LightSeed expects that maraviroc and/or vicriviroc can be used in combination with these therapies to synergistically reduce cardiotoxicity as the reformulation mechanism of action is primarily through reducing the amount of Doxorubicin that reaches the heart.** The DOX reformulation agents we will study herein, were selected based on their approval status (FDA or EMA, European Medicines Evaluation Agency) or stage of development (clinical phase II/III trial). LightSeed plans to pursue marketing and licensing agreements with these alternative formulation companies upon successful clinical trials.

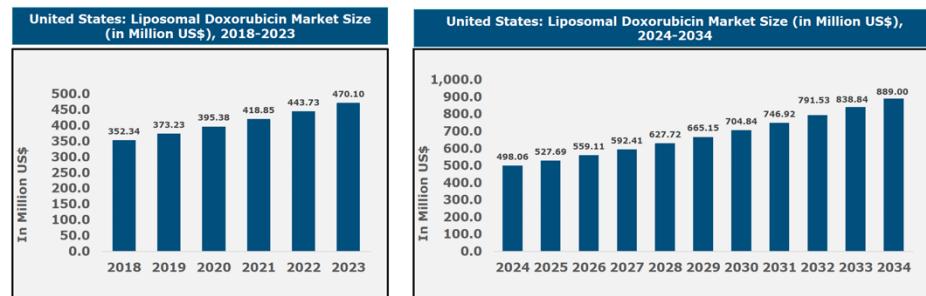


Figure 5. The USA liposomal Doxorubicin market size current (left hand panel) and forecast (right hand panel).

Table 2. Doxorubicin Reformulation technologies

Potential Partner	Structure /Source	Approval status
Doxil	PEGylated liposomal DOX, Baxter	FDA 1995
Caelyx	PEGylated liposomal DOX, MSK-Janssen in USA	Marketing approval 2005
JNS002	PEGylated liposomal DOX/Janssen	Phase III clinical trial (no longer manufactured)
Myocet	non-PEGylated liposome (Zeneus, Takeda)	EMA approval 2000, lower incidence of hand foot syndrome
SP1049C	polymeric micelle/ Supratek Pharma. Inc., Canada	ODD 2008
NK911	polymeric micelle/Nippon Kayaku Pharmaceutical Co	Well tolerated (Phase II (no longer manufactured)
LivaTag	polymeric nanoparticle /Valerio Therapeutics	On FDA fast track for liver cancer (not approved did not meet efficacy in liver cancer study).

D. Intellectual Property (IP) Protection

D.1. Established IP—LightSeed is building a multi-dimensional IP strategy through patents, trade secrets, internal expertise, and relationships with key leaders to protect and expand its market position (**Table 3**). Currently, LightSeed has a pending patent around the use case of drugs to reduce cardiotoxicity with cancer therapies. A patent for CCR5 inhibitors in the treatment of cancer and cancer metastasis belongs solely to Dr. Pestell. LightSeed is currently expanding our IP protection strategy and plans to submit additional protections for current and future applications of this technology. LightSeed also has current proprietary unique therapies that are currently trade secrets. We will explore patent protection in the future upon further evaluation of these trade secrets. Furthermore, LightSeed conducted a freedom to operate (FTO) search in June 2023 through Global Prior Art Inc, indicating the lack of competitor technology or patents, and will continue to conduct FTO evaluations in the future. Seed funding will be used to expand our patent protection efforts including the 10 new compounds we have identified.

Table 3. IP Relevant to This Project

Patent #	Title	Inventor	Application # Filing Date	Patent # Issue Date	Status
US20230035491A1	Methods, kits and compositions for reducing cardiotoxicity associated with cancer therapies	Richard G. Pestell Anthony Wayne Ashton	2020-12-15	N/A	Pending
US 9,453,836	Use of modulators of CCR5 in the treatment of cancer and cancer metastasis	Richard G. Pestell	2013-05-14	2016-09-27	Active

E. Finance Plan

E.1. Financing—As previously stated, LightSeed has raised approximately \$10 million in funding to support our proof-of-concept efforts. Currently, we are raising \$2 million in Seed funding, as well as this SBIR Phase II project, to complete preclinical data, start IND-enabling studies, as well as expand our patent protection efforts. Completion of these efforts will allow us to pursue a \$5 million Series A round that will fund our Phase I clinical trials. Completion of Phase I clinical trials will allow LightSeed to pursue licensing agreements with large pharmaceutical companies to advance maraviroc.

E.2. Exit Strategy—LightSeed plans to become a self-sustaining biotechnology company through license of the technology to two types of companies (1). Reformulation companies by showing enhanced efficacy. Companies that have invested heavily in alternative formulations of doxorubicin to reduce cardiotoxicity, (a predicted market of \$600 million by 2025.) would benefit from a competitive edge. If the combination of these doxorubicin formulations with maraviroc and/or vicriviroc pretreatment reduces cardiotoxicity further, any licensee will maintain a further competitive edge in the market. These companies include Sun Pharmaceuticals (Lipodox), Pfizer, Cadila Pharmaceuticals, Ranbaxy Laboratories, SRS Pharmaceuticals, Myocet, NantKwest, and Bristol-Myer Squibb. Our use-case patent includes different formulations of doxorubicin. These companies would require a license from LightSeed to market the combination therapy. (2). Potential licensees also include companies in the supportive care industry, such as Helsinn Group and Ipsen, pharmaceutical companies focused on developing medicines to reduce the burden of patients.

F. Production and Marketing Plan

F.1. Production—LightSeed plans to purchase the drugs directly from the manufacturers who already manufacture these drugs for patients. Maraviroc is currently produced by Pfizer (ViiV Healthcare) and we will work with them to acquire clinical-grade maraviroc for clinical trials. Alternatively, we will look at other companies making generic versions of maraviroc, including Camber Pharmaceuticals, Hetero Labs, and i3 Pharmaceuticals, to acquire clinical grade maraviroc. Purchasing maraviroc from these companies, who already produce maraviroc for patient-use, will likely be the most straightforward method for production. GMP-grade DOX can be acquired from manufacturers like BroadPharm, MedicaPharma, Biosynth, and more. Vicriviroc is produced by Merck and we will work with them to acquire clinical-grade vicriviroc for clinical trials. For DOX reformulations, we will reach out to the direct manufacturers to purchase the drug outright. In the event that these companies do not allow us to outright buy their DOX reformulations, we will seek out a use-license to acquire reformulated DOX. Alternatively, we could pursue working with a CMO/CDMO to produce maraviroc, vicriviroc, DOX, and DOX reformulations, but we predict this will be more difficult and costly. Therefore, our first strategy will be to acquire the drugs directly from the manufacturers.

F.2. Marketing—Our main marketing strategies include: (1). incorporating maraviroc into the NCCN guidelines for treating breast cancer. This will be the best way to market maraviroc, by establishing it as the standard of care. (2). presenting our findings at large key clinical oncology and cardiology meetings. (3) publishing our findings in peer reviewed journals to influence key opinion leaders. Because our goal is to license out maraviroc and vicriviroc to companies either in the oncology support industry and the doxorubicin formulations industry, LightSeed's marketing strategy is to quickly identify and partner with potential licensees. To do this, LightSeed will pursue presenting our results at conferences such as the American Association for Cancer Research, American Society for Clinical Oncology, Global Cardio Oncology Summit, and more, where we can constantly update our preclinical and clinical results. Furthermore, LightSeed will also work with key opinion leaders for conducting clinical trials with maraviroc and providing testimony to other clinicians and corporations. This will be the best method for marketing maraviroc as a viable strategy for treating doxorubicin-induced cardiotoxicity. Once licensing agreements are formed, LightSeed will look to leverage the established customer base of companies licensing maraviroc and vicriviroc. This customer base will primarily be clinicians prescribing doxorubicin to their patients.

G. Revenue Stream

G.1. General Revenue Estimates and Strategy—LightSeed's revenue stream is expected to be exclusively from licensing agreements with partner companies. Our strategy is to license our technology after Phase I clinical trials. LightSeed will seek an upfront fee, additional milestones payout for successful clinical trials, and a percentage of royalties based on sales of the licensed product in our term sheets. Furthermore, LightSeed will seek non-exclusive licensing agreements, which will allow us to license our technology to multiple companies and multiple doxorubicin formulations. Upon successful completion of a CCR5 inhibitor to reduce DOX-induced

cardiotoxicity in breast cancer patients, we will then pursue indications in other cancers, such as ovarian cancer and leukemia. We will also pursue using CCR5 inhibitors to counteract other anthracycline-induced cardiotoxicities, further expanding our potential revenue. Furthermore, we will also pursue pediatric indications of CCR5i for DOX-induced cardiotoxicity.

G.2. Revenue Projections—Revenue projections are presented in **Table 4** for using our CCR5i to treat doxorubicin-induced cardiotoxicity in new US breast cancer patients. This is based on assumptions that there are 300,000 new breast cancer patients in the US per year with no current growth,³⁵ consistent growth in market penetration, and that the drug cost per patient is \$500. This price estimate is based on the current price for maraviroc today. Revenue predictions are similar to historical sales of dexamoxane in the US to treat doxorubicin-induced cardiotoxicity in metastatic breast cancer patients (See Error! Reference source not found.). We expect our CCR5i to outpace dexamoxane after Year 5, assuming superior benefits of our CCR5i than dexamoxane. Because our estimates only encompass new breast cancer patients, we believe this to be a fairly conservative revenue projection. Upon additional cancer indications, we expect revenue to be able to grow immensely.

Table 4. US Projections for Revenue in Breast Cancer

	Year 1	Year 2	Year 3	Year 4	Year 5
New US Breast cancer patients per year	300,000	300,000	300,000	300,000	300,000
% Market Penetration	5%	7.5%	10%	12.5%	15%
Price per patient	\$500	\$500	\$500	\$500	\$500
Revenue	\$7,500,000	\$11,250,000	\$15,000,000	\$18,750,000	\$22,500,000

COMMERCIALIZATION PLAN REFERENCES

1. McGowan JV, Chung R, Maulik A, Piotrowska I, Walker JM, Yellon DM. Anthracycline Chemotherapy and Cardiotoxicity. *Cardiovasc Drugs Ther.* 2017 Feb;31(1):63–75. PMCID: PMC5346598
2. Lipshultz SE, Lipsitz SR, Sallan SE, Dalton VM, Mone SM, Gelber RD, Colan SD. Chronic progressive cardiac dysfunction years after doxorubicin therapy for childhood acute lymphoblastic leukemia. *J Clin Oncol.* 2005 Apr 20;23(12):2629–2636. PMID: 15837978
3. Cardinale D, Colombo A, Bacchiani G, Tedeschi I, Meroni CA, Veglia F, Civelli M, Lamantia G, Colombo N, Curieliano G, Fiorentini C, Cipolla CM. Early detection of anthracycline cardiotoxicity and improvement with heart failure therapy. *Circulation.* 2015 Jun 2;131(22):1981–1988. PMID: 25948538
4. Cardinale D, Iacopo F, Cipolla CM. Cardiotoxicity of Anthracyclines. *Front Cardiovasc Med.* 2020;7:26. PMCID: PMC7093379
5. Shaikh F, Dupuis LL, Alexander S, Gupta A, Mertens L, Nathan PC. Cardioprotection and Second Malignant Neoplasms Associated With Dexrazoxane in Children Receiving Anthracycline Chemotherapy: A Systematic Review and Meta-Analysis. *J Natl Cancer Inst.* 2016 Apr;108(4):djv357. PMID: 26598513
6. Chow EJ, Aplenc R, Vrooman LM, Doody DR, Huang YSV, Aggarwal S, Armenian SH, Baker KS, Bhatia S, Constine LS, Freyer DR, Kopp LM, Leisenring WM, Asselin BL, Schwartz CL, Lipshultz SE. Late health outcomes after dexrazoxane treatment: A report from the Children's Oncology Group. *Cancer.* 2022 Feb 15;128(4):788–796. PMCID: PMC8792306
7. Parkin DM, Fernández LMG. Use of statistics to assess the global burden of breast cancer. *Breast J.* 2006;12 Suppl 1:S70-80. PMID: 16430400
8. Jiao X, Nawab O, Patel T, Kossenkov AV, Halama N, Jaeger D, Pestell RG. Recent Advances Targeting CCR5 for Cancer and Its Role in Immuno-Oncology. *Cancer Res.* 2019 Oct 1;79(19):4801–4807. PMCID: PMC6810651
9. Batist G, Barton J, Chaikin P, Swenson C, Welles L. Myocet (liposome-encapsulated doxorubicin citrate): a new approach in breast cancer therapy. *Expert Opin Pharmacother.* 2002 Dec;3(12):1739–1751. PMID: 12472371
10. Early Breast Cancer Trialists' Collaborative Group (EBCTCG). Effects of chemotherapy and hormonal therapy for early breast cancer on recurrence and 15-year survival: an overview of the randomised trials. *Lancet.* 2005 May 14;365(9472):1687–1717. PMID: 15894097
11. Velasco-Velázquez M, Jiao X, De La Fuente M, Pestell TG, Ertel A, Lisanti MP, Pestell RG. CCR5 antagonist blocks metastasis of basal breast cancer cells. *Cancer Res.* 2012 Aug 1;72(15):3839–3850. PMID: 22637726
12. Avila MS, Siqueira SRR, Ferreira SMA, Bocchi EA. Prevention and Treatment of Chemotherapy-Induced Cardiotoxicity. *Methodist Debakey Cardiovasc J.* 2019;15(4):267–273. PMCID: PMC6977564
13. Mouabbi JA, Kiluk JV. Breast Cancer Treatment Protocols: Treatment of Noninvasive Breast Cancer, Neoadjuvant and/or Adjuvant Therapy for ER-Positive Early-Stage Breast Cancer, Neoadjuvant and/or Adjuvant Therapy for Triple-Negative Early-Stage Breast Cancer. *Medscape* [Internet]. 2023 Dec 4 [cited 2023 Dec 6]; Available from: <https://emedicine.medscape.com/article/2006464-overview>
14. Breast Cancer Treatment (PDQ®) - NCI [Internet]. 2023 [cited 2023 Dec 10]. Available from: <https://www.cancer.gov/types/breast/hp/breast-treatment-pdq>
15. Florescu DR, Nistor DE. Therapy-induced cardiotoxicity in breast cancer patients: a well-known yet unresolved problem. *Discoveries (Craiova).* 7(1):e89. PMCID: PMC7093073
16. Kaboré EG, Macdonald C, Kaboré A, Didier R, Arveux P, Meda N, Boutron-Ruault MC, Guenancia C. Risk Prediction Models for Cardiotoxicity of Chemotherapy Among Patients With Breast Cancer: A Systematic Review. *JAMA Network Open.* 2023 Feb 23;6(2):e230569.
17. Research C for DE and. M3(R2) Nonclinical Safety Studies for the Conduct of Human Clinical Trials and Marketing Authorization for Pharmaceuticals [Internet]. FDA; 2020 [cited 2023 Dec 28]. Available from: <https://www.fda.gov/regulatory-information/search-fda-guidance-documents/m3r2-nonclinical-safety-studies-conduct-human-clinical-trials-and-marketing-authorization>
18. Pfizer's SelzentryTM (Maraviroc) Tablets, Novel Treatment for HIV, Approved by FDA | Pfizer [Internet]. [cited 2023 Dec 28]. Available from: https://www.pfizer.com/news/press-release/press-release-detail/pfizer_s_selzentrytm_maraviroc_tablets_novel_treatment_for_hivApproved_by_fda

19. Caseiro MM, Nelson M, Diaz RS, Gathe J, de Andrade Neto JL, Slim J, Solano A, Netto EM, Mak C, Shen J, Greaves W, Dunkle LM, Vilchez RA, Zeinecker J. Vicriviroc plus optimized background therapy for treatment-experienced subjects with CCR5 HIV-1 infection: final results of two randomized phase III trials. *J Infect*. 2012 Oct;65(4):326–335. PMID: 22634184
20. Pestell RG, Taylor RR. Effect of cigarette smoking on the frequency of ventricular premature complexes in normal subjects. *Clin Exp Pharmacol Physiol*. 1989 Aug;16(8):647–650. PMID: 2477179
21. Bouzahzah B, Yurchenko V, Nagajyothi F, Hulit J, Sadofsky M, Braunstein VL, Mukherjee S, Weiss H, Machado FS, Pestell RG, Lisanti MP, Tanowitz HB, Albanese C. Regulation of host cell cyclin D1 by Trypanosoma cruzi in myoblasts. *Cell Cycle*. 2008 Feb 15;7(4):500–503. PMID: 18239452
22. Huang H, Petkova SB, Cohen AW, Bouzahzah B, Chan J, Zhou J nian, Factor SM, Weiss LM, Krishnamachary M, Mukherjee S, Wittner M, Kitsis RN, Pestell RG, Lisanti MP, Albanese C, Tanowitz HB. Activation of transcription factors AP-1 and NF-kappa B in murine Chagasic myocarditis. *Infect Immun*. 2003 May;71(5):2859–2867. PMCID: PMC153290
23. Huang H, Yanagisawa M, Kisanuki YY, Jelicks LA, Chandra M, Factor SM, Wittner M, Weiss LM, Pestell RG, Shtutin V, Shirani J, Tanowitz HB. Role of cardiac myocyte-derived endothelin-1 in chagasic cardiomyopathy: molecular genetic evidence. *Clin Sci (Lond)*. 2002 Aug;103 Suppl 48:263S-266S. PMID: 12193100
24. Huang H, Petkova SB, Pestell RG, Bouzahzah B, Chan J, Magazine H, Weiss LM, Christ GJ, Lisanti MP, Douglas SA, Shtutin V, Halonen SK, Wittner M, Tanowitz HB. Trypanosoma cruzi infection (Chagas' disease) of mice causes activation of the mitogen-activated protein kinase cascade and expression of endothelin-1 in the myocardium. *J Cardiovasc Pharmacol*. 2000 Nov;36(5 Suppl 1):S148-150. PMID: 11078362
25. Petkova SB, Huang H, Factor SM, Pestell RG, Bouzahzah B, Jelicks LA, Weiss LM, Douglas SA, Wittner M, Tanowitz HB. The role of endothelin in the pathogenesis of Chagas' disease. *Int J Parasitol*. 2001 May 1;31(5–6):499–511. PMID: 11334935
26. Petkova SB, Tanowitz HB, Magazine HI, Factor SM, Chan J, Pestell RG, Bouzahzah B, Douglas SA, Shtutin V, Morris SA, Tsang E, Weiss LM, Christ GJ, Wittner M, Huang H. Myocardial expression of endothelin-1 in murine Trypanosoma cruzi infection. *Cardiovasc Pathol*. 2000;9(5):257–265. PMID: 11064272
27. Ashton AW, Watanabe G, Albanese C, Harrington EO, Ware JA, Pestell RG. Protein kinase C δ inhibition of S-phase transition in capillary endothelial cells involves the cyclin-dependent kinase inhibitor p27(Kip1). *J Biol Chem*. 1999 Jul 23;274(30):20805–20811. PMID: 10409620
28. Hassan GS, Mukherjee S, Nagajyothi F, Weiss LM, Petkova SB, de Almeida CJ, Huang H, Desruisseaux MS, Bouzahzah B, Pestell RG, Albanese C, Christ GJ, Lisanti MP, Tanowitz HB. Trypanosoma cruzi infection induces proliferation of vascular smooth muscle cells. *Infect Immun*. 2006 Jan;74(1):152–159. PMCID: PMC1346667
29. Pestell RG, Barr AL, Brand G. Vitamin C and congestive cardiac failure. *Med J Aust*. 1987 Aug 3;147(3):153–154. PMID: 3600482
30. Cardiovascular Drugs Market Development Strategy, And Growth Opportunities 2023 To 2032 [Internet]. [cited 2024 Feb 9]. Available from: <https://www.linkedin.com/pulse/cardiovascular-drugs-market-development-strategy-growth-alena-sharma-njif/>
31. Global Industry Analysts, Inc. Doxorubicin - Global Strategic Business Report - Research and Markets [Internet]. Research and Markets. 2023 [cited 2023 Dec 6]. Available from: <https://www.researchandmarkets.com/reports/5302837/doxorubicin-global-strategic-business-report>
32. Doxorubicin Market Size & Share | Global Industry Report, 2018 - 2024 [Internet]. Grand View Research. 2020 [cited 2023 Dec 6]. Available from: <https://www.grandviewresearch.com/industry-analysis/doxorubicin-market>
33. Zhao N, Woodle MC, Mixson AJ. Advances in delivery systems for doxorubicin. *J Nanomed Nanotechnol*. 2018;9(5):519. PMCID: PMC6319900
34. Chatterjee K, Zhang J, Honbo N, Karliner JS. Doxorubicin Cardiomyopathy. *Cardiology*. 2010 Jan;115(2):155–162. PMCID: PMC2848530
35. Breast Cancer Facts & Stats | Incidence, Age, Survival, & More [Internet]. National Breast Cancer Foundation. [cited 2023 Dec 6]. Available from: <https://www.nationalbreastcancer.org/breast-cancer-facts>

36. Gradishar WJ, Moran MS, Abraham J, Aft R, Agnese D, Allison KH, Anderson B, Burstein HJ, Chew H, Dang C, Elias AD, Giordano SH, Goetz MP, Goldstein LJ, Hurvitz SA, Isakoff SJ, Jankowitz RC, Javid SH, Krishnamurthy J, Leitch M, Lyons J, Mortimer J, Patel SA, Pierce LJ, Rosenberger LH, Rugo HS, Sitapati A, Smith KL, Smith ML, Soliman H, Stringer-Reasor EM, Telli ML, Ward JH, Wisinski KB, Young JS, Burns J, Kumar R. Breast Cancer, Version 3.2022, NCCN Clinical Practice Guidelines in Oncology. *Journal of the National Comprehensive Cancer Network*. National Comprehensive Cancer Network; 2022 Jun 1;20(6):691–722.
37. Eneh C, Lekkala MR. Dexrazoxane. StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2023 [cited 2023 Dec 10]. Available from: <http://www.ncbi.nlm.nih.gov/books/NBK560559/> PMID: 32809394
38. Bansal N, Adams MJ, Ganatra S, Colan SD, Aggarwal S, Steiner R, Amdani S, Lipshultz ER, Lipshultz SE. Strategies to prevent anthracycline-induced cardiotoxicity in cancer survivors. *Cardiooncology*. 2019 Dec 2;5:18. PMCID: PMC7048046
39. Witteles RM, Bosch X. Myocardial Protection During Cardiotoxic Chemotherapy. *Circulation*. American Heart Association; 2015 Nov 10;132(19):1835–1845.
40. Vuong JT, Stein-Merlob AF, Cheng RK, Yang EH. Novel Therapeutics for Anthracycline Induced Cardiotoxicity. *Frontiers in Cardiovascular Medicine* [Internet]. 2022 [cited 2023 Dec 28];9. Available from: <https://www.frontiersin.org/articles/10.3389/fcvm.2022.863314>
41. Hafez HM, Hassanein H. Montelukast ameliorates doxorubicin-induced cardiotoxicity via modulation of p-glycoprotein and inhibition of ROS-mediated TNF- α /NF- κ B pathways. *Drug Chem Toxicol*. 2022 Mar;45(2):548–559. PMID: 32106718
42. Pogorzelska A, Mazur M, Świdławska M, Wietrzyk J, Sigorski D, Fronczyk K, Wiktorska K. Anticancer effect and safety of doxorubicin and nutraceutical sulforaphane liposomal formulation in triple-negative breast cancer (TNBC) animal model. *Biomedicine & Pharmacotherapy*. 2023 May 1;161:114490.
43. Das A, Hovsepian S, Das S, Samidurai A, Mauro AG, Cain C, Kraskauskas D, Corral L, Fung L, Sullivan R, Chan KW, Swindlehurst CA, Salloum FN. Abstract 17055: Novel Dual mTOR Inhibitor/AMPK Activator Mitigates Doxorubicin Cardiotoxicity and Potentiates Its Chemotherapeutic Efficacy Against Triple Negative Breast Cancer. *Circulation*. American Heart Association; 2020 Nov 17;142(Suppl_3):A17055–A17055.
44. Bradley JM, Spaletta P, Li Z, Sharp TE, Goodchild TT, Corral LG, Fung L, Chan KWH, Sullivan RW, Swindlehurst CA, Lefer DJ. A novel fibroblast activation inhibitor attenuates left ventricular remodeling and preserves cardiac function in heart failure. *Am J Physiol Heart Circ Physiol*. 2018 Sep 1;315(3):H563–H570. PMCID: PMC6172635
45. Auransa. Auransa Enters into Exclusive Licensing Agreement with China Oncology Focus Limited, an Affiliate of Lee's Pharmaceutical Holdings, for Rights to AU018 in Greater China and Southeast Asia [Internet]. GlobeNewswire News Room. 2018 [cited 2023 Dec 28]. Available from: <https://www.globenewswire.com/news-release/2018/12/19/1669377/0/en/Auransa-Enters-into-Exclusive-Licensing-Agreement-with-China-Oncology-Focus-Limited-an-Affiliate-of-Lee-s-Pharmaceutical-Holdings-for-Rights-to-AU018-in-Greater-China-and-Southeast.html>
46. Dempke WCM, Zielinski R, Winkler C, Silberman S, Reuther S, Priebe W. Anthracycline-induced cardiotoxicity — are we about to clear this hurdle? *European Journal of Cancer*. Elsevier; 2023 May 1;185:94–104. PMID: 36966697

PHS 398 Cover Page Supplement

1. Vertebrate Animals Section

Are vertebrate animals euthanized? Yes No

If "Yes" to euthanasia

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

Yes No

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

.....

2. *Program Income Section

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

Yes No

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

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

3. Human Embryonic Stem Cells Section

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

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

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

Cell Line(s) (Example: 0004):

4. Human Fetal Tissue Section

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

If "yes" then provide the HFT Compliance Assurance

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

5. Inventions and Patents Section (Renewal applications)

*Inventions and Patents: Yes No

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

*Previously Reported: Yes No

6. Change of Investigator/Change of Institution Section

Change of Project Director/Principal Investigator

Name of former Project Director/Principal Investigator

Prefix:

*First Name:

Middle Name:

*Last Name:

Suffix:

Change of Grantee Institution

*Name of former institution:

PHS 398 Research Plan

Introduction	
1. Introduction to Application (for Resubmission and Revision applications)	
Research Plan Section	
2. Specific Aims	SpecificAims_LightSeed_20240329.pdf
3. Research Strategy*	Research_LightSeed_20240329.pdf
4. Progress Report Publication List	PRPL_No_publications.pdf
Other Research Plan Section	
5. Vertebrate Animals	VAS_LightSeed_20240328.pdf
6. Select Agent Research	
7. Multiple PD/PI Leadership Plan	
8. Consortium/Contractual Arrangements	LOI_SOW_LightSeed_20240328.pdf
9. Letters of Support	LOS_LightSeed_20240328.pdf
10. Resource Sharing Plan(s)	Resource_Sharing_LightSeed_20240328.pdf
11. Other Plan(s)	DMSP_LightSeed_20240328.pdf
12. Authentication of Key Biological and/or Chemical Resources	Authentication_Plan_LightSeed_20240328.pdf
Appendix	
13. Appendix	

SPECIFIC AIMS

Doxorubicin (DOX) is one of the most widely used chemotherapeutic agent for cancer in the world, but it remains a serious cause of morbidity and mortality in children and adults due to dose-dependent cardiomyopathy.¹ LightSeed has identified the anti-retroviral drug maraviroc, which also inhibits cysteine-cysteine chemokine receptor 5 (CCR5, a.k.a. CD195), to elicit a dual function by limiting DOX-induced cardio toxicity while enhancing DOX-induced cancer cell killing. DOX is an anthracycline widely used to treat lymphomas, myelomas, sarcoma, breast, ovarian, gastric, lung, thyroid, and pediatric cancers.² About 10% of patients treated with DOX experience cardio toxicity within the first year, increasing to 25% over 5 years,³ with the risk of congestive heart failure (CHF) increasing with the cumulative dose of DOX. Dexrazoxane is the only cardio protectant currently approved by the FDA, with the specific indication to treat women with metastatic breast cancer who have received a cumulative dose of 300 mg/m² of DOX. Dexrazoxane can cause myelotoxicity⁴ and, less likely, secondary malignancies.⁵ Alternative strategies to minimize DOX-induced cardio toxicity include antioxidants, which were ineffective,^{6,7} and new DOX liposomal formulations currently under development, including Myocet,⁸ which causes mucositis and hand-foot syndrome.⁹ Thus, there is a clear unmet need for the development of novel cardio protectants that diminish or eliminate DOX-induced cardio toxicity.

LightSeed aims to improve the therapeutic potential of anthracyclines by providing both cardio protection and enhanced cancer cell killing. Partially funded by a Phase I award (1R43HL164131-01A1), we have identified several small molecules that 1) protected from DOX-induced toxicity in cardiac cells, and 2) enhanced cancer cell killing by DOX, including the CCR5 inhibitors maraviroc and viceriviroc. CCR5 is overexpressed in pediatric and adult cancers, including approximately 50% of breast cancer patients,¹⁰ where it induces cancer stemness, cell survival, and DNA repair. Maraviroc 1) achieved cardiac protection in a mouse model of chronic DOX cardio toxicity, in which DOX treatment was shown to enhance expression of CCR5; and 2) prevented lung metastasis in a xenograft model (MDA-MB-231) and a syngeneic model (Py8119) of triple negative breast cancer (TNBC). This Phase II renewal will identify the optimal combination of small molecule cardio protectant and DOX formulation with enhanced anti-TNBC activity *in vivo* and limited or no cardiotoxicity:

Aim 1. Identify the optimal combination of cardio protectant and DOX formulation that protects from DOX-induced cardiotoxicity. We will test the combination of the most advanced cardio protectants maraviroc and viceriviroc with several DOX formulations, including **PEGylated liposomes** (Doxil), **non-pegylated liposomes** (Myocet), **polymeric micelles** (PM) (SP1049C), and **polymeric nanoparticles** (PNP) (LivaTag), in a murine model of DOX-induced cardiotoxicity. We will then assess the combination of the optimal DOX formulation and sequence of administration with ten (10) new small molecules identified in our Phase I proposal that protect from DOX-induced cardiac cell killing *in vitro*. **Milestones:** 1) These studies will select the most effective combination and sequence of administration of cardio protectant and DOX formulation.

Aim 2. Assess the anticancer efficacy of the optimal combination of cardio protectant and DOX formulation in a syngeneic TNBC model. Based on the best cardio protectant/DOX formulation and sequence of administration established in aim 1, we will assess the functional synergy in a syngeneic TNBC model of C57BL/6 mice implanted with orthotopic Py8119 tumors. Tumor-bearing mice (n=10/group) will be treated with the optimal DOX formulation/cardio protectant combination. Mice will be treated with vehicle, DOX+Dexrazoxane or drug alone as controls. Tumor volume (caliper) and body weight will be measured 3 times per week; daily clinical assessments will be performed as early signs of toxicity. Overall survival will be recorded for each treatment group. At endpoint, animals will be sacrificed; blood will be collected for analytical chemistry. Tumors and major organs will be collected for histopathological evaluation. Cardiac function will be monitored under anesthesia by electrocardiogram and echocardiography. **Milestones:** Demonstrate that the combination inhibits tumor growth by ≥60% compared to vehicle, more efficiently than either drug alone, with limited or no cardiotoxicity.

Aim 3. Cardiotoxicity study with the combination of DOX and CCR5i in SD rats. We will work with Charles River Labs to perform an 8-week DOX cardiotoxicity study with 14-days treatment in Sprague Dawley (SD) rats, followed by an 8-week cardiotoxicity study of the combination of maraviroc (or another cardio protectant) with DOX (or the optimal DOX formulation identified in Aim 1). **Milestones:** Maraviroc, or another cardio protectant, will prevent DOX-induced cardiotoxicity by at least 80%.

Successful completion of this Phase II proposal will achieve IND enabling studies demonstrating that maraviroc (or viceriviroc) is an effective cardio protectant that prevents DOX-induced cardio toxicity and establishes the optimal sequencing of administration to advance towards First-in-Human clinical trials.

SIGNIFICANCE

*Doxorubicin (DOX) is an anthracycline that is widely used in cancer treatment, but it remains a serious cause of morbidity and mortality in children and adults due to dose-dependent cardiomyopathy.¹ LightSeed has identified the anti-retroviral drugs maraviroc and vicriviroc, which also inhibit cysteine-cysteine chemokine receptor 5 (CCR5, a.k.a. CD195), that elicit a dual function by limiting DOX-induced cardio toxicity while enhancing DOX-induced cancer cell killing. Ten (10) additional cardio protectants have been identified as part of the Phase I award (1R43HL164131-01A1). This Phase II proposal will 1) test the cardio protectant activity of maraviroc and vicriviroc in combination with several DOX formulations currently under development to identify the optimal combination and sequence of administration that achieve maximum cardio protection and cancer cell killing activity *in vivo*, and 2) perform preliminary safety studies in Sprague Dawley (SD) rats.*

DOX is widely used to treat lymphomas, myelomas, sarcoma, breast, ovarian, gastric, lung, thyroid, and pediatric cancers.² DOX-induced cardio toxicity is the leading cause of discontinuation of treatment and can lead to cardiovascular-associated death.¹¹ DOX remains a serious cause of morbidity and mortality in children and adults by inducing a dose-dependent cardiomyopathy, which is often lethal or requires heart transplantation.¹ About 10% of patients treated with DOX experience cardio toxicity within the first year, increasing to 25% over 5 years in pediatric and elderly populations,³ with the risk of congestive heart failure (CHF) increasing with the cumulative dose of DOX. DOX-induced cardio toxicity is the leading cause of discontinuation of treatment and can lead to cardiovascular-associated death.¹¹ Breast cancer is the leading cause of cancer death in women, second only to cardiovascular disease as a cause of death worldwide.⁶ About 30% of breast cancer patients in the U.S. receive DOX, with a recommended dose of DOX ~240 mg/m²; it is estimated that >50,000 women/year will develop severe cardiotoxicity. With 19 million cancer survivors estimated in the USA by 2025, DOX-induced cardiotoxicity is considered part of the "cardio-oncology epidemic."

The mechanisms mediating DOX-induced cardiotoxicity involve direct (histone eviction)¹² and indirect (inflammation)¹³ cardiac effects. DOX binds to DNA and topoisomerase IIb (Topo IIb), inducing double-stranded DNA breaks¹⁴ and causing structural changes to rRNA, impairing ribosomal function and activating the ribosomal stress response.¹⁵ A series of events, including nucleolar stress with suppression of new pre-ribosomal RNA synthesis, activation of autophagy,^{16,17} inhibition of mammalian Target of Rapamycin (mTOR),¹⁸ disruption of mitochondrial electron transport,¹⁹ activation of proteases,²⁰ induction of cellular senescence,²¹ and damage to cardiac "stem cells". DNA damage, causing double-strand breaks (DSBs) following the poisoning of Topo IIb, and chromatin damage (epigenomic and transcriptional alterations and attenuated DSB repair) induced by histone eviction, are required for cardiotoxicity and therapy-related tumorigenesis.²² As indirect mechanisms, DOX-induced ribotoxic stress and reduction of ribosomal biogenesis, and activation of JNK and p38 MAPK, promotes the release of IL1 β , inflammatory cytokines, chemokines, and growth factors (TNF α , IL-6, CXCL1/Gro α , CCL2/MCP-1, granulocyte colony-stimulating factor (GCSF), and CXCL10/IP-10) from bone marrow-derived macrophages.²³ In addition, DOX enhances the activity of natural killer cells and cytotoxic T-lymphocytes and the differentiation of macrophages. The expression of cardiomyocyte cell surface membrane death receptors is induced by DOX,^{23,24} and activates toll-like receptors (TLR).²⁵

Dexrazoxane is the only cardio protectant currently approved by the FDA, with the limited indication to treat women with metastatic breast cancer who have received a cumulative dose of 300 mg/m² of DOX. Dexrazoxane can cause myelotoxicity⁴ and, less likely, secondary malignancies.⁵ In a large meta-analysis, dexrazoxane was largely ineffective at reducing chronic cardiac toxicity in off-label use in the pediatric population, providing incomplete cardio protection and no impact on overall survival.^{26,27} Alternative strategies to minimize DOX-induced cardio toxicity include antioxidants, which were ineffective,^{6,7} and new DOX liposomal formulations currently under development, including Myocet,⁸ which causes mucositis and hand-foot syndrome.⁹ In preclinical studies, inhibition of PI3K γ ,²⁸ ginsenoside Rg3 micelles,²⁹ and inhibitors of PARP³⁰ reduce DOX-induced cardiotoxicity; however, none of these agents are being clinically used as cardio protectants. Thus, there is a clear unmet need for the development of novel cardio protectants to reduce or eliminate DOX-induced cardio toxicity.

LightSeed aims to improve the therapeutic potential of anthracyclines by providing both cardio protection and enhanced cancer cell killing. Partially funded by a Phase I award (1R43HL164131-01A1), we have identified maraviroc and vicriviroc, two CCR5 inhibitors (CCR5i, **Figure 1**), that 1) provide cardiac protection in a mouse model of chronic DOX cardio toxicity and 2) enhance cancer cell killing by DOX in TNBC cell lines. CCR5 is overexpressed in pediatric and adult cancers, including approximately 50% of breast cancer patients,¹⁰ where it induces cancer stemness, cell survival, and DNA repair. DOX treatment induced CCR5 and its ligands in a murine model of DOX-induced cardiac toxicity and in the hearts of patients undergoing cardiac transplantation.

Both maraviroc and virciviroc have been deemed safe, but virciviroc was never approved due to lack of efficacy; both compounds have a “dual function” and protect from DOX induced cardiomyopathy while enhancing DOX-induced cancer cell killing.³¹

Rigor of the prior work. DOX induced ferroptosis,^{32,33} inhibits ribosomal biogenesis, induces cardiac cell death and inflammation.^{17,34,35} The G-protein coupled receptor CCR5 is present on the same pediatric and adult malignancies treated with DOX; small molecule CCR5i^{10,36} or a humanized monoclonal antibody to CCR5,³⁷ reduce tumor growth and metastasis. Herein CCR5i protect from DOX-induced cardiac toxicity, reverse DOX-induced ferroptosis, ribosomal stress, induce Akt/mTORC1/mTOR/p70S6K (Figure. 2). DOX also induces DNA damage and histone eviction, which is required for cell death²² and herein CCR5i reduce DOX-induced histone eviction. TLR4 participates in cardiac cell hypertrophy and death.³⁸ A plausible, cell type-specific mechanism by which CCR5i provide cardioprotection is via direct effects on the cardiac myocyte, inducing Akt, mTORC1 which reduces ferroptosis (Figure 2).

CCR5i also convey indirect effects on inflammation (reducing the influx of CCR5⁺/CX3CR1⁺ atypical monocytes) and resident cardiac M1 macrophages to enhance the strength of cardiac remodeling. Maraviroc increases M1 vs. M2 macrophage in tumors,³⁹ which is associated with increased tumor immune surveillance and cancer cell killing.⁴⁰⁻⁴² Increased M1 macrophages contribute to DOX-induced cardiac toxicity.⁴³⁻⁴⁵ CCL5 inhibition reduces the recruitment of CCR2⁺ classical monocytes,⁴⁶ which contribute to the pool of resident cardiac macrophages⁴⁷ and are the primary source of cardiac damaging M1 macrophage influx.⁴³⁻⁴⁵ Cell-autonomous mechanisms show that CCR5 ligands impair the contractile function of isolated cardiomyocytes.⁴⁸

DOX stimulates immunogenic cell death of cancer cells, and enhances anti-tumor immune responses (CD11b⁺ myeloid cells, interferons, dendritic like-cells).⁴⁹ We have shown that 1) Two CCR5 small molecule inhibitors (maraviroc, virciviroc) block metastasis of human TNBC xenografts to the lungs in immune-deficient mice;^{10,50} 2) The humanized antibody to CCR5 leronlimab reduced (TNBC) metastasis by >90%, enhanced chemotherapy-induced BCa cell killing, and reduced established TNBC metastasis;³⁷ 3) CCR5i enhanced (up to 300%) cell killing of eight distinct BCa cell lines by DNA damaging agents (DOX) and radiation;³¹ and 4) CCR5⁺ cells within breast tumors have markers of cancer stem cells (CSC), grow as mammospheres, and give rise to new tumors, whereas the CCR5⁻ cells do not.³¹

Strength of the research team. **Xuanmao Jiao, PhD** (Senior Researcher at LightSeed, Associate Professor at Baruch S. Blumberg Institute—BSBI) will be the PI for this application and will oversee the whole project, including experimental design, performing *in vitro* and *in vivo* experiments proposed in Aims 1 and 2, and interpretation of results. He conducted the cell culture experiments, including the library screen and has worked with Dr Pestell for ~15 years. **Richard Pestell, MD, PhD** (founder and President of LightSeed; Distinguished Professor, BSBI), co-Investigator (PI of subaward); this application has been built based on >15 years of work on CCR5 in his laboratory, and he will work closely with the PI in all experimental design and data interpretation. **Zhiping Li, PhD** (Associate Professor, BSBI), Scientist, will closely work with the Subaward PI in experiments

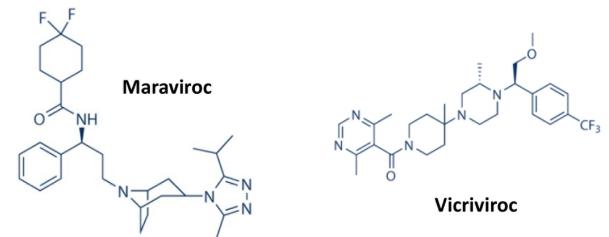


Figure 1. Structures of maraviroc and vicriviroc.

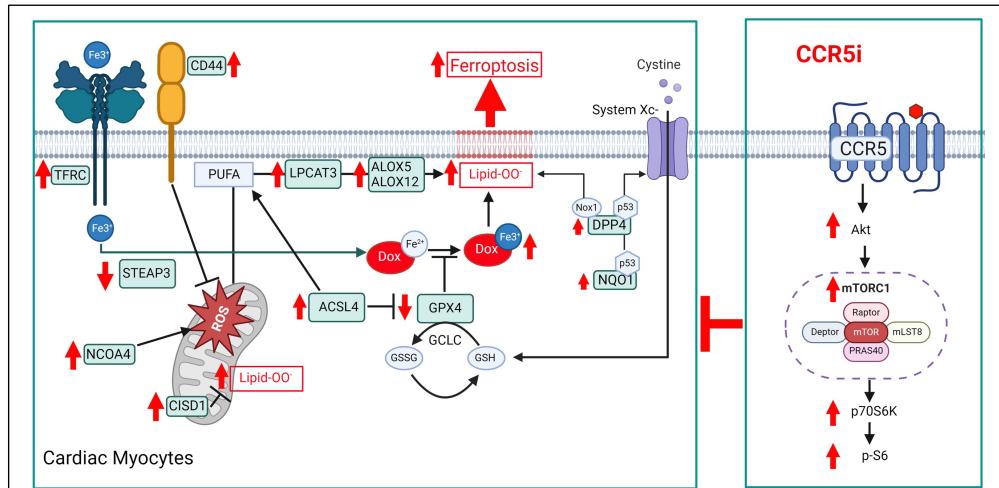


Figure 2. DOX induction of ferroptosis is reversed by CCR5i. Schematic representation of ferroptosis signaling and gene expression in cardiac myocytes. We conducted single cell gene expression analysis of mice treated with a chronic cardiotoxic dose of DOX vs DOX + CCR5i (maraviroc). DOX regulated ferroptosis genes were reversed by Maraviroc (red shows direction of gene expression induced by DOX associated with induction of ferroptosis, reversed by Maraviroc (in green). Right hand panel shows impact of CCR5i (maraviroc) in the cardiac myocytes on mTORC1 signaling, an inhibitor of ferroptosis.

proposed in Aims 1 and 2. **Anthony W. Ashton, PhD** (Associate Professor, Lankenau Institute for Medical Research), Collaborator; who conducted the echocardiography for the preliminary data, has coauthored multiple papers with Dr. Pestell in cardiac cell death over the last 20 years and will perform echocardiography with DOX formulations and cardio protectants in mice and will advise on data interpretation. **Alexander V. Kabanov, PhD** (Director, Center for Nanotechnology in Drug Delivery and Carolina Institute for Nanomedicine, UNC Eshelman School of Pharmacy), Consultant; as an expert on polymeric drug delivery systems for small molecules, co-developer of DOX-poloxamer polymeric micelles (SP1049C), he will provide SP1049C and advise on the use of DOX formulations and data interpretation. **Javid J. Moslehi, MD** (Distinguished Professor in Cardiology, Cardiovascular Research Institute, UCSF School of Medicine), Consultant; he is a cardio oncologist with expertise on chemotherapy-induced cardiotoxicity. **Edward T.H. Yeh, MD** (Professor of Medicine, Chair Department of Internal Medicine, College of Medicine, University of Arkansas), Key Opinion Leader; is an expert on anthracycline-induced cardiotoxicity. The team is also supported by **Randall N. Hyer, MD, PhD** (President, BSBI) and **Dario C Altieri, MD** (President and CEO, Director Caplan Cancer Center).

INNOVATION

In the Phase I application screening of FDA approved compound libraries made the novel finding that CCR5 inhibitors are cardio protectants from DOX induced cardio toxicity. Secondly, we developed the novel finding that these compounds have “dual function” to prevent DOX-induced cardio toxicity and enhance DOX-dependent cancer cell killing. The use of CCR5 inhibitors to abrogate DOX cardio toxicity is novel. As part of the Phase I application, we developed a unique and highly relevant model system with immune knock-in mice to define novel conceptual advances in anti-tumor responses and DOX cardiotoxicity. This heterozygous murine breast cancer model has immune cells with normal function and distinct fluorescent markers knocked-in into relevant loci (*Ccr2*^{RFP}*Cx3cr1*^{GFP}) and can be implanted with isogenic Py8119 breast cancer cells labeled with *Luc2-Aqua-FP-NLS* to define the impact of CCR5i on the immune response in the tumor and the myocardium.

The CCR5 inhibitor maraviroc has been validated as “dual function” compound and shown to prevent lung metastasis in MDA-MB-231 mouse model and in Py8119 syngeneic model of TNBC. Since our original studies,¹⁰ others have shown that CCR5i reduce tumorigenesis in xenografts of pediatric and adult malignancies (acute lymphocytic leukemia, ovarian cancer, multiple myeloma, Kaposi Sarcoma, leukemia, bone sarcoma, endometrial, gastric, liver, prostate, breast, and kidney cancers).^{36,37,51,52}

Our screening has also identified ten additional small molecule cardio protectants, some of which have been approved by the FDA for various indications. The most active cardio protectants, memantine and piceatannol have been shown to inhibit cancer cell growth in numerous preclinical studies.^{53,54} In addition to the molecules identified in our Phase I study, NovoMedix is developing a series of AMPK activators with dual mTORC1 inhibitory activity (NM043, NM1155, and NM922) that attenuate DOX-induced cardiotoxicity while synergizing with DOX in preventing MDA-MB-231 tumor growth.^{55,56} The CCR5 inhibitors maraviroc and vicriviroc, and the new molecules found to have cardio protectant function have unique MOAs that are independent of AMPK activation/mTORC1 inhibition and various of these have the advantage that have been proven safe in humans; many have been FDA approved while others are still under clinical development.

Intellectual Property. LightSeed has a pending patent around the use case of drugs to reduce cardiotoxicity with cancer therapies (US20230035491A1, filed in December 15, 2020). A patent entitled “Use of modulators of CCR5 in the treatment of cancer” (US 9,453,836) was issued in 2016. LightSeed is currently expanding its IP protection strategy, with the ten new compounds, and plans to submit additional protections for current and future applications of this technology.

APPROACH

PHASE I REPORT FOR 1R43HL164131-01A1

The ultimate goal of the Phase I application was to identify and repurpose FDA-approved drugs with a dual function: 1) enhance DOX-induced cancer cell killing and 2) protect from DOX-induced cardiotoxicity. The following aims have been completed.

Aim 1: Use HTS to identify FDA approved and other compounds that reduce DOX cardiotoxicity. We first characterized DOX-induced cardio toxicity using two cardiomyocyte cell lines, human AC16 and rat H9C2. We demonstrated that DOX induced dose-dependent inhibition of cell growth by methylene blue, and treatment with 5 μ M DOX for 24 h induced apoptosis in primary myocytes and in AC16 cells, as demonstrated by flow cytometry. Maraviroc, a small molecule inhibitor of CCR5, prevented DOX-induced apoptosis in AC16 cells and

induced CCR5 expression, and reversed DOX-induced apoptosis in primary myocytes (Figure 3).

Screening of the Bioactive Compound Library Max identified compound C34 (2-acetamidopyranoside isopropyl-2-[acetyl-amino]-2-deoxy α D-glucopyranoside 3,4,6-triacetate) as a candidate cardio protectant in primary canine myocytes and AC16 cells. C34 is a Toll-like receptor (TLR4) inhibitor that reduces systemic inflammation in models of endotoxemia and necrotizing enterocolitis. As TLR2 and TLR4 deficient mice are resistant to DOX-induced cardio toxicity,^{34,57} we assessed the impact of C34 in cardiac myocytes cell lines. Twenty-four hours incubation with DOX induced caspase 3 activity in a dose-dependent manner in AC16 and H9C2 cells. In the presence of 50 μ M dexamzoxane inhibited DOX-induced apoptosis in AC16 cells, but had the opposite effect (enhanced DOX-induced apoptosis) in H9C2 cells (Figure 4). Maraviroc and C34 compound both reduced DOX-induced apoptosis by ~95% in AC16 and H9C2 cells.

Screening of additional libraries has identified ten more small molecules that protected AC16 cells against DOX-induced apoptosis (Table 1). AC16 cells were incubated with 5 μ M DOX for 24 h in the absence or presence of 10 μ M of compounds, when apoptosis was measured by flow cytometry. AC16 cells were maintained in DMEM/F12 (50/50) medium supplemented with 10% FBS, 100 IU/mL penicillin, and 100 μ g/ml streptomycin. 5000 cells/100 ml/well were plated into 96-well plates, allowed to adhere overnight, pre-treated with the 50 μ M of each drug in the library in duplicate for 24 hours, and then treated with 2.5 μ M of doxorubicin to induce cell death. The cell survival was detected with CellTiter-Glo Luminescent Cell Viability Assay (Promega, Madison, WI). The bioluminescence was read by Biotek Synergy2 plate reader. The relative percentage of cell survival was calculate based on following equation: $(BLI_{\text{drug}} - BLI_{\text{DMSO}}) / (BLI_{\text{doxorubicin}} - BLI_{\text{DMSO}}) \times 100$. The most active cardio protectants identified include memantine (44.3% rescue), piceatannol (43.1%), and triamcinolone (40.5%). Memantine is a known N-methyl-D-aspartate (NMDA) receptor antagonist approved for use in several neuropsychiatric disorders⁵⁸ that also slows the progression of moderate-to-severe Alzheimer's disease.⁵⁹ It has been shown to induce apoptosis in LNCaP prostate cancer cells.⁵³ Piceatannol is a natural stilbene that is well documented to inhibit growth of various cancer cell lines in preclinical studies.⁵⁴

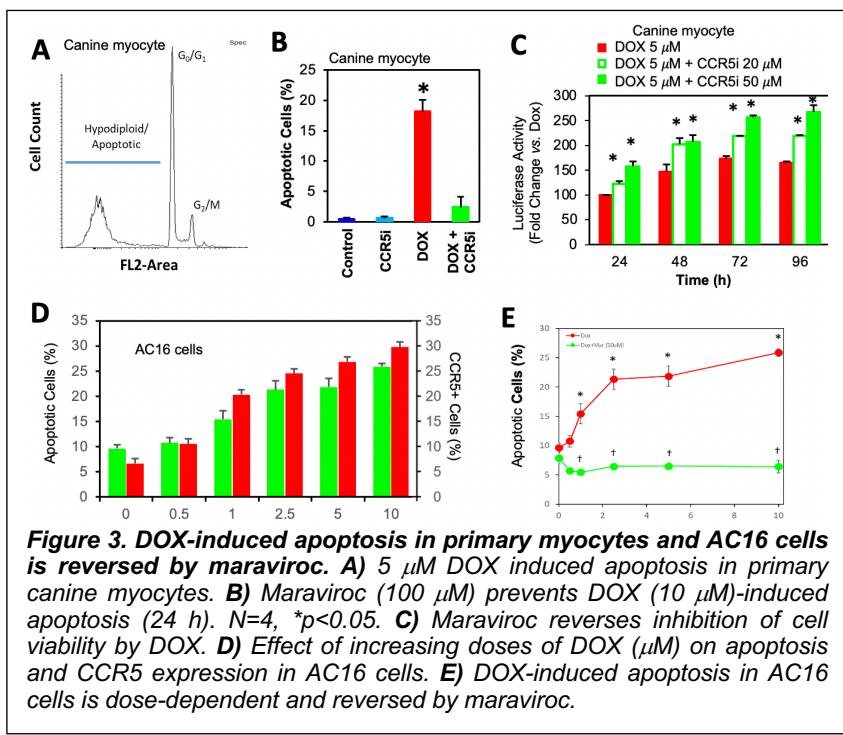


Figure 3. DOX-induced apoptosis in primary myocytes and AC16 cells is reversed by maraviroc. **A)** 5 μ M DOX induced apoptosis in primary canine myocytes. **B)** Maraviroc (100 μ M) prevents DOX (10 μ M)-induced apoptosis (24 h). N=4, *p<0.05. **C)** Maraviroc reverses inhibition of cell viability by DOX. **D)** Effect of increasing doses of DOX (μ M) on apoptosis and CCR5 expression in AC16 cells. **E)** DOX-induced apoptosis in AC16 cells is dose-dependent and reversed by maraviroc.

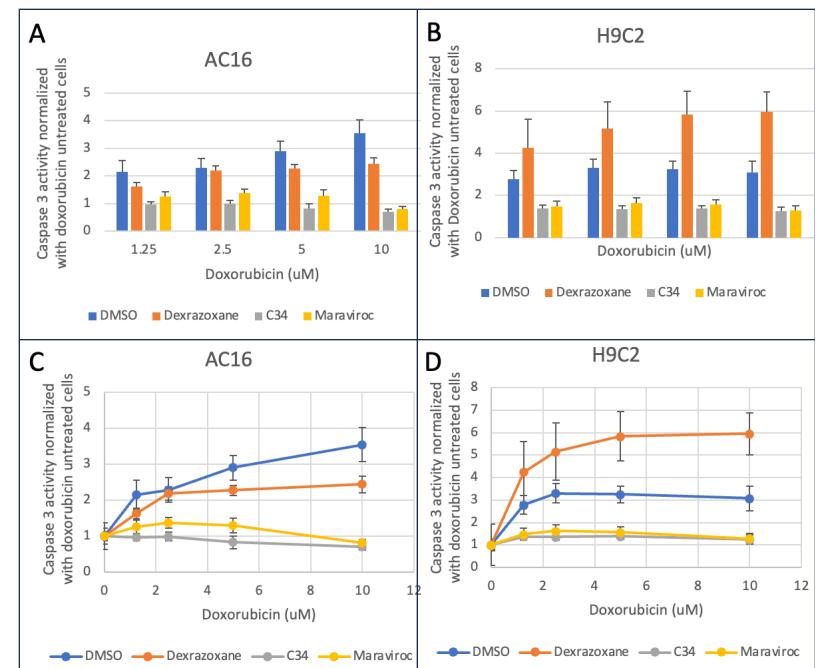


Figure 4. C34 attenuates DOX-induced apoptosis in AC16 and H9C2 cells. AC 16 (A,C) or H9C2 (B,D) cells were placed into 96-well plate at 5000 cells/well one day before the treatment. The cells were pretreated with Dexrazoxane (50 μ M), C34 (25 μ M) and Maraviroc (50 μ M) for 24 hours. Then Doxorubicin was added for another 24 hours and the caspase 3 activity was measured with ENZChek Caspase 3 Assay kit from Invitrogen following the manufacturer's instruction

Table 1. Novel molecules that protect AC16 cells from DOX-induced cell death.

Compound	MOA	Rescue (%)	Indication	FDA approved
Memantine hydrochloride	NMDA receptor antagonist.	44.3 ± 0.7	Moderate to severe dementia in Alzheimer's patients.	YES
Piceatannol	Anti-oxidant.	43.1 ± 2.3	Supplement, natural product	NO
Triamcinolone	Corticosteroid with anti-inflammatory properties.	40.5 ± 10.4	Reduce or suppress inflammation, used to treat skin disease, allergies, and rheumatic disorders.	YES
Nitisinone	Hydroxyphenyl-piruvate dioxygenase inhibitor.	37.8 ± 6.7	Adult and pediatric patients with hereditary tyrosinemia type 1 (HT-1) in combination with dietary restriction of tyrosine and phenylalanine.	YES
L-745,870 hydrochloride	Dopamine receptor antagonist selective for the D4 subtype.	36.7 ± 0.1		NO
4-Amino-1,8-naphthalimide	PARP inhibitor	32.6 ± 2.5		NO
NSC405020	MMP14 Inhibitor.	31.2 ± 2.2		NO
(±)-Octopamine hydrochloride	α-adrenoceptor agonist.	27.8 ± 1.1	Temporary relief of bloating, gas, indigestion, occasional headache, or lethargy due to sensitivity to phenolic compounds in food.	YES
CyPPA	Positive modulator of the small-conductance calcium-activated potassium channels KCa2.2/SK2 and KCa2.3/SK3.	27.7 ± 4.6		NO
Metoclopramide hydrochloride	Dopamine 2 receptor antagonist	26.4 ± 2.6	Gastroesophageal reflux disease, gastroparesis, and severe chemotherapy-induced nausea.	YES

We then applied a strategic analysis to the top 20 compounds excluding compounds that had been disclosed through publication as cardioprotective, shown to promote tumorigenesis or were potentially unsafe based on clinical studies to derive a list of top 10 compounds and their relevant solvent. Nitisinone, (8 mg/kg, oral),⁶⁰ 4-amino-1,8-naphthalimide (3 mg/kg, IP),⁶¹ NSC405020 (0.5 mg/kg, intratumoral),⁶² CyPPA (40 mg/kg, IP),⁶³ Metoclopramide hydrochloride (5-10 mg/kg, IV),⁶⁴ Pitstop 2 (2.8 g/kg in rats, stereotaxic injection),⁶⁵ HA155 (4 mg/kg, IP),⁶⁶ JW74 (150-300 mg/kg, IP),⁶⁷ BIX (2.6 mg/kg, intravitreal),⁶⁸ O-(Carboxymethyl)hydroxylamine hemihydrochloride (5 mg/kg, IP).⁶⁹

Aim 2. Determine functional synergy between novel compounds and DNA damage inducing chemotherapy in cardiac myocytes from iPSCs vs BCa cells. We performed a dose response of C34 compound to define the optimal cardio protectant dose. AC16 cells were incubated with 5 μM DOX for 24 h in the presence of increasing concentrations of C34 compound and cell number was assessed by methylene blue staining and found that C34 completely reversed DOX inhibitory activity at 25 μM (Figure 5).

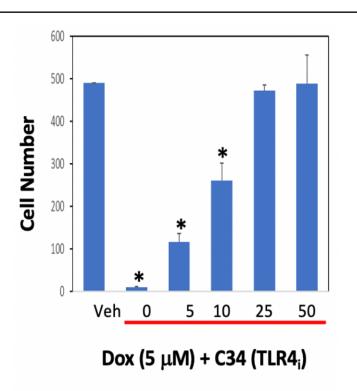


Figure 5. C34 dose response in AC16 cells. AC16 cells were incubated with 5 μM DOX in the absence or the presence of increasing concentrations of C34 compound. Cell number was determined by methylene blue after 24 h of treatment.

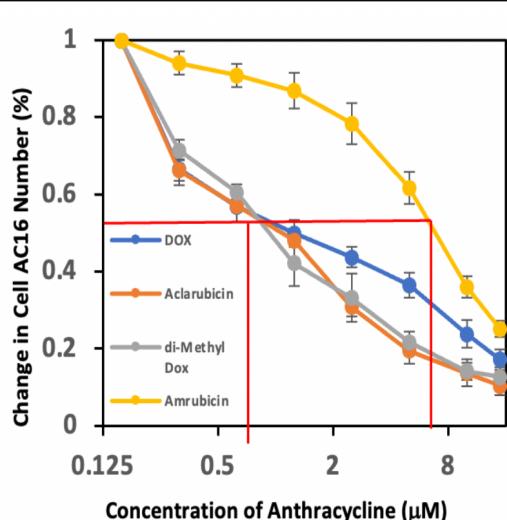
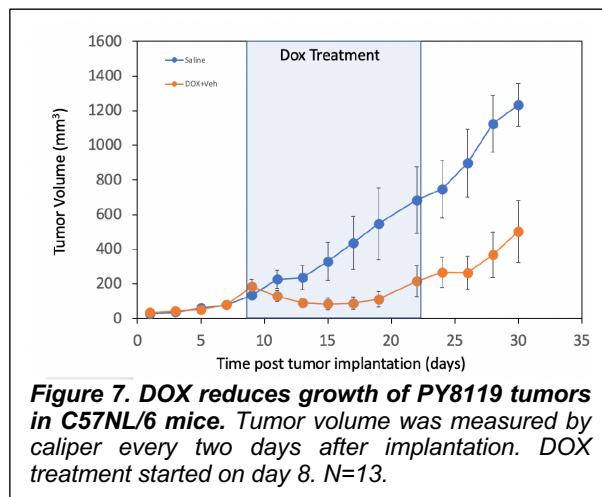


Figure 6. Anthracycline analogs that induce histone eviction induce AC16 cell killing. AC 16 cells were placed into 96-well plate at 5000 cells/well one day before the treatment. Anthracycline was added to treat the cells for 24 hours. Cell numbers was measured by Methylene blue assay.

TLR4 binds damage-associated molecular patterns (DAMPs) and is activated by evicted histones released in response to DNA damage. Histones evicted into the extracellular space can bind TLR4.⁷⁰ Anthracyclines like DOX that induce both DNA damage and histone eviction cause cardiomyocyte cell death. We found that anthracycline analogs that cause histone eviction but not DNA damage, aclarubicin, are effective killers of AC16 cells, whereas amrubicin, which induces DNA damage but not histone eviction was >10-fold less effective in killing AC16 cells (Figure 6). These data indicate a critical role for histone eviction in DOX-induced cardio toxicity.

Aim 3. Determine functional synergy between novel compounds and DNA damage inducing chemotherapy in mice for cardio protection and enhanced breast cancer cell killing. We have established two syngeneic tumor models in which Py230 and Py8119 breast cancer cells were implanted in the third mammary fat pad of 10-12 weeks old C57BL/6J mice dual

fluorescent reporter mice. While Py230 tumors grew slowly, Py8119 tumor growth was ideal for assessing DOX-induced cardiotoxicity. We confirmed the tumor growth inhibitory activity of 8 intraperitoneal injections of 3 mg/kg DOX (Figure 7), validating this model for testing cardio protectant drugs.



DOX-induced cardiomyopathy increases CCR5 protein and CCL5 mRNA expression, and the CCR5i maraviroc reverses fibrosis. The expression of CCR5 in normal myocardium is very low and confined to the microvascular endothelium, endocardium, and resident monocytes (Figure 8A, C). Expression of CCR5 is enhanced in myocytes of patients undergoing heart transplant due to DOX cardio toxicity (Figure 8B) and in mice treated with DOX (Figure 8D). Fibrosis staining demonstrated that DOX-induced fibrosis in mice (2.2 \pm 0.07 fold) was reversed by maraviroc (0.8 \pm 0.06 fold) (Figure 8E-G).

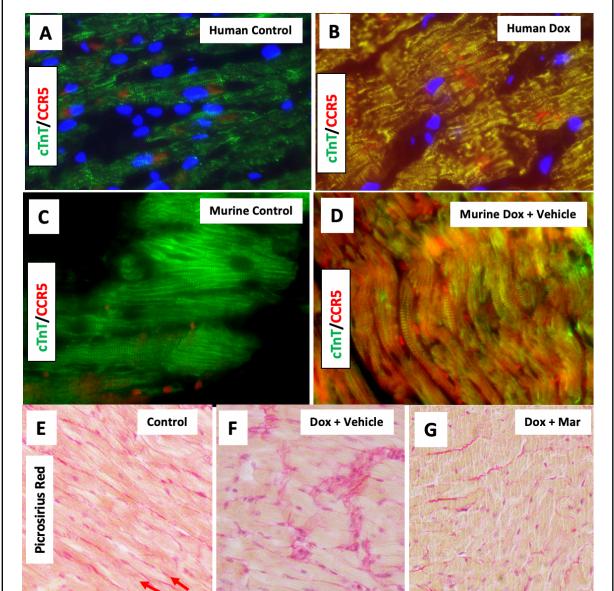
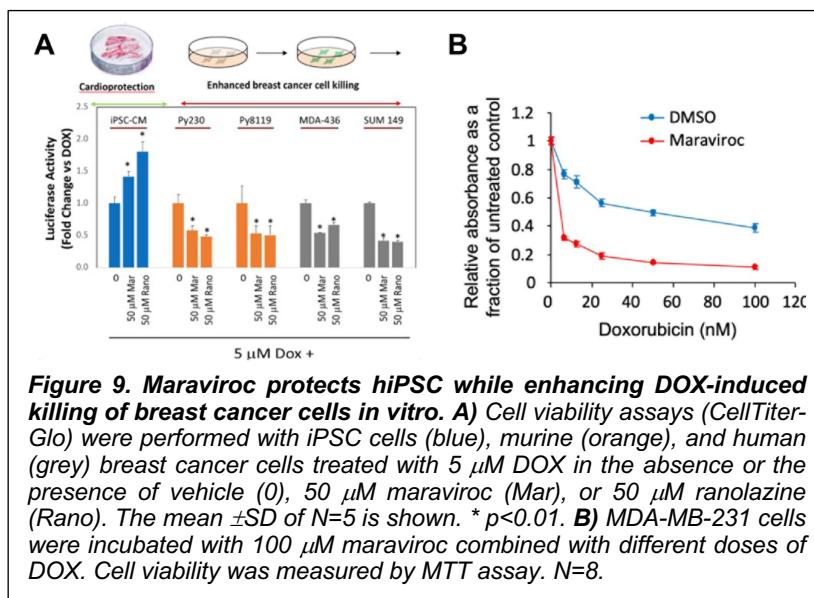


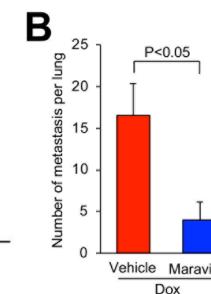
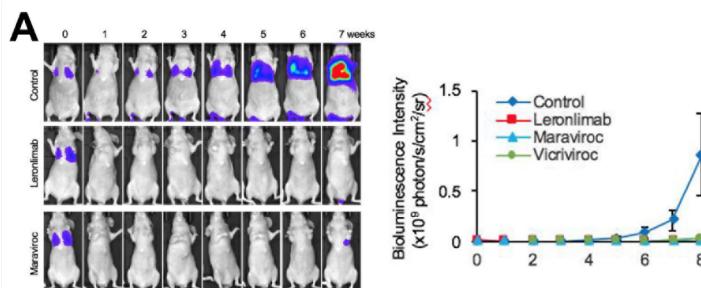
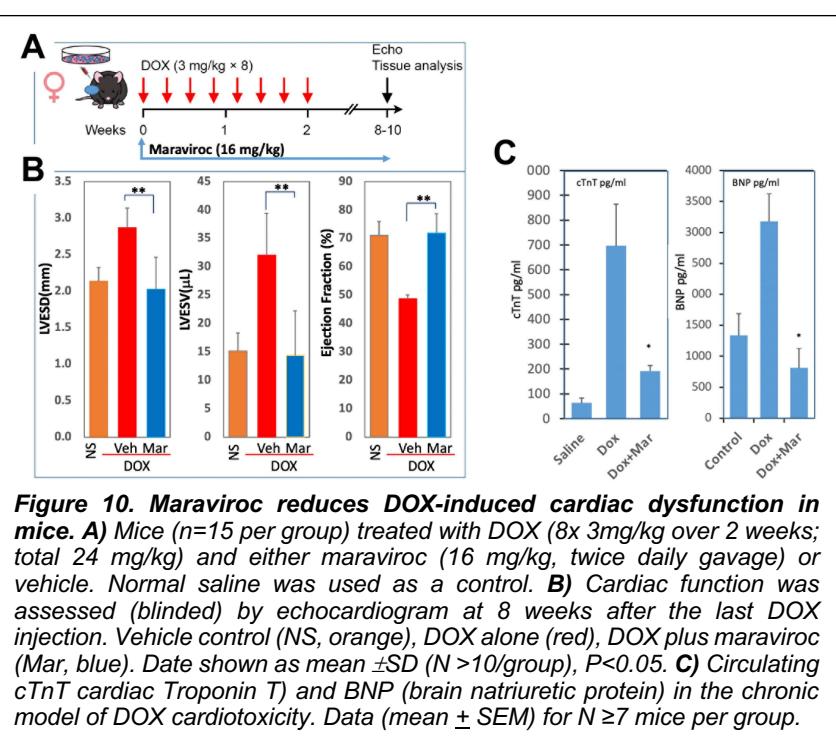
Figure 8. DOX-induced CCR5 myocardial expression. Expression of CCR5 in hearts from healthy patients A) and patients undergoing cardiac transplantation due to DOX-cardiotoxicity B). CTNT, cardiac troponin T (green). C) Murine heart control. D) chronic DOX treatment. E-G) Fibrosis was detected by picro-sirius red stain in hearts of mice treated with vehicle (control), DOX, or DOX + maraviroc (Mar). N=5.



CCR5 inhibitors protect cardiac myocytes from DOX-induced dysfunction and enhance DOX-induced breast cancer cell killing. 100 μ M maraviroc prevented DOX-induced apoptosis in canine cardiac myocytes from 17% to 3% (N=4, p<0.05) (Figure 3B). Likewise, maraviroc and the NaV1.5 channel inhibitor ranolazine dihydrochloride, protected human induced pluripotent stem cells (hiPSC) from DOX-induced killing, while enhancing DOX-induced cell killing of a panel of breast cancer cell lines (Figure 9).

Maraviroc diminishes DOX-induced cardiac dysfunction in mice. C57BL/6 mice were treated with a clinically relevant dose of DOX (8 IP injections of 3 mg/kg DOX over a period of 2 weeks, 24 mg/kg total)^{71,72} alone or in the presence of 16 mg/kg, BID, maraviroc (Figure 10A). After six weeks, histopathology and serial echocardiography were performed to detect characteristic features of DOX-induced chronic cardiotoxicity. Echocardiograms showed that DOX treatment induced a time-dependent reduction in left ventricular ejection fraction (below 50%), reduced fractional shortening, increased ESD (end-systolic dimension), and end-systolic volume (ESV) compared to vehicle control mice (Figure 10B). Treatment in the presence of maraviroc normalized these responses (Figure 10B). Circulating blood levels of cTNT and BNP that were induced by DOX, were also reduced in the presence of maraviroc treatment (Figure 10C).

The CCR5 inhibitors maraviroc and vicriviroc inhibit lung metastasis in MDA-MB-231 and Py8119 mouse models. In the human TNBC model, 8-week-old female athymic nu/nu nude mice were injected into the tail vein with MDA-MB-231 cells expressing Luc2-eGFP (10^6 per mice). The day before tumor cell injection, treatment was started with 2 mg/kg leronlimab (IP twice weekly),⁷³ maraviroc or vicriviroc, (8 mg/kg oral BID).¹⁰ Untreated mice were used as control. Lung metastasis was monitored once weekly for up to 7 weeks by bioluminescence imaging (BLI) following IP injection of 30 mg/ml D-luciferin.³⁷ Our data show that all CCR5 inhibitors completely prevented lung metastasis in the MDA-MB-231 model (Figure 11A). In the mouse syngeneic model, 10-12 weeks old female C57BL/6J mice dual fluorescent reporter mice were implanted with Py8119 breast cancer cells in the third mammary fat pad. Animals were treated with 8 cycles of DOX (3 mg/kg, IP every 2 days, total 24 mg/kg), maraviroc (16 mg/kg, oral daily), or vehicle as control. Tumor metastasis number and size was monitored weekly and at sacrifice. We show that maraviroc significantly inhibited the number of lung metastases (Figure 11B).



In summary, our Phase I proposal has successfully identified two CCR5 inhibitors, maraviroc and vicriviroc, as potent inhibitors of DOX-induced cardiotoxicity protectants that enhance DOX-dependent breast cancer cell killing *in vitro* and prevent lung metastasis in TNBC mouse models. In addition to the CCR5i, we also identified ten (10) other small molecules with significant cardio protectant activity in human cardiomyocytes (AC16 cells). Some of these molecules are known to have anticancer activity,^{53,54} and this Phase II renewal will identify the optimal combination of cardio protectant and DOX formulation, as well as sequence of addition, that achieves maximal cardio protection and anticancer activity in mouse models. To complete this proposal, we will carry out preliminary safety studies in SD rats in preparation to full IND studies.

STUDY DESIGN

Sex as a Biological Variable. Since breast cancer predominantly affects women, all animal studies proposed in this application will use only female mice, except cardiotoxicity studies that will use both male and females.

Safety considerations and Biohazards. All work involving biohazards will be handled according to BSL-2 laboratory practices in compliance with NIH Guidelines and the CDC/NIH Biosafety in Microbiological and Biomedical Laboratories (BMBL) practices. LightSeed, its collaborators, and contracted CROs will follow

established institutional guidelines, which are available for all technical personnel.

Scientific Rigor and Reproducibility. All experiments will employ methodological approaches that have been previously utilized by LightSeed and its collaborators to ensure scientific rigor, reproducibility, and transparency. Data will be reported as mean \pm SE values. Measurement of all endpoints will be completed in a blinded fashion to achieve robust, unbiased, and reproducible results. Experiments will include appropriate controls and reference immunotherapies. Detailed methodology and data will be published to ensure transparency.

Statistical Considerations. All statistical analysis will be performed with Graph Prism software. Statistical significance will be defined as a p value <0.05 . For *in vivo* efficacy studies, we propose using group sizes of 10 female mice per group to achieve 80% power to significantly detect ($\alpha=0.05$) group differences of 70% reduction in tumor size, measured as total photon flux or by tumor volume. Differences in tumor incidence and growth between groups will be assessed by ANOVA followed by Tukey post hoc test or by Student's *t* test as appropriate.

Specific Aim 1. Identify the optimal combination of cardio protectant and DOX formulation that protects from DOX-induced cardiotoxicity.

Table 2. Aim 1 Milestones.

Milestone	Analysis Method	Quantitative Metric
1.1 Identify the optimal CCR5i-DOX combination.	Cardiac function (echocardiogram). Circulating cTnT levels (ELISA, qRT-PCR).	Identify the optimal CCR5i and DOX formulation combination that produces none or limited cardiotoxicity.
1.2 Determine the optimal order of addition to achieve maximum cardio protection.	Proliferation, cell death, DNA damage response, inflammation (immunohistochemistry).	

Rationale. Based on our hypothesis that CCR5 upregulation by DOX in the heart causes cardiac dysfunction and damage, and CCR5 upregulation in BCa creates DNA damage-resistant cells, we will first assess the cardioprotective activity of both maraviroc and vicriviroc using a murine model of DOX-induced cardiotoxicity (Table 2). We will test several DOX formulations currently under development (a) **PEGylated liposome** (Doxil); (b) **non-pegylated liposome** (Myocet); (c) **polymeric micelle** (PM) (SP1049C); and (d) **polymeric nanoparticle** (PNP) (LivaTag)) in combination with the CCR5i and different sequence of addition. Meta analysis showed a trend towards reduced cardiotoxicity with PEGylated liposome Doxil;⁷⁴⁻⁷⁶ however Doxil-induced cardiotoxicity (4%), dose-dependent cardiac events (10%),⁷⁶ and the prevalence of Doxil-induced palmar plantar erythrodysesthesia (48%),⁷⁶ limit its utility. Residual cardiotoxicity with liposomal and pegylated Doxorubicin remains problematic.⁷⁵ Our studies will select the optimal DOX formulation and determine the most effective sequencing of administration to assess alternate cardio protectants identified in Table 1, to find the optimal combination for maximum cardio protection. The dose of maraviroc herein is the bioequivalent dose approved as safe and effective by the FDA for treatment of HIV- treatment which targets/inhibits HIV binding to the CCR5 receptor. The dose has been shown to be effective to reduce cancer metastasis/growth^{10,36} and to provide cardioprotection (Figure 10). The dose of Dexrazoxane used as control is the same as that used in the preclinical studies used for the IND. The dose of DOX was shown to induce cardiotoxicity in mice.⁷⁷

1.1.- Identify the optimal DOX formulation to combine with CCR5i to achieve maximal cardio protection. Mice will be treated with vehicle, CCR5i, DOX, or CCR5i+DOX according to Table 3. DOX and CCR5i will be given concurrently; DOX will be given by IP injection (8 injections during two weeks); CCR5i will be given orally for 8 weeks, until endpoint. Cardiac function will be measured at 2- (immediately after the last DOX injection), 6-, and 8-weeks (see below).

1.2.- Determine the optimal timing of CCR5i addition to achieve maximum cardio protection.

1.2.- Determine the optimal timing of CCR5i addition to achieve maximum cardio protection. To define the optimal timing to maximize cardio protection, we will deliver maraviroc or vicriviroc to female mice (16 or 30 mg/kg based on previous studies) either simultaneously, 72 hrs before, or 4 weeks after the last dosing of DOX treatment (IP, 8x 3 mg/kg, total dose of 24mg/kg).⁷⁷ Saline will be used as a control for the DOX, and the diluents for maraviroc/vicriviroc will be used as vehicle control. The effect of CCR5i on DOX-induced cardiac function, proliferation,

Table 3. Combination of CCR5i and DOX formulation for maximal cardio protection.

Group	DOX	IP dose	CCR5i	Oral Dose	Mice
1	Saline		Vehicle		10 F
2	Saline		Maraviroc	30 mg/kg	10 F
3	Saline		Vicriviroc	30 mg/kg	10 F
4	DOX	8x 3 mg/kg during 2 weeks	Vehicle		10 F
5			Maraviroc	16 mg/kg	10 F
6			Maraviroc	30 mg/kg	10 F
7			Vicriviroc	16 mg/kg	10 F
8			Vicriviroc	30 mg/kg	10 F
9-13	Doxil	Same as above (groups 4-8)			50 F
14-18	Myocet	Same as above (groups 4-8)			50 F
19-23	SP1049C	Same as above (groups 4-8)			50 F
24-28	LivaTag	Same as above (groups 4-8)			50 F

cell death, DNA damage and immune responses will be assessed as before. The optimal combination will produce minimal cardiac damage.

1.3.- Test other cardio protectants identified in Phase 1 in combination with the optimal DOX formulation (1.1) and sequence of administration (1.2). Using the same protocol, we will combine the best DOX formulation selected in 1.1 with all alternate cardio protectants identified in **Table 1**.

Methods. We will use female C57BL/6J mice basically as described in preliminary studies (**Figure 10**). Studies will be conducted in a manner blinded to the treatment (echocardiography, histopathology). Blood collected at sacrifice will be analyzed for established markers of acute myocardial injury with lactate dehydrogenase (LDH, CK-MB),⁷⁸ necrosis, cardiac troponin T (cTnT),⁷⁹ and heart failure with brain natriuretic protein (BNP).⁸⁰

Cardiac function will be determined at 2 weeks (immediately following the last DOX injection), 6 and 8 weeks (to monitor progression of cardiac remodeling) using antemortem transthoracic echocardiogram. Professor Anthony Ashton is highly experienced with echocardiography, performed all the echocardiograms in the preliminary data and will conduct the analysis proposed in this application. Heart and body weight will be recorded. Protein and RNA will be isolated from the heart apex and the rest of the heart is processed for histological analysis.

Histological sections will be analyzed for a limited number of proteins that will inform the mechanisms by which CCR5i impact cardiac survival including (i) the signal pathways we hypothesize govern CR5-mediated cardiotoxicity (Fig. 2), (2) cell death pathways (phospho- γ H2AX, TdT-mediated dUTP nick end labeling (TUNEL) and loss of the chromatin-binding protein HMGB1 and myocardial DNA fragmentation) and inflammation.

Proliferation: PCNA (Cat# 56), Ki-67, MAPKs: JNK Total/T^{hr183 tyr185P}, MAPK, MAPK^{Thr202/Tyr204P}, p38 MAPK. Cell Death: Bcl2 (Cat. 7382), Bax (Cat. 20067), cleaved Caspase 3 (Cat. 56053), and PARP cleavage (Cat. 56196).

The (3) DNA damage response: γ H2AX (Cat# 517348), DNA-PKcs^{T2609, S2056}, TopIIa, TopIIb. Chromatin Damage: chromatin-binding protein HMGB1, H2A, and γ H2A accumulation in the cytosol by IHC. Autophagy: microtubule-associated protein light-chain 3 (LC3) puncta. (4) Ribotoxic Stress (Fig. 2): p70RSK^{Thr 389/412}, Akt (Cat # sc-5298), Akt1/2/3p^{S473} (Cat #sc-7985), ARF translocation, (p-) EIF2a, mTOR, p-mTOR^{Ser2481}, P-mTOR^{Ser2448}, total eIF4G, eIF4E^{Ser209}, ZAK α ^{S657P} and p53 induction. (5) We will assess the impact of CCR5i on cardiac Inflammation using markers for: Monocytes (CD14; Cat# 1182), T-cells (CD3/CD4; Cat# 20079), B-cells (CD20; Cat# 271183), TIMD4 (resident cardiac macrophages) and comparison to current atlases. IHC will be performed with heat-based antigen retrieval at pH9 using ImmPress HRP polymer second antibodies (Cat. #ZF0906) and ImmPACT NovaRed substrate (Cat. ZF0905) as previously described.

Statistical analysis. Power calculations showed that n=30 would enable identification of significant differences (\geq 1.3-fold change) in aspects of cardiac function to be detected with at least 95% power. Kaplan-Meier analysis and Log-rank (Mantel-Cox) test will be used to evaluate the statistical significance for the comparison of echocardiograms and survival curves. Western blot and confocal data will be quantified using ImageJ software. The student's t-test will distinguish two groups of independent samples. One-way ANOVA will be used to reach more than two groups of independent samples. Two-way ANOVA with repeated measure analysis will be used to compare the response of two drugs over time.

Expected outcomes, pitfalls, and alternative approaches: reformulation and mechanism of action rationale. Our previous studies demonstrated that CCR5i reduced breast cancer growth in mice and enhanced breast cancer cell killing by DOX and radiation *in vitro*.^{7,10,31,81,82} The “dual function” compounds provided cardio protection. We anticipate that maraviroc and/or vicriviroc given contemporaneously will prevent DOX-induced cardiac dysfunction, reduce circulating markers of cardiac damage (cTnT and BNP), and diminish cardiac cell death. The methodology for all the experiments proposed in Aim 1 is standard in the laboratories of Drs. Pestell for more than 20 years and we don't anticipate major technical challenges to complete the proposed experiments. The optimal combination of CCR5i (maraviroc or vicriviroc) and DOX formulation and time/order of addition that offers maximum cardio protection will be selected for subsequent studies in Aim 2. LivaTag passed safety criterion in humans but did not reach its phase III clinical trial endpoint.^{83,84} LivaTag consists of DOX-loaded PNP formed using alkyl cyanoacrylate (PACA) and covalently linked to squalene. LivaTag aims to promote the penetration of DOX into tumor cells and enhance the contact between target DNA and DOX, LivaTag does not have reduced cardiotoxicity but rather enhanced cancer cell killing. We will assess synergy with CCR5i in cell killing and cardioprotection.

Specific Aim 2. Assess the anticancer efficacy of the optimal combination of cardio protectant and DOX formulation in a syngeneic TNBC model.

Table 4. Aim 2 Milestones.

Milestone	Analysis Method	Quantitative Metric
-----------	-----------------	---------------------

2.1 Demonstrate inhibition of tumor growth.	Tumor volume (caliper, BLI). Clinical signs of toxicity.	Demonstrate inhibition of tumor growth by ≥60% compared to vehicle, in the absence of overt toxicity.
---	---	---

Rationale. Based on the sequencing of administration established in aim 1, we will assess the functional synergy between DOX and the CCR5 inhibitors in C57BL/6 mice implanted with orthotopic Py8119 tumors (**Table 4**). Tumor-bearing mice (n=10/group) will be treated with DOX or the optimal formulation identified in Aim 1, 10-30 mg/kg maraviroc (or virciviroc), and the combination of both; vehicle will be given to control group. Tumor volume (caliper) and body weight will be measured 3 times per week; daily clinical assessments will be performed as early signs of toxicity. Overall survival will be recorded for each treatment group. At endpoint, animals will be sacrificed; blood will be collected for analytical chemistry. Tumors and major organs will be collected for histopathological evaluation. Cardiac function will be monitored under anesthesia by EKG and echocardiography.

Methods. Isogenic (C57BL/6J) genetically engineered mouse model (GEMM)-derived breast cancer cell lines (Py8119) will be used to demonstrate the efficacy of CCR5i/DOX combination. The breast cancer cells have been stably transduced with **Luc2-Aqua-FP** (Aquamarine-NLS) to enable sequential *in vivo* live imaging as previously described by us.^{10,36} The **Luc2-Aqua-FP**-NLS cells will be implanted orthotopically, as this method results in reliable metastasis,⁸⁵ into the 4th mammary fat pad (10⁵ cells) at ten weeks of age.⁸⁶⁻⁸⁹ Thirty animals will be used in each study arm. Treatment will begin when tumors are 0.5 cm diameter. Tumor-bearing mice will be treated with saline (control) or DOX (3mg/kg, (24 mg/kg total dose)) by IV injection. Contemporaneous treatment with maraviroc, virciviroc (16 mg/kg, or 30 mg/kg daily given twice daily by gavage) or vehicle control will be provided (N=10 in each study arm). Metastasis (in liver and lungs) will be determined using photometric quantitation from the LUC2 gene³⁷, IHC and Western blot for BCa keratins and the BCa cell **Aqua**-FP protein. Cardiotoxicity measurements of cardiac tissue will be conducted as described in Aim 1 with priority given to markers of cell death and damage (cTnT from the blood, markers of fibrosis and cell death in cardiac tissue).

An initial experiment will be performed to test the combination of maraviroc with all DOX formulations tested in Aim 1. We will test the following orders of addition: 1) Add CCR5i first, 3 days before adding DOX; 2) Add both CCR5i and DOX formulation together; and 3) Treat with DOX formulation first, then follow with CCR5i 3 days later. The rationale to test the latest option is that DOX induces expression of CCR5, such that maraviroc effects could be better defined. To define the optimal timing to maximize cardio protection, we will deliver maraviroc to female mice (16 or 30 mg/kg based on optimal dose found in Aim 1) either simultaneously, 72 hrs. before, or after 3 days of DOX treatment. DOX will be administered by intravenous (IV) injection, 8 doses of 3 mg/kg each, total 24mg/kg.⁷⁷ Saline is the control for DOX, and the diluents for maraviroc will be used as vehicle control.

The effect of CCR5i on DOX-induced cardiac function, primary tumor size, proliferation, cell death, DNA damage and tumor and immune responses will be assessed per Aim 1. Metastasis (liver, lungs) will be determined using IHC for BCa keratins and the BCa cell **Aqua**-FP protein. The optimal combination will produce BCa cell killing and minimal cardiac damage.

Expected outcomes, pitfalls, and alternative approaches. We do not anticipate any technical difficulties to achieve our goals, as the methodology for the experiments proposed in Aim 1 have been standard in the laboratory of Dr. Pestell for over 20 years. The doses of DOX are within a range that induce cardiotoxicity and kills BCa cells (24 mg/kg total).⁷² Intravenous DOX administration is uncommonly problematic (vein sclerosant and occlusions). The maraviroc gavage feeding will ensure the maraviroc is received with greater certainty and accuracy than by adding to the animal's food. Tumor size will be compared to previous studies with these lines,⁹⁰ the development of which appears to be unaffected by Luc2,⁹¹ as may be seen in some circumstances.⁹²

Specific Aim 3. Cardiotoxicity study with the combination of DOX and CCR5i in SD rats.

Table 5. Aim 3 Milestones.		
Milestone	Analysis Method	Quantitative Metric
3.1.- 8-week DOX cardiotoxicity study in SD rats with 14-day treatment (CRL) (non-GLP).	Plasma toxicokinetics, HPLC/UPLC. Mortality/moribundity. Body weight, food consumption. Detailed clinical observations. Echocardiography. cTn and NT-proBNP for myocardial ischemia. Histopathology (hearts).	<ul style="list-style-type: none"> No evidence of inflammation, damage or other adverse events. No unexpected or unexplained changes in clinical chemistry/hematology in treated groups relative to control. AUC, C_{max}/T_{max} reported for each study group.
3.2.- 8-week DOX cardiotoxicity study with maraviroc in SD rats (CRL) (non-GLP).		

Rationale. In order to begin the process towards a clinical trial we will work with Charles River Labs (CRL) to perform a non-GLP 8-weeks DOX (or DOX formulation) cardiotoxicity study that includes 14-days of treatment,

followed by an 8-weeks cardiotoxicity study of DOX in combination with maraviroc (or viceriviroc, or other cardio protectant identified in Aim 1). Studies will be conducted in SD rats as this is still the advised approach for regulatory toxicology testing (**Table 5**).

Development and validation of HPLC and LC/MS/MS methods for the quantification of DOX and maraviroc in rat plasma. CRL will first develop and validate bioanalytical methods to measure dose formulation concentration and homogeneity determination of DOX and maraviroc (and/or viceriviroc and other cardio protectants identified in Aim 1) in rat plasma, with a targeted analytical range in ng/ml; in addition, we will validate analytical methods to detect the drug candidate in extracts obtained from heart. A liquid chromatography method, either HPLC or UPLC, will be developed with 3 different sets of formulations to support toxicology studies.

3.1.- 8-Week DOX cardiotoxicity study in SD rats (non-GLP). We will first carry out a pilot study with DOX administered for 14 days followed by 6-weeks recovery (8-weeks total). SD naïve rats, 6-8 weeks old, equal numbers of male and female (**Table 6**) will be given vehicle or DOX (2.5 mg/kg, IP, 3x per week for 2 weeks, with a total dose of 15 mg/kg).

Alternatively, rats will be treated with the DOX formulation identified in Aim 1 that achieves most benefits when in combination with CCR5i (or other cardio protectants). Groups of animals will be terminated on Days 1, 21, or 42 after the last dose (Day 15, 35, and 56, respectively).

Table 6. 8-Week DOX cardiotoxicity study in SD rats (non-GLP).

Group	Treatment	Treatment duration	Route and frequency	Endpoint (Day)	SD rats		
1	Vehicle	14 days	IP 3 times per week	15	3 M / 3 F		
				35	3 M / 3 F		
				56	3 M / 3 F		
	2.5 mg/kg DOX			15	6 M / 6 F		
				35	6 M / 6 F		
				56	6 M / 6 F		

In-life procedures, observations, and measurements include **1) mortality/moribundity** will be performed twice daily during the course of the experiment. **2) Cage-side observations**, once daily during and post-treatment. **3) Detailed clinical observations**, including body weight and food consumption, will be recorded once weekly throughout the experiment. **4) Echocardiography** will be performed on anesthetized animals once prior to treatment and then on Days 15, 35, and 56, just before necropsy; transthoracic echocardiography will be performed using a commercially available ultrasound system. The following parameters will be determined: end diastolic and systolic diameters (LVEDD and LVESD), diastolic posterior wall thicknesses (dPWth). LV end diastolic and systolic volumes (LVEDV and LVESV) will be calculated to assess LV ejection fraction (LVEF), whereas LV shortening fraction (LVSF) will be calculated from LVEDD and LVESD. LV diastolic function parameters will be derived from pulsed-wave trans-mitral inflow pattern and tissue doppler imaging. Peak of E wave velocities, the isovolumic relaxation time (IVRT), the mean of peak velocities of basal septal and lateral walls (pulsed wave TDI) during systole (Sa) and in early diastole (Ea) to calculate E/Ea ratio. Radial 2D strain analyses will be performed using 2D speckle-tracking method on every medial myocardial segment. **5) Clinical pathology**: cardiac troponin T (cTn) and N-terminal pro-B-type natriuretic peptides (NT-proBNP) will be measured in blood collected once before treatment, and on Days 15, 35, and 56 after echocardiography and just prior to necropsy. **Necropsy**: on Days 15, 35, and 56, groups of animals will be euthanized following echocardiography using KCl to arrest the heart in the diastolic phase; brains and hearts will be collected and weighted. Macroscopic observations will be recorded, and hearts will be processed for histopathological analysis (hematoxylin and eosin staining and cleaved caspase 3 by immunohistochemistry, as marker for apoptosis). If animals are found dead at unscheduled dates or are found moribund and are euthanized for humane reasons, a full necropsy will be completed. Depending on the cardio protectant being used in the study, if additional target tissues are identified during the course of this proposal, we will evaluate these tissues as well at necropsy.

3.2.- 8-Week DOX cardiotoxicity study in combination with maraviroc (non-GLP). We will follow with an 8-week DOX cardiotoxicity study in combination with maraviroc (note that an alternate DOX formulation or cardio protectant will be used in this study based on results of Aim 1). SD naïve rats, 6-8 weeks old, equal numbers of male and female, will be given 2.5 mg/kg DOX by

Table 7. 8-Week cardiotoxicity study with DOX and maraviroc (non-GLP).

Group	DOX	Route	Maraviroc	Route	SD rats
Main	1 N/A	N/A	Vehicle	N/A	3 M / 3 F
	2 N/A	N/A	High	Oral	3 M / 3 F
	3 2.5 mg/kg x6	IP	Vehicle	N/A	6 M / 6 F
	4				6 M / 6 F
	5				6 M / 6 F
	6 2.5 mg/kg x6		Low	Oral	6 M / 6 F
	7		Medium		6 M / 6 F
	8		High		6 M / 6 F
TK	9 NA	N/A	High		3 M / 3 F
	10 2.5 mg/kg x6	IP	Vehicle	N/A	3 M / 3 F
	11		Low	Oral	3 M / 3 F
	12		Medium		3 M / 3 F
	13		High		3 M / 3 F

IP injection, week, for 6 weeks (total dose 15 mg/kg) alone or in combination with vehicle or 3 levels (Low, Medium, High) or daily oral doses of maraviroc (**Table 7**). Studies will be conducted in SD rats as this is still the advised approach for regulatory toxicology testing.⁹³ This dose of doxorubicin is consistent with the higher range of doses used in rats to induce chronic DOX cardiotoxicity.⁹⁴⁻¹⁰⁵ Animals in Main group will undergo the same in-life procedures, observations, and measurements as described in 3.1. Hearts and brains will be collected from all animals in Main group and processed as before (Aim 3.1). Blood will be collected from all animals in the toxicokinetic (TK) group at 6 different time points on days 1 (after 1st dose) and 14 (after last dose). Drug concentration in plasma will be measured using methods developed and validated by CRL as described above. Non-compartmental pharmacokinetic / toxicokinetic analysis will be performed using Phoenix WinNonlin software to calculate standard PK parameters.

Expected outcomes, pitfalls, and alternative approaches. Successful completion of this GLP-compliant toxicokinetic study on the combination of DOX + CCR5i performed by CRL will meet all preset safety and tolerability criteria to move into a Phase 1 clinical trial. As part of a successful IND-enabling program, we anticipate that our drug candidate will be safe. CRL is a world renowned CRO with decades of experience in providing full-service solutions covering GLP-compliant preclinical safety, and we do not anticipate problems in completing all studies during the proposed timeline. Should we find unsatisfactory safety concerns with the combination of DOX and CCR5i, we will explore alternative DOX formulations and/or the combination with vircriviroc, provided this was also effective in aims 1 and 2.

TIMELINE

Milestone	Year 1				Year 2			
	1	2	3	4	5	6	7	8
1.1—Test the effect of various DOX formulations with CCR5i and different order of addition on cardio protection.	■	■						
1.2—Test the combination of alternate cardio protectants with the selected DOX formulation and optimal sequence of administration			■	■	■			
2.1—Test the antitumor efficacy of the optimal DOX formulation in combination with maraviroc or vircriviroc.					■	■		
3.1—8-week DOX cardiotoxicity study in rats, with 14-days treatment.					■	■	■	
3.2—8-week cardiotoxicity study of DOX with maraviroc in rats.					■	■	■	■

SUMMARY AND FUTURE STUDIES

Successful completion of this proposal will identify which combination of DOX formulation and CCR5i (maraviroc or vircriviroc) or other of the newly discovered cardio protectants, as well as which sequence of addition, is most effective in preventing cardiotoxicity while enhancing potent anticancer activity. The preliminary safety/toxicology study will set the basis for a pre-IND meeting with the FDA to discuss all necessary requirements to complete IND studies and file an IND package to advance CCR5i/DOX combination for the treatment of solid tumors. The oral bioavailability of Maraviroc and safety was established during the FDA approval process and subsequent safety studies.^{106,107} Safety profile of CCR5i have been carefully evaluated, and active clinical trials in cancer (breast cancer, colon cancer, pancreatic cancer) were cleared by FDA (NCT03274804, NCT03838367, NCT04504942, NCT03631407, NCT04721301).

PROGRESS REPORT PUBLICATION LIST

No publications to report.

PHS Human Subjects and Clinical Trials Information

OMB Number: 0925-0001

Expiration Date: 01/31/2026

Use of Human Specimens and/or Data

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

Yes

No

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

Are Human Subjects Involved

Yes

No

Is the Project Exempt from Federal regulations?

Yes

No

Exemption Number

1 2 3 4 5 6 7 8

Other Requested Information

Delayed Onset Studies

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

VERTEBRATE ANIMALS

The proposed studies will use mice to assess DOX-induced cardiotoxicity and antitumor efficacy. Mice will be housed at the Lankenau Institute for Medical Research. We will also work with Charles River Labs to carry out cardiotoxicity studies in rats. This proposal was approval of institutional animal care at LIMR.

Table 1: Vertebrate Animals used in this project.

Aim	Description	C57BL/6 mice	SD rats
Exp. 1	Find optimal DOX formulation with MAR/VIC	280 F	
Exp. 2	Efficacy study in Py8119 syngeneic model	60F	
Exp. 3	Cardiotoxicity of DOX ± MAR/VIC in rats		84 M/84 F
Total		340 F	84 M/84 F

Sex as a biological variable (SABV). The mouse studies will be performed in female mice which will be implanted with Py8119 breast cancer cells. Aim 3 will carry out cardiotoxicity studies in male and female rats to comply with NIH and FDA guidelines on SABV by using equal numbers of male and female animals.

Statistical Analysis. For the mouse studies proposed in aims 1 and 2, we propose group sizes of 10 (female) mice per group, which will achieve 80% power to significantly ($\alpha=0.05$) detect group differences of $\geq 70\%$ reduction in tumor burden as measured by BLI. Differences in tumor incidence, growth, and metastasis progression between groups will be assessed by ANOVA followed by Tukey post hoc test or by Student's *t* test as appropriate. P-values <0.05 will be considered statistically significant.

1. Description of Procedures

1.1.- Identify the optimal DOX formulation to combine with CCR5i to achieve maximal cardio protection. Mice will be treated with vehicle, CCR5i, DOX, or CCR5i+DOX. DOX and CCR5i will be given concurrently; DOX will be given by IP injection (8 injections during two weeks); CCR5i will be given orally for 8 weeks, until endpoint. Cardiac function will be measured at 2- (immediately after the last DOX injection), 6-, and 8-weeks (see below).

1.2.- Determine the optimal timing of CCR5i addition to achieve maximum cardio protection. To define the optimal timing to maximize cardio protection, we will deliver maraviroc or vircriviroc to female mice (16 or 30 mg/kg based on previous studies) either simultaneously, 72 hrs before, or 4 weeks after the last dosing of DOX treatment (IP, 8x 3 mg/kg, total dose of 24mg/kg). Saline will be used as a control for the DOX, and the diluents for maraviroc/vircriviroc will be used as vehicle control. The effect of CCR5i on DOX-induced cardiac function, proliferation, cell death, DNA damage and immune responses will be assessed as before. The optimal combination will produce minimal cardiac damage.

1.3.- Test other cardio protectants identified in Phase I in combination with the optimal DOX formulation (1.1) and sequence of administration (1.2). Blood collected at sacrifice will be analyzed for established markers of acute myocardial injury with lactate dehydrogenase (LDH, CK-MB), necrosis with or without cardiac troponin T (cTnT), and heart failure with brain natriuretic protein (BNP).

Endpoints:

Cardiac function will be determined at 2 weeks (immediately following the last DOX injection), 6 and 8 weeks (to monitor progression of cardiac remodeling) using antemortem transthoracic echocardiogram. Heart and body weight will be recorded. Protein and RNA will be isolated from the heart apex and the rest of the heart is processed for histological analysis.

Histological sections will be analyzed for signal transduction and cell death pathways, proliferation, cell death, DNA damage response, autophagy, and inflammation.

2.- Assess the anticancer efficacy of the optimal combination of cardio protectant and DOX formulation in a syngeneic TNBC model. Isogenic (C57BL/6J) genetically engineered mouse model (GEMM)-derived breast cancer cell lines (Py8119) will be used to demonstrate the efficacy of CCR5i/DOX combination. Py8119 cells will be implanted orthotopically, as this method results in reliable metastasis, into the 4th mammary fat pad (10^5 cells) at ten weeks of age. Thirty animals will be used in each study arm. Treatment will begin when tumors are 0.5 cm diameter. Tumor-bearing mice will be treated with saline (control) or DOX (3mg/kg, (24 mg/kg total dose)) by IV injection. Contemporaneous treatment with maraviroc, vircriviroc (16 mg/kg, or 30 mg/kg daily given twice daily

by gavage) or vehicle control will be provided (N=10 in each study arm). Metastasis (in liver and lungs) will be determined using IHC for BCa keratins and the BCa cell [Aqua-FP](#) protein.

The effect of CCR5i on DOX-induced cardiac function, primary tumor size, proliferation, cell death, DNA damage and tumor and immune responses will be assessed per Aim 1. Metastasis (liver, lungs) will be determined using IHC for BCa keratins and the BCa cell [Aqua-FP](#) protein. The optimal combination will produce BCa cell killing and minimal cardiac damage.

3. Cardiotoxicity study with the combination of DOX and CCR5i in SD rats.

3.1.- 8-Week DOX cardiotoxicity study in SD rats (non-GLP). We will first carry out a pilot study with DOX administered for 14 days followed by 6-weeks recovery (8-weeks total). SD naïve rats, 6-8 weeks old, equal numbers of male and female will be given vehicle or DOX (2.5 mg/kg, IP, 3x per week for 2 weeks, with a total dose of 15 mg/kg). Alternatively, rats will be treated with the DOX formulation identified in Aim 1 that achieves most benefits when in combination with CCR5i (or other cardio protectants). Groups of animals will be terminated on Days 1, 21, or 42 after the last dose (Day 15, 35, and 56, respectively).

3.2.- 8-Week DOX cardiotoxicity study in combination with maraviroc (non-GLP). We will follow with an 8-week DOX cardiotoxicity study in combination with maraviroc (note that an alternate DOX formulation or cardio protectant will be used in this study based on results of Aim 1).

SD naïve rats, 6-8 weeks old, equal numbers of male and female, will be given 2.5 mg/kg DOX by IP injection, 3x per week, for 2 weeks (total dose 15 mg/kg) alone or in combination with vehicle or 3 levels (Low, Medium, High) or daily oral doses of maraviroc. Animals in Main group will undergo the same in-life procedures, observations, and measurements as described in 3.1. Hearts and brains will be collected from all animals in Main group. Blood will be collected from all animals in the toxicokinetic (TK) group at 6 different time points on days 1 (after 1st dose) and 14 (after last dose). Drug concentration in plasma will be measured using methods developed and validated by CRL as described above. Non-compartmental pharmacokinetic / toxicokinetic analysis will be performed using Phoenix WinNonlin software to calculate standard PK parameters.

Endpoints:

- 1) mortality/moribundity will be performed twice daily during the course of the experiment.
- 2) Cage-side observations, once daily during and post-treatment.
- 3) Detailed clinical observations, including body weight and food consumption, will be recorded once weekly throughout the experiment.
- 4) Echocardiography will be performed on anesthetized animals once prior to treatment and then on Days 15, 35, and 56, just before necropsy;
- 5) Clinical pathology: cardiac troponin T (cTn) and N-terminal pro-B-type natriuretic peptides (NT-proBNP) will be measured in blood collected once before treatment, and on Days 15, 35, and 56 after echocardiography and just prior to necropsy.
- 6) Necropsy: on Days 15, 35, and 56, groups of animals will be euthanized following echocardiography using KCl to arrest the heart in the diastolic phase; brains and hearts will be collected and weighted.

2. Justification for the conduct of the studies described and the use of animals

In this proposal, we are studying the role of CCR5 in the onset and progression of DOX-induced cardiac failure and breast cancer therapy. Examination of these effects in humans is not possible and is broadly considered unethical, therefore the use of transgenic mice or tumor implantation is necessary to address these clinically relevant biological questions.

3. Minimization of Pain and Distress

Expected adverse reactions following our surgical procedures could include loss of appetite, infection, gastrointestinal upset, diarrhea and skin irritation after shaving. It is possible these side effects could be lethal. For post-surgical pain we will treat animals with appropriate analgesics (e.g. buprenorphine) in accordance with the LIMR Veterinarian recommendations. Animals that exhibit overt indications of infection or other severe complications will be sacrificed immediately. Animals that clearly exhibit severe adverse effects (e.g. immobile even when touched or severe hunched posture affecting gait) will be euthanized prior to an experimental end to

avoid undue suffering. Pain and discomfort following our implantation procedure may result. Non-malignant human cells injected into the mice are carried in mouse extracellular matrix, are slow-growing and therefore we do not anticipate any compression from cellular expansion, nor do we expect any rejection reaction in this immune compromised strain following the implant. Redness and swelling at the injection site are signs of infection. If we observe overt signs of pain or discomfort in the mouse after surgery, we will closely observe the mouse for the signs of infection. If upon consult with a LIMR Veterinarian we determine the infection is localized and can be treated with a topical antibiotic we will apply ointment to the wound. If the infection is more extensive and systemic, we will immediately euthanize the mouse and collect tissues for subsequent analysis. If we detect a mouse with an unexpected and unusually high tumor burden, we will immediately euthanize the mouse. We follow a policy for end stage illness and humane endpoints. All invasive procedures will be carried out under anesthesia. Inhalation anesthesia is induced by brief inhalation of isoflurane in a closed chamber. The depth of anesthesia is monitored by spontaneous motion, respiratory rate, eye-blink, and response to stimulation. Properly anaesthetized mice are unresponsive to stimuli but recover fully within five minutes. Procedures requiring longer anesthesia will utilize a cocktail of Ketamine and Xylazine. For example, for cardiac punctures to draw oxygenated arterial blood, the mouse will be anesthetized with a Ketamine/Xylazine solution after first scrubbing the anterior chest wall with an aseptic betadine solution. All post-surgery animals will be closely monitored for signs of morbidity. Animals which exhibit morbidity or distress, will be promptly sacrificed by cervical dislocation or bilateral pneumothorax subsequent to CO₂ asphyxiation following isoflurane anesthesia administration as described below.

4. Methods of Euthanasia

At the end of the experiment, animals are euthanized painlessly by methods consistent with the recommendations of the AVMA Panel of Euthanasia. The specific method, described above as part of each procedure, was approved by our attending veterinarian and ACUC. The mice are obtained from approved commercial sources and housed comfortably in our AAALAC-accredited central animal facility for approximately 5 days. They are then brought to a specific room where they are anesthetized with isoflurane inhalation or carbon dioxide inhalation or administration of an anesthetic by injection for anesthesia and killed painlessly by cervical dislocation, only by laboratory members proficient in this procedure. Tissues are quickly excised and sections prepared for further analysis. This method is consistent with the recommendations of the Panel on Euthanasia of the American Veterinary Medical Association (AVMA) and has been chosen because it is safe, fast, and effective. Anesthesia prior to euthanasia will ensure that the mice will not suffer any pain or discomfort during this procedure. No significant changes or additions to the above procedures will be implemented until reviewed and approved by our Institutional Animal Care and Use Committee (IACUC). These procedures are not unnecessarily duplicative of previous experiments. Discomfort and injury will be limited to that which is unavoidable in the conduct of scientifically valuable research. Analgesic, anesthetic, and tranquilizing drugs will be used where indicated and appropriate to minimize discomfort and pain to animals as described above as part of each procedure. The animals will be housed in our central AAALAC-accredited animal facility at all times where they are observed daily. They will not be deprived of water or food at any time nor be subjected to prolonged physical restraint. Any animals that become moribund (as evidenced by observation of hunched posture, ruffled fur, slow movement, loss of appetite, etc.) will be euthanized painlessly.

LITERATURE CITED

- McGowan JV, Chung R, Maulik A, Piotrowska I, Walker JM, Yellon DM. Anthracycline Chemotherapy and Cardiotoxicity. *Cardiovasc Drugs Ther.* 2017 Feb;31(1):63–75. PMCID: PMC5346598
- Lipshultz SE, Lipsitz SR, Sallan SE, Dalton VM, Mone SM, Gelber RD, Colan SD. Chronic progressive cardiac dysfunction years after doxorubicin therapy for childhood acute lymphoblastic leukemia. *J Clin Oncol.* 2005 Apr 20;23(12):2629–2636. PMID: 15837978
- Cardinale D, Iacopo F, Cipolla CM. Cardiotoxicity of Anthracyclines. *Front Cardiovasc Med.* 2020;7:26. PMCID: PMC7093379
- Shaikh F, Dupuis LL, Alexander S, Gupta A, Mertens L, Nathan PC. Cardioprotection and Second Malignant Neoplasms Associated With Dexrazoxane in Children Receiving Anthracycline Chemotherapy: A Systematic Review and Meta-Analysis. *J Natl Cancer Inst.* 2016 Apr;108(4):djv357. PMID: 26598513
- Chow EJ, Aplenc R, Vrooman LM, Doody DR, Huang YSV, Aggarwal S, Armenian SH, Baker KS, Bhatia S, Constine LS, Freyer DR, Kopp LM, Leisenring WM, Asselin BL, Schwartz CL, Lipshultz SE. Late health outcomes after dexrazoxane treatment: A report from the Children's Oncology Group. *Cancer.* 2022 Feb 15;128(4):788–796. PMCID: PMC8792306
- Parkin DM, Fernández LMG. Use of statistics to assess the global burden of breast cancer. *Breast J.* 2006;12 Suppl 1:S70-80. PMID: 16430400
- Jiao X, Nawab O, Patel T, Kossenkov AV, Halama N, Jaeger D, Pestell RG. Recent Advances Targeting CCR5 for Cancer and Its Role in Immuno-Oncology. *Cancer Res.* 2019 Oct 1;79(19):4801–4807. PMCID: PMC6810651
- Batist G, Barton J, Chaikin P, Swenson C, Welles L. Myocet (liposome-encapsulated doxorubicin citrate): a new approach in breast cancer therapy. *Expert Opin Pharmacother.* 2002 Dec;3(12):1739–1751. PMID: 12472371
- Early Breast Cancer Trialists' Collaborative Group (EBCTCG). Effects of chemotherapy and hormonal therapy for early breast cancer on recurrence and 15-year survival: an overview of the randomised trials. *Lancet.* 2005 May 14;365(9472):1687–1717. PMID: 15894097
- Velasco-Velázquez M, Jiao X, De La Fuente M, Pestell TG, Ertel A, Lisanti MP, Pestell RG. CCR5 antagonist blocks metastasis of basal breast cancer cells. *Cancer Res.* 2012 Aug 1;72(15):3839–3850. PMID: 22637726
- Cardinale D, Colombo A, Bacchiani G, Tedeschi I, Meroni CA, Veglia F, Civelli M, Lamantia G, Colombo N, Curigliano G, Fiorentini C, Cipolla CM. Early detection of anthracycline cardiotoxicity and improvement with heart failure therapy. *Circulation.* 2015 Jun 2;131(22):1981–1988. PMID: 25948538
- Zhang S, Liu X, Bawa-Khalfe T, Lu LS, Lyu YL, Liu LF, Yeh ETH. Identification of the molecular basis of doxorubicin-induced cardiotoxicity. *Nat Med.* 2012 Nov;18(11):1639–1642. PMID: 23104132
- Pecoraro M, Del Pizzo M, Marzocco S, Sorrentino R, Ciccarelli M, Iaccarino G, Pinto A, Popolo A. Inflammatory mediators in a short-time mouse model of doxorubicin-induced cardiotoxicity. *Toxicol Appl Pharmacol.* 2016 Feb 15;293:44–52. PMID: 26780402
- Lyu YL, Kerrigan JE, Lin CP, Azarova AM, Tsai YC, Ban Y, Liu LF. Topoisomerase IIbeta mediated DNA double-strand breaks: implications in doxorubicin cardiotoxicity and prevention by dexrazoxane. *Cancer Res.* 2007 Sep 15;67(18):8839–8846. PMID: 17875725
- Avitabile D, Bailey B, Cottage CT, Sundararaman B, Joyo A, McGregor M, Gude N, Truffa S, Zarrabi A, Konstandin M, Khan M, Mohsin S, Völkers M, Toko H, Mason M, Cheng Z, Din S, Alvarez R, Fischer K, Sussman MA. Nucleolar stress is an early response to myocardial damage involving nucleolar proteins nucleostemin and nucleophosmin. *Proc Natl Acad Sci U S A.* 2011 Apr 12;108(15):6145–6150. PMCID: PMC3076816
- Xu X, Chen K, Kobayashi S, Timm D, Liang Q. Resveratrol attenuates doxorubicin-induced cardiomyocyte death via inhibition of p70 S6 kinase 1-mediated autophagy. *J Pharmacol Exp Ther.* 2012 Apr;341(1):183–195. PMCID: PMC3310694
- Li DL, Wang ZV, Ding G, Tan W, Luo X, Criollo A, Xie M, Jiang N, May H, Kyrychenko V, Schneider JW, Gillette TG, Hill JA. Doxorubicin Blocks Cardiomyocyte Autophagic Flux by Inhibiting Lysosome Acidification. *Circulation.* 2016 Apr 26;133(17):1668–1687. PMCID: PMC4856587

18. Zhu W, Soonpaa MH, Chen H, Shen W, Payne RM, Liechty EA, Caldwell RL, Shou W, Field LJ. Acute doxorubicin cardiotoxicity is associated with p53-induced inhibition of the mammalian target of rapamycin pathway. *Circulation*. 2009 Jan 6;119(1):99–106. PMCID: PMC2630181
19. Ichikawa Y, Ghanefar M, Bayeva M, Wu R, Khechaduri A, Naga Prasad SV, Mutharasan RK, Naik TJ, Ardehali H. Cardiotoxicity of doxorubicin is mediated through mitochondrial iron accumulation. *J Clin Invest*. 2014 Feb;124(2):617–630. PMCID: PMC3904631
20. Antoniak S, Tatsumi K, Schmedes CM, Grover SP, Pawlinski R, Mackman N. Protease-activated receptor 1 activation enhances doxorubicin-induced cardiotoxicity. *J Mol Cell Cardiol*. 2018 Sep;122:80–87. PMCID: PMC6173317
21. Mitry MA, Laurent D, Keith BL, Sira E, Eisenberg CA, Eisenberg LM, Joshi S, Gupte S, Edwards JG. Accelerated cardiomyocyte senescence contributes to late-onset doxorubicin-induced cardiotoxicity. *Am J Physiol Cell Physiol*. 2020 Feb 1;318(2):C380–C391. PMCID: PMC7052608
22. Qiao X, van der Zanden SY, Wander DPA, Borràs DM, Song JY, Li X, van Duikeren S, van Gils N, Rutten A, van Herwaarden T, van Tellingen O, Giacomelli E, Bellin M, Orlova V, Tertoolen LGJ, Gerhardt S, Akkermans JJ, Bakker JM, Zuur CL, Pang B, Smits AM, Mummery CL, Smit L, Arens R, Li J, Overkleeft HS, Neefjes J. Uncoupling DNA damage from chromatin damage to detoxify doxorubicin. *Proc Natl Acad Sci U S A*. 2020 Jun 30;117(26):15182–15192. PMCID: PMC7334570
23. Sauter KAD, Wood LJ, Wong J, Iordanov M, Magun BE. Doxorubicin and daunorubicin induce processing and release of interleukin-1 β through activation of the NLRP3 inflammasome. *Cancer Biol Ther*. 2011 Jun 15;11(12):1008–1016. PMCID: PMC3142364
24. Zhao L, Zhang B. Doxorubicin induces cardiotoxicity through upregulation of death receptors mediated apoptosis in cardiomyocytes. *Sci Rep*. 2017 Mar 16;7:44735. PMCID: PMC5353581
25. Pop-Moldovan AL, Trofenciu NM, Dărăbanțiu DA, Precup C, Branea H, Christodorescu R, Pușchiță M. Customized laboratory TLR4 and TLR2 detection method from peripheral human blood for early detection of doxorubicin-induced cardiotoxicity. *Cancer Gene Ther*. 2017 May;24(5):203–207. PMID: 28256509
26. Smith LA, Cornelius VR, Plummer CJ, Levitt G, Verrill M, Canney P, Jones A. Cardiotoxicity of anthracycline agents for the treatment of cancer: systematic review and meta-analysis of randomised controlled trials. *BMC Cancer*. 2010 Jun 29;10:337. PMCID: PMC2907344
27. Getz KD, Sung L, Alonzo TA, Leger KJ, Gerbing RB, Pollard JA, Cooper T, Kolb EA, Gamis AS, Ky B, Aplenc R. Effect of Dexrazoxane on Left Ventricular Systolic Function and Treatment Outcomes in Patients With Acute Myeloid Leukemia: A Report From the Children's Oncology Group. *J Clin Oncol*. 2020 Jul 20;38(21):2398–2406. PMCID: PMC7367546
28. Li M, Sala V, De Santis MC, Cimino J, Cappello P, Pianca N, Di Bona A, Margaria JP, Martini M, Lazzarini E, Pirozzi F, Rossi L, Franco I, Bornbaum J, Heger J, Rohrbach S, Perino A, Tocchetti CG, Lima BHF, Teixeira MM, Porporato PE, Schulz R, Angelini A, Sandri M, Ameri P, Sciarretta S, Lima-Júnior RCP, Mongillo M, Zaglia T, Morello F, Novelli F, Hirsch E, Ghigo A. Phosphoinositide 3-Kinase Gamma Inhibition Protects From Anthracycline Cardiotoxicity and Reduces Tumor Growth. *Circulation*. 2018 Aug 14;138(7):696–711. PMID: 29348263
29. Li L, Ni J, Li M, Chen J, Han L, Zhu Y, Kong D, Mao J, Wang Y, Zhang B, Zhu M, Gao X, Fan G. Ginsenoside Rg3 micelles mitigate doxorubicin-induced cardiotoxicity and enhance its anticancer efficacy. *Drug Deliv*. 2017 Nov;24(1):1617–1630. PMCID: PMC8241051
30. Pacher P, Liaudet L, Mabley JG, Czirák A, Haskó G, Szabó C. Beneficial effects of a novel ultrapotent poly(ADP-ribose) polymerase inhibitor in murine models of heart failure. *Int J Mol Med*. 2006 Feb;17(2):369–375. PMCID: PMC2245862
31. Jiao X, Velasco-Velázquez MA, Wang M, Li Z, Rui H, Peck AR, Korkola JE, Chen X, Xu S, DuHadaway JB, Guerrero-Rodriguez S, Addya S, Sicoli D, Mu Z, Zhang G, Stucky A, Zhang X, Cristofanilli M, Fatastis A, Gray JW, Zhong JF, Prendergast GC, Pestell RG. CCR5 Governs DNA Damage Repair and Breast Cancer Stem Cell Expansion. *Cancer Res*. 2018 Apr 1;78(7):1657–1671. PMCID: PMC6331183
32. Fang X, Wang H, Han D, Xie E, Yang X, Wei J, Gu S, Gao F, Zhu N, Yin X, Cheng Q, Zhang P, Dai W, Chen J, Yang F, Yang HT, Linkermann A, Gu W, Min J, Wang F. Ferroptosis as a target for protection against cardiomyopathy. *Proc Natl Acad Sci U S A*. 2019 Feb 12;116(7):2672–2680. PMCID: PMC6377499

33. Tadokoro T, Ikeda M, Ide T, Deguchi H, Ikeda S, Okabe K, Ishikita A, Matsushima S, Koumura T, Yamada KI, Imai H, Tsutsui H. Mitochondria-dependent ferroptosis plays a pivotal role in doxorubicin cardiotoxicity. *JCI Insight*. 2020 May 7;5(9):e132747, 132747. PMCID: PMC7253028
34. Riad A, Bien S, Gratz M, Escher F, Westermann D, Heimesaat MM, Bereswill S, Krieg T, Felix SB, Schultheiss HP, Kroemer HK, Tschope C. Toll-like receptor-4 deficiency attenuates doxorubicin-induced cardiomyopathy in mice. *Eur J Heart Fail*. 2008 Mar;10(3):233–243. PMID: 18321777
35. Yang Y, Lv J, Jiang S, Ma Z, Wang D, Hu W, Deng C, Fan C, Di S, Sun Y, Yi W. The emerging role of Toll-like receptor 4 in myocardial inflammation. *Cell Death Dis*. 2016 May 26;7(5):e2234. PMCID: PMC4917669
36. Sicoli D, Jiao X, Ju X, Velasco-Velazquez M, Ertel A, Addya S, Li Z, Andò S, Fatis A, Paudyal B, Cristofanilli M, Thakur ML, Lisanti MP, Pestell RG. CCR5 receptor antagonists block metastasis to bone of v-Src oncogene-transformed metastatic prostate cancer cell lines. *Cancer Research*. 2014;74(23):7103–7114. PMID: 25452256
37. Jiao X, Wang M, Zhang Z, Li Z, Ni D, Ashton AW, Tang HY, Speicher DW, Pestell RG. Leronlimab, a humanized monoclonal antibody to CCR5, blocks breast cancer cellular metastasis and enhances cell death induced by DNA damaging chemotherapy. *Breast Cancer Res*. 2021 Jan 23;23(1):11. PMCID: PMC7825185
38. Ha T, Li Y, Hua F, Ma J, Gao X, Kelley J, Zhao A, Haddad GE, Williams DL, William Browder I, Kao RL, Li C. Reduced cardiac hypertrophy in toll-like receptor 4-deficient mice following pressure overload. *Cardiovasc Res*. 2005 Nov 1;68(2):224–234. PMID: 15967420
39. Halama N, Zoernig I, Berthel A, Kahlert C, Klupp F, Suarez-Carmona M, Suetterlin T, Brand K, Krauss J, Lasitschka F, Lerchl T, Luckner-Minden C, Ulrich A, Koch M, Weitz J, Schneider M, Buechler MW, Zitvogel L, Herrmann T, Benner A, Kunz C, Luecke S, Springfield C, Grabe N, Falk CS, Jaeger D. Tumoral Immune Cell Exploitation in Colorectal Cancer Metastases Can Be Targeted Effectively by Anti-CCR5 Therapy in Cancer Patients. *Cancer Cell*. 2016 Apr 11;29(4):587–601. PMID: 27070705
40. Mills CD. M1 and M2 Macrophages: Oracles of Health and Disease. *Crit Rev Immunol*. 2012;32(6):463–488. PMID: 23428224
41. Mills CD, Lenz LL, Harris RA. A Breakthrough: Macrophage-Directed Cancer Immunotherapy. *Cancer Res*. 2016 Feb 1;76(3):513–516. PMCID: PMC4738030
42. Ley K. M1 Means Kill; M2 Means Heal. *J Immunol*. 2017 Oct 1;199(7):2191–2193. PMID: 28923980
43. Johnson TA, Singla DK. PTEN inhibitor VO-OHpic attenuates inflammatory M1 macrophages and cardiac remodeling in doxorubicin-induced cardiomyopathy. *Am J Physiol Heart Circ Physiol*. 2018 Nov 1;315(5):H1236–H1249. PMCID: PMC6297808
44. Singla DK, Johnson TA, Tavakoli Dargani Z. Exosome Treatment Enhances Anti-Inflammatory M2 Macrophages and Reduces Inflammation-Induced Pyroptosis in Doxorubicin-Induced Cardiomyopathy. *Cells*. 2019 Oct 9;8(10):1224. PMCID: PMC6830113
45. Zhang N, Shou B, Chen L, Lai X, Luo Y, Meng X, Liu R. Cardioprotective Effects of Latifolin Against Doxorubicin-Induced Cardiotoxicity by Macrophage Polarization in Mice. *J Cardiovasc Pharmacol*. 2020 Jun;75(6):564–572. PMCID: PMC7266001
46. Alard JE, Ortega-Gomez A, Wichapong K, Bongiovanni D, Horckmans M, Megens RTA, Leoni G, Ferraro B, Rossaint J, Paulin N, Ng J, Ippel H, Suylen D, Hinkel R, Blanchet X, Gaillard F, D'Amico M, von Hundelshausen P, Zarbock A, Scheiermann C, Hackeng TM, Steffens S, Kupatt C, Nicolaes GAF, Weber C, Soehnlein O. Recruitment of classical monocytes can be inhibited by disturbing heteromers of neutrophil HNP1 and platelet CCL5. *Sci Transl Med*. 2015 Dec 9;7(317):317ra196. PMID: 26659570
47. Lavine KJ, Pinto AR, Epelman S, Kopecky BJ, Clemente-Casares X, Godwin J, Rosenthal N, Kovacic JC. The Macrophage in Cardiac Homeostasis and Disease: JACC Macrophage in CVD Series (Part 4). *J Am Coll Cardiol*. 2018 Oct 30;72(18):2213–2230. PMCID: PMC6209119
48. Kelly KM, Tocchetti CG, Lyashkov A, Tarwater PM, Bedja D, Graham DR, Beck SE, Metcalf Pate KA, Queen SE, Adams RJ, Paolocci N, Mankowski JL. CCR5 inhibition prevents cardiac dysfunction in the SIV/macaque model of HIV. *J Am Heart Assoc*. 2014 Apr 2;3(2):e000874. PMCID: PMC4187513
49. Ma Y, Adjemian S, Mattarollo SR, Yamazaki T, Aymeric L, Yang H, Portela Catani JP, Hannani D, Duret H, Steegh K, Martins I, Schlemmer F, Michaud M, Kepp O, Sukkurwala AQ, Menger L, Vacchelli E, Droin N, Galluzzi L, Krzysiek R, Gordon S, Taylor PR, Van Endert P, Solary E, Smyth MJ, Zitvogel L, Kroemer

- G. Anticancer chemotherapy-induced intratumoral recruitment and differentiation of antigen-presenting cells. *Immunity*. 2013 Apr 18;38(4):729–741. PMID: 23562161
50. Velasco-Velázquez M, Pestell RG. The CCL5/CCR5 axis promotes metastasis in basal breast cancer. *Oncoimmunology*. 2013 Apr 1;2(4):e23660. PMCID: PMC3654591
51. Zi J, Yuan S, Qiao J, Zhao K, Xu L, Qi K, Xu K, Zeng L. Treatment with the C-C chemokine receptor type 5 (CCR5)-inhibitor maraviroc suppresses growth and induces apoptosis of acute lymphoblastic leukemia cells. *Am J Cancer Res*. 2017;7(4):869–880. PMCID: PMC5411794
52. Pervaiz A, Zepp M, Mahmood S, Ali DM, Berger MR, Adwan H. CCR5 blockage by maraviroc: a potential therapeutic option for metastatic breast cancer. *Cell Oncol (Dordr)*. 2019 Feb;42(1):93–106. PMID: 30456574
53. Albayrak G, Konac E, Dikmen AU, Bilen CY. Memantine induces apoptosis and inhibits cell cycle progression in LNCaP prostate cancer cells. *Hum Exp Toxicol*. 2018 Sep;37(9):953–958. PMID: 29226720
54. Banik K, Ranaware AM, Harsha C, Nitesh T, Girisa S, Deshpande V, Fan L, Nalawade SP, Sethi G, Kunnumakkara AB. Piceatannol: A natural stilbene for the prevention and treatment of cancer. *Pharmacol Res*. 2020 Mar;153:104635. PMID: 31926274
55. Das A, Samidurai A, Corral LG, Fung L, Sullivan RW, Chan KW, Swindlehurst CA, Salloum FN. Abstract 15913: Novel Dual mTOR Inhibitors/AMPK Activators Attenuate Doxorubicin-Induced Cardiotoxicity. *Circulation*. American Heart Association; 2019 Nov 19;140(Suppl_1):A15913–A15913.
56. Das A, Hovsepian S, Das S, Samidurai A, Mauro AG, Cain C, Kraskauskas D, Corral L, Fung L, Sullivan R, Chan KW, Swindlehurst CA, Salloum FN. Abstract 17055: Novel Dual mTOR Inhibitor/AMPK Activator Mitigates Doxorubicin Cardiotoxicity and Potentiates Its Chemotherapeutic Efficacy Against Triple Negative Breast Cancer. *Circulation*. American Heart Association; 2020 Nov 17;142(Suppl_3):A17055–A17055.
57. Sumneang N, Tanajak P, Oo TT. Toll-like Receptor 4 Inflammatory Perspective on Doxorubicin-Induced Cardiotoxicity. *Molecules*. 2023 May 24;28(11):4294. PMCID: PMC10254273
58. Lu S, Nasrallah HA. The use of memantine in neuropsychiatric disorders: An overview. *Ann Clin Psychiatry*. 2018 Aug;30(3):234–248. PMID: 30028898
59. Lv X, Li Q, Mao S, Qin L, Dong P. The protective effects of memantine against inflammation and impairment of endothelial tube formation induced by oxygen-glucose deprivation/reperfusion. *Aging (Albany NY)*. 2020 Nov 7;12(21):21469–21480. PMCID: PMC7695423
60. Onojafe IF, Megan LH, Melch MG, Aderemi JO, Alur RP, Abu-Asab MS, Chan CC, Bernardini IM, Albert JS, Cogliati T, Adams DR, Brooks BP. Minimal Efficacy of Nitisinone Treatment in a Novel Mouse Model of Oculocutaneous Albinism, Type 3. *Invest Ophthalmol Vis Sci*. 2018 Oct 1;59(12):4945–4952. PMCID: PMC6181301
61. Bae S, Siu PM, Choudhury S, Ke Q, Choi JH, Koh YY, Kang PM. Delayed activation of caspase-independent apoptosis during heart failure in transgenic mice overexpressing caspase inhibitor CrmA. *Am J Physiol Heart Circ Physiol*. 2010 Nov;299(5):H1374–1381. PMCID: PMC2993210
62. Remacle AG, Golubkov VS, Shiryaev SA, Dahl R, Stebbins JL, Chernov AV, Cheltsov AV, Pellecchia M, Strongin AY. Novel MT1-MMP small-molecule inhibitors based on insights into hemopexin domain function in tumor growth. *Cancer Res*. 2012 May 1;72(9):2339–2349. PMCID: PMC3342448
63. Skarra DV, Cornwell T, Solodushko V, Brown A, Taylor MS. CyPPA, a positive modulator of small-conductance Ca^{2+} -activated K^{+} channels, inhibits phasic uterine contractions and delays preterm birth in mice. *Am J Physiol Cell Physiol*. 2011 Nov;301(5):C1027–1035. PMID: 21795518
64. Hernández-Lozano I, Mairinger S, Sauberer M, Stanek J, Filip T, Wanek T, Ciarimboli G, Tournier N, Langer O. Influence of Cation Transporters (OCTs and MATEs) on the Renal and Hepatobiliary Disposition of $[11C]$ Metoclopramide in Mice. *Pharm Res*. 2021 Jan;38(1):127–140. PMCID: PMC7902338
65. Paksoy A, Hoppe S, Dörflinger Y, Horstmann H, Sätzler K, Körber C. Effects of the clathrin inhibitor Pitstop-2 on synaptic vesicle recycling at a central synapse in vivo. *Front Synaptic Neurosci*. 2022;14:1056308. PMCID: PMC9714552

66. Fisher N, Edwards MG, Hemming R, Allin SM, Wallis JD, Bulman Page PC, Mckenzie MJ, Jones SM, Elsegood MRJ, King-Underwood J, Richardson A. Synthesis and Activity of a Novel Autotaxin Inhibitor-Icodextrin Conjugate. *J Med Chem.* 2018 Sep 13;61(17):7942–7951. PMID: 30059212
67. Waaler J, Machon O, von Kries JP, Wilson SR, Lundenes E, Wedlich D, Gradi D, Paulsen JE, Machonova O, Dembinski JL, Dinh H, Krauss S. Novel synthetic antagonists of canonical Wnt signaling inhibit colorectal cancer cell growth. *Cancer Res.* 2011 Jan 1;71(1):197–205. PMID: 21199802
68. Inokuchi Y, Nakajima Y, Shimazawa M, Kurita T, Kubo M, Saito A, Sajiki H, Kudo T, Aihara M, Imaizumi K, Araie M, Hara H. Effect of an inducer of BiP, a molecular chaperone, on endoplasmic reticulum (ER) stress-induced retinal cell death. *Invest Ophthalmol Vis Sci.* 2009 Jan;50(1):334–344. PMID: 18757512
69. Yang B, Su Y, Han S, Chen R, Sun R, Rong K, Long F, Teng H, Zhao J, Liu Q, Qin A. Aminoxyacetic acid hemihydrochloride inhibits osteoclast differentiation and bone resorption by attenuating oxidative phosphorylation. *Front Pharmacol.* 2022;13:980678. PMCID: PMC9561130
70. Silk E, Zhao H, Weng H, Ma D. The role of extracellular histone in organ injury. *Cell Death Dis.* 2017 May 25;8(5):e2812. PMCID: PMC5520745
71. Baklaushev VP, Grinenko NF, Yusubalieva GM, Gubskii IL, Burenkov MS, Rabinovich EZ, Ivanova NV, Chekhonin VP. Mono- and Combined Therapy of Metastasizing Breast Carcinoma 4T1 with Zoledronic Acid and Doxorubicin. *Bull Exp Biol Med.* 2016 Aug;161(4):580–586. PMID: 27590765
72. Amgalan D, Garner TP, Pekson R, Jia XF, Yanamandala M, Paulino V, Liang FG, Corbalan JJ, Lee J, Chen Y, Karagiannis GS, Sanchez LR, Liang H, Narayanagari SR, Mitchell K, Lopez A, Margulets V, Scarlata M, Santulli G, Asnani A, Peterson RT, Hazan RB, Condeelis JS, Oktay MH, Steidl U, Kirshenbaum LA, Gavathiotis E, Kitsis RN. A small-molecule allosteric inhibitor of BAX protects against doxorubicin-induced cardiomyopathy. *Nat Cancer.* 2020 Mar;1(3):315–328. PMCID: PMC7413180
73. Burger DR, Parker Y, Quinta K, Lindner D. PRO 140 Monoclonal Antibody to CCR5 Prevents Acute Xenogeneic Graft-versus-Host Disease in NOD-scid IL-2R^γ null Mice. *Biol Blood Marrow Transplant.* 2018 Feb;24(2):260–266. PMID: 29128556
74. Yamaguchi N, Fujii T, Aoi S, Kozuch PS, Hortobagyi GN, Blum RH. Comparison of cardiac events associated with liposomal doxorubicin, epirubicin and doxorubicin in breast cancer: a Bayesian network meta-analysis. *Eur J Cancer.* 2015 Nov;51(16):2314–2320. PMID: 26343314
75. Ghasemi K, Vaseghi G, Mansourian M. Pharmacological interventions for preventing anthracycline-induced clinical and subclinical cardiotoxicity: A network meta-analysis of metastatic breast cancer. *J Oncol Pharm Pract.* 2021 Mar;27(2):414–427. PMID: 33081570
76. O'Brien MER, Wigler N, Inbar M, Rosso R, Grischke E, Santoro A, Catane R, Kieback DG, Tomczak P, Ackland SP, Orlandi F, Mellars L, Allard L, Tendler C, CAELYX Breast Cancer Study Group. Reduced cardiotoxicity and comparable efficacy in a phase III trial of pegylated liposomal doxorubicin HCl (CAELYX/Doxil) versus conventional doxorubicin for first-line treatment of metastatic breast cancer. *Ann Oncol.* 2004 Mar;15(3):440–449. PMID: 14998846
77. Desai VG, Herman EH, Moland CL, Branham WS, Lewis SM, Davis KJ, George NI, Lee T, Kerr S, Fusco JC. Development of doxorubicin-induced chronic cardiotoxicity in the B6C3F1 mouse model. *Toxicol Appl Pharmacol.* 2013 Jan 1;266(1):109–121. PMID: 23142469
78. Pelsers MMAL, Hermens WT, Glatz JFC. Fatty acid-binding proteins as plasma markers of tissue injury. *Clin Chim Acta.* 2005 Feb;352(1–2):15–35. PMID: 15653098
79. Mair J, Dienstl F, Puschendorf B. Cardiac troponin T in the diagnosis of myocardial injury. *Crit Rev Clin Lab Sci.* 1992;29(1):31–57. PMID: 1388708
80. Gülgün M, Fidancı K, Genç FA, Kesik V. Natriuretic peptide and cardiac troponin levels in doxorubicin-induced cardiotoxicity. *Anatol J Cardiol.* 2016 Apr;16(4):299. PMCID: PMC5368444
81. Upadhyaya C, Jiao X, Ashton A, Patel K, Kossenkov AV, Pestell RG. The G protein coupled receptor CCR5 in cancer. *Adv Cancer Res.* 2020;145:29–47. PMCID: PMC7755305
82. Velasco-Velázquez M, Xolalpa W, Pestell RG. The potential to target CCL5/CCR5 in breast cancer. *Expert Opin Ther Targets.* 2014 Nov;18(11):1265–1275. PMID: 25256399
83. Mura S, Fattal E, Nicolas J. From poly(alkyl cyanoacrylate) to squalene as core material for the design of nanomedicines. *J Drug Target.* 2019;27(5–6):470–501. PMID: 30720372

84. Ekladious I, Colson YL, Grinstaff MW. Polymer-drug conjugate therapeutics: advances, insights and prospects. *Nat Rev Drug Discov.* 2019 Apr;18(4):273–294. PMID: 30542076
85. Yang Y, Yang HH, Hu Y, Watson PH, Liu H, Geiger TR, Anver MR, Haines DC, Martin P, Green JE, Lee MP, Hunter KW, Wakefield LM. Immunocompetent mouse allograft models for development of therapies to target breast cancer metastasis. *Oncotarget.* 2017 May 9;8(19):30621–30643. PMCID: PMC5458155
86. Aslakson CJ, Miller FR. Selective events in the metastatic process defined by analysis of the sequential dissemination of subpopulations of a mouse mammary tumor. *Cancer Res.* 1992 Mar 15;52(6):1399–1405. PMID: 1540948
87. Takai K, Le A, Weaver VM, Werb Z. Targeting the cancer-associated fibroblasts as a treatment in triple-negative breast cancer. *Oncotarget.* 2016 Dec 13;7(50):82889–82901. PMCID: PMC5341254
88. Steenbrugge J, Breyne K, Denies S, Dekimpe M, Demeyere K, De Wever O, Vermeulen P, Van Laere S, Sanders NN, Meyer E. Comparison of the Adipose and Luminal Mammary Gland Compartment as Orthotopic Inoculation Sites in a 4T1-Based Immunocompetent Preclinical Model for Triple-Negative Breast Cancer. *J Mammary Gland Biol Neoplasia.* 2016 Dec;21(3–4):113–122. PMID: 27714576
89. Paschall AV, Liu K. An Orthotopic Mouse Model of Spontaneous Breast Cancer Metastasis. *J Vis Exp.* 2016 Aug 14;(114):54040. PMCID: PMC5091834
90. Biswas T, Gu X, Yang J, Ellies LG, Sun LZ. Attenuation of TGF- β signaling supports tumor progression of a mesenchymal-like mammary tumor cell line in a syngeneic murine model. *Cancer Lett.* 2014 Apr 28;346(1):129–138. PMCID: PMC3947668
91. Zuo H, Yang D, Yang Q, Tang H, Fu YX, Wan Y. Differential regulation of breast cancer bone metastasis by PARP1 and PARP2. *Nat Commun.* 2020 Mar 27;11(1):1578. PMCID: PMC7101362
92. Grzelak CA, Goddard ET, Lederer EE, Rajaram K, Dai J, Shor RE, Lim AR, Kim J, Beronja S, Funnell APW, Ghajar CM. Elimination of fluorescent protein immunogenicity permits modeling of metastasis in immune-competent settings. *Cancer Cell.* 2022 Jan 10;40(1):1–2. PMCID: PMC9668376
93. Harvey WD. Species Selection for Pharmaceutical Toxicity Studies. In: Hock FJ, Gralinski MR, Pugsley MK, editors. *Drug Discovery and Evaluation: Safety and Pharmacokinetic Assays* [Internet]. Cham: Springer International Publishing; 2022. p. 1–31. Available from: https://doi.org/10.1007/978-3-030-73317-9_133-1
94. Villani F, Galimberti M, Zunino F, Monti E, Rozza A, Lanza E, Favalli L, Poggi P. Prevention of doxorubicin-induced cardiomyopathy by reduced glutathione. *Cancer Chemother Pharmacol.* 1991;28(5):365–369. PMID: 1914080
95. Sacco G, Bigioni M, Evangelista S, Goso C, Manzini S, Maggi CA. Cardioprotective effects of zofenopril, a new angiotensin-converting enzyme inhibitor, on doxorubicin-induced cardiotoxicity in the rat. *Eur J Pharmacol.* 2001 Feb 23;414(1):71–78. PMID: 11230997
96. Sayed-Ahmed MM, Khattab MM, Gad MZ, Osman AM. Increased plasma endothelin-1 and cardiac nitric oxide during doxorubicin-induced cardiomyopathy. *Pharmacol Toxicol.* 2001 Sep;89(3):140–144. PMID: 11589785
97. Rahimi Balaei M, Momeny M, Babaeikelishomi R, Ejtemaei Mehr S, Tavangar SM, Dehpour AR. The modulatory effect of lithium on doxorubicin-induced cardiotoxicity in rat. *Eur J Pharmacol.* 2010 Sep 1;641(2–3):193–198. PMID: 20534381
98. Hydock DS, Lien CY, Jensen BT, Parry TL, Schneider CM, Hayward R. Rehabilitative exercise in a rat model of doxorubicin cardiotoxicity. *Exp Biol Med (Maywood).* 2012 Dec;237(12):1483–1492. PMID: 23354407
99. Hole LD, Larsen TH, Fossan KO, Limé F, Schjøtt J. A short-time model to study relevant indices of cardiotoxicity of doxorubicin in the rat. *Toxicol Mech Methods.* 2013 Jul;23(6):412–418. PMID: 23379389
100. Toblli JE, Rivas C, Cao G, Giani JF, Funk F, Mizzen L, Dominici FP. Ferric carboxymaltose-mediated attenuation of Doxorubicin-induced cardiotoxicity in an iron deficiency rat model. *Chemother Res Pract.* 2014;2014:570241. PMCID: PMC4022115
101. Kang Y, Wang W, Zhao H, Qiao Z, Shen X, He B. Assessment of Subclinical Doxorubicin-induced Cardiotoxicity in a Rat Model by Speckle-Tracking Imaging. *Arq Bras Cardiol.* 2017 Jul 10;109(2):0. PMCID: PMC5576117

102. Medeiros-Lima DJM, Carvalho JJ, Tibirica E, Borges JP, Matsuura C. Time course of cardiomyopathy induced by doxorubicin in rats. *Pharmacol Rep.* 2019 Aug;71(4):583–590. PMID: 31174019
103. Wang P, Wang L, Lu J, Hu Y, Wang Q, Li Z, Cai S, Liang L, Guo K, Xie J, Wang J, Lan R, Shen J, Liu P. SESN2 protects against doxorubicin-induced cardiomyopathy via rescuing mitophagy and improving mitochondrial function. *J Mol Cell Cardiol.* 2019 Aug;133:125–137. PMID: 31199952
104. Chakouri N, Farah C, Matecki S, Amedro P, Vincenti M, Saumet L, Vergely L, Sirvent N, Lacampagne A, Cazorla O. Screening for in-vivo regional contractile defaults to predict the delayed Doxorubicin Cardiotoxicity in Juvenile Rat. *Theranostics.* 2020;10(18):8130–8142. PMCID: PMC7381739
105. Sharma A, Parikh M, Shah H, Gandhi T. Modulation of Nrf2 by quercetin in doxorubicin-treated rats. *Helix.* 2020 Apr;6(4):e03803. PMCID: PMC7177035
106. FDA notifications. Maraviroc approved as a CCR5 co-receptor antagonist. *AIDS Alert.* 2007 Sep;22(9):103. PMID: 18411462
107. Wasmuth JC, Rockstroh JK, Hardy WD. Drug safety evaluation of maraviroc for the treatment of HIV infection. *Expert Opin Drug Saf.* 2012 Jan;11(1):161–174. PMID: 22118500

Form Approved Through 02/28/2023

OMB No. 0925-0001

<p>Department of Health and Human Services Public Health Services</p> <p>Grant Application</p> <p><i>Do not exceed character length restrictions indicated.</i></p>		<p>LEAVE BLANK—FOR PHS USE ONLY.</p> <table border="1" style="width: 100%; border-collapse: collapse;"> <tr> <td style="width: 33%;">Type</td> <td style="width: 33%;">Activity</td> <td style="width: 33%;">Number</td> </tr> <tr> <td>Review Group</td> <td colspan="2">Formerly</td> </tr> <tr> <td colspan="2">Council/Board (Month, Year)</td> <td>Date Received</td> </tr> </table>		Type	Activity	Number	Review Group	Formerly		Council/Board (Month, Year)		Date Received
Type	Activity	Number										
Review Group	Formerly											
Council/Board (Month, Year)		Date Received										
1. TITLE OF PROJECT (<i>Do not exceed 81 characters, including spaces and punctuation.</i>)												
Improving Outcomes in Cancer Treatment-Related Cardiotoxicity												
2. RESPONSE TO SPECIFIC REQUEST FOR APPLICATIONS OR PROGRAM ANNOUNCEMENT OR SOLICITATION <input type="checkbox"/> NO <input type="checkbox"/> YES (If "Yes," state number and title)												
Number: PA-23-230 Title: Omnibus Solicitation of the NIH, CDC and FDA for Small Business Inn												
3. PROGRAM DIRECTOR/PRINCIPAL INVESTIGATOR												
3a. NAME (Last, first, middle) Pestell, Richard G.		3b. DEGREE(S) MD, PhD	3h. eRA Commons User Name RPESTELL									
3c. POSITION TITLE Professor		3d. MAILING ADDRESS (Street, city, state, zip code) 100 East Lancaster Avenue Wynnewood, PA 19096-3450										
3e. DEPARTMENT, SERVICE, LABORATORY, OR EQUIVALENT PA Cancer & Regenerative Med Center												
3f. MAJOR SUBDIVISION												
3g. TELEPHONE AND FAX (Area code, number and extension) TEL: 267-402-0545 FAX:		E-MAIL ADDRESS: richard.pestell@bblumberg.org										
4. HUMAN SUBJECTS RESEARCH <input checked="" type="checkbox"/> No <input type="checkbox"/> Yes		4a. Research Exempt <input type="checkbox"/> No <input type="checkbox"/> Yes If "Yes," Exemption No.										
4b. Federal-Wide Assurance No.		4c. Clinical Trial <input type="checkbox"/> No <input type="checkbox"/> Yes	4d. NIH-defined Phase III Clinical Trial <input type="checkbox"/> No <input type="checkbox"/> Yes									
5. VERTEBRATE ANIMALS <input type="checkbox"/> No <input checked="" type="checkbox"/> Yes		5a. Animal Welfare Assurance No. D16-00003										
6. DATES OF PROPOSED PERIOD OF SUPPORT (month, day, year—MM/DD/YY)		7. COSTS REQUESTED FOR INITIAL BUDGET PERIOD		8. COSTS REQUESTED FOR PROPOSED PERIOD OF SUPPORT								
From 12/01/2024	Through 11/30/2026	7a. Direct Costs (\$) \$221,478	7b. Total Costs (\$) \$354,365	8a. Direct Costs (\$) \$450,249								
				\$720,399								
9. APPLICANT ORGANIZATION Name Baruch S. Blumberg Institute Address 3805 Old Easton Road Doylestown PA 18902-8400		10. TYPE OF ORGANIZATION Public: → <input type="checkbox"/> Federal <input type="checkbox"/> State <input type="checkbox"/> Local Private: → <input checked="" type="checkbox"/> Private Nonprofit For-profit: → <input type="checkbox"/> General <input type="checkbox"/> Small Business <input type="checkbox"/> Woman-owned <input type="checkbox"/> Socially and Economically Disadvantaged										
		11. ENTITY IDENTIFICATION NUMBER NAYCKSJ7F68										
		DUNS NO. 167281851	Cong. District PA-001									
12. ADMINISTRATIVE OFFICIAL TO BE NOTIFIED IF AWARD IS MADE		13. OFFICIAL SIGNING FOR APPLICANT ORGANIZATION										
Name Patti McAlloon		Name Patti McAlloon										
Title VP of Finance		Title VP of Finance										
Address 3805 Old Easton Road Doylestown PA 18902-8400		Address 3805 Old Easton Road Doylestown PA 18902-8400										
Tel: 215-489-4911 FAX:		Tel: 215-489-4911 FAX:										
E-Mail: patti.mcalloon@bblumberg.org		E-Mail: patti.mcalloon@bblumberg.org										
14. APPLICANT ORGANIZATION CERTIFICATION AND ACCEPTANCE: I certify that the statements herein are true, complete and accurate to the best of my knowledge, and accept the obligation to comply with Public Health Services terms and conditions if a grant is awarded as a result of this application. I am aware that any false, fictitious, or fraudulent statements or claims may subject me to criminal, civil, or administrative penalties.		SIGNATURE OF OFFICIAL NAMED IN 13. <i>(In ink. Please sign. Signature not acceptable.)</i> Patti McAlloon		DATE 3/12/2024								

<p>Department of Health and Human Services Public Health Services</p> <p>Grant Application</p> <p><i>Do not exceed character length restrictions indicated.</i></p>		<p>LEAVE BLANK—FOR PHS USE ONLY.</p> <table border="1" style="width: 100%; border-collapse: collapse;"> <tr> <td style="width: 33%;">Type</td> <td style="width: 33%;">Activity</td> <td style="width: 33%;">Number</td> </tr> <tr> <td>Review Group</td> <td colspan="2">Formerly</td> </tr> <tr> <td colspan="2">Council/Board (Month, Year)</td> <td>Date Received</td> </tr> </table>		Type	Activity	Number	Review Group	Formerly		Council/Board (Month, Year)		Date Received
Type	Activity	Number										
Review Group	Formerly											
Council/Board (Month, Year)		Date Received										
<p>1. TITLE OF PROJECT <i>(Do not exceed 81 characters, including spaces and punctuation.)</i> Improving Outcomes in Cancer Treatment-Related Cardiotoxicity</p>												
<p>2. RESPONSE TO SPECIFIC REQUEST FOR APPLICATIONS OR PROGRAM ANNOUNCEMENT OR SOLICITATION <input type="checkbox"/> NO <input checked="" type="checkbox"/> YES <i>(If "Yes," state number and title)</i></p> <p>Number: PA-20-230 Title:</p>												
<p>3. PROGRAM DIRECTOR/PRINCIPAL INVESTIGATOR</p>												
<p>3a. NAME (Last, first, middle) Ashton, Anthony W</p>		<p>3b. DEGREE(S) PhD</p>	<p>3h. eRA Commons User Name AWASHTON</p>									
<p>3c. POSITION TITLE Professor</p>		<p>3d. MAILING ADDRESS <i>(Street, city, state, zip code)</i> 100 Lancaster Avenue Wynnewood, PA 19096</p>										
<p>3e. DEPARTMENT, SERVICE, LABORATORY, OR EQUIVALENT</p>												
<p>3f. MAJOR SUBDIVISION</p>												
<p>3g. TELEPHONE AND FAX <i>(Area code, number and extension)</i> TEL: 484-476-2888 FAX: 484-476-2205</p>		<p>E-MAIL ADDRESS: AshtonA@mlhs.org</p>										
<p>4. HUMAN SUBJECTS RESEARCH <input type="checkbox"/> No <input checked="" type="checkbox"/> Yes</p>		<p>If "Yes," Exemption No. <input type="checkbox"/> No <input type="checkbox"/> Yes</p>										
<p>4b. Federal-Wide Assurance No. 000001169</p>		<p>4c. Clinical Trial <input type="checkbox"/> No <input type="checkbox"/> Yes</p>	<p>4d. NIH-defined Phase III Clinical Trial <input type="checkbox"/> No <input type="checkbox"/> Yes</p>									
<p>5. VERTEBRATE ANIMALS <input type="checkbox"/> No <input checked="" type="checkbox"/> Yes</p>		<p>5a. Animal Welfare Assurance No.</p>										
<p>6. DATES OF PROPOSED PERIOD OF SUPPORT <i>(month, day, year—MM/DD/YY)</i> From 12/1/2024 Through 11/30/2025</p>		<p>7. COSTS REQUESTED FOR INITIAL BUDGET PERIOD 7a. Direct Costs (\$) 82,203</p>		<p>8. COSTS REQUESTED FOR PROPOSED PERIOD OF SUPPORT 8a. Direct Costs (\$) 166,075 8b. Total Costs (\$) 279,006</p>								
<p>9. APPLICANT ORGANIZATION Name Lankenau Institute for Medical Research Address 100 Lancaster Avenue Wynnewood, PA 19096</p>		<p>10. TYPE OF ORGANIZATION Public: → <input type="checkbox"/> Federal <input type="checkbox"/> State <input type="checkbox"/> Local Private: → <input type="checkbox"/> Private Nonprofit For-profit: → <input type="checkbox"/> General <input type="checkbox"/> Small Business <input type="checkbox"/> Woman-owned <input type="checkbox"/> Socially and Economically Disadvantaged</p> <p>11. ENTITY IDENTIFICATION NUMBER 1232175626A1 DUNS NO. 125797084 Cong. District PA-002</p>										
<p>12. ADMINISTRATIVE OFFICIAL TO BE NOTIFIED IF AWARD IS MADE Name Sam Diianni Title Director of Finance Address 100 Lancaster Avenue Wynnewood, PA 19096 Tel: 484-476-2755 FAX: 484-476-8533 E-Mail: diianniS@mlhs.org</p>		<p>13. OFFICIAL SIGNING FOR APPLICANT ORGANIZATION Name George C. Prendergast, Ph.D. Title President & CEO Address 100 Lancaster Avenue Tel: 484-476-8429 FAX: 484-476-8533 E-Mail: prendergast@limr.org</p>										
<p>14. APPLICANT ORGANIZATION CERTIFICATION AND ACCEPTANCE: I certify that the statements herein are true, complete and accurate to the best of my knowledge, and accept the obligation to comply with Public Health Services terms and conditions if a grant is awarded as a result of this application. I am aware that any false, fictitious, or fraudulent statements or claims may subject me to criminal, civil, or administrative penalties.</p>		<p>SIGNATURE OF OFFICIAL NAMED IN 13. <i>(In ink. "Per" signature not acceptable.)</i>  George Prendergast (Mar 1, 2024 17:24 EST)</p>		<p>DATE Mar 1, 2024</p>								

LANKENAU INSTITUTE FOR MEDICAL RESEARCH (LIMR)

A. Statement of Work

Anthony Ashton, Ph.D. (Co-Principal Investigator, 3 calendar months effort or ~20% effort). Dr. Ashton is an Professor, at the Lankenau Institute of Medical Research who is also involved directly with project management and bench research for this proposal. Dr. Ashton has a broad published background in the mechanisms of cell death, angiogenesis, immunology and G protein coupled receptor signaling. He has experience with echocardiography and in vivo imaging of tumors and will conduct measurements of the cardiac response and tumor response to Doxorubicin in the presence of the reformulations of doxorubicin with and without CCR5 antagonists *in vivo* and *in vitro*. His efforts will remain directed to the characterization of cardiac remodeling in Aim 1 and Aim 2. The studies outlined are highly labor intensive and we are keenly aware of the critical importance of Dr. Ashton's involvement in this project. Salary support for his effort and reagent costs as pertain to completion of the assigned aims are requested for all years of the proposal.

Fringe Benefits consist of FICA, Health Insurance, Dental Insurance, Vision Insurance, Life Insurance, Accidental Death & Dismemberment Insurance, Workers Compensation, Retirement, Short and Long-Term Disability Insurance.

LETTERS OF SUPPORT

In support of this Phase II Renewal proposal, we include letters of support from several key stakeholders

1.	Richard G. Pestell, MD, PhD	Subaward PI	Blumberg Distinguished Professor, Baruch S. Blumberg Institute
2.	Anthony W. Ashton, PhD	Subaward PI	Head, Cardiovascular Remodeling Program, Lankenau Institute for Medical Research
3.	Randall N. Hyer, MD, PhD, MPH	Institutional Support	President, Baruch S. Blumberg Institute
4.	George C. Prendergast, PhD	Institutional Support	President & CEO, Lankenau Institute for Medical Research
5.	Alexander V. Kabanov, PhD, D.Sc.	Collaborator	Director, Center for Nanotechnology in Drug Delivery and Carolina Institute for Nanomedicine, UNC Eshelman School of Pharmacy
6.	Dawn E. Bowles, PhD	Collaborator	Assistant Professor, Department of Surgery, Division of Surgical Sciences, Co-director, Duke Human Heart Repository
7.	Sean Lal, PhD	Collaborator	Clinical Academic Cardiologist, University of Sydney, Department of Cardiology, Royal Prince Alfred Hospital
8	Edward T.H. Yeh, MD	Consultant	Nolan Family Distinguished Chair in Internal Medicine, Professor of Medicine, Chair, Department of Internal Medicine College of Medicine, University of Arkansas for Medical Science
9	Richard N. Kitsis, MD	Consultant	Professor of Medicine and Cell Biology; The Dr. Gerald and Myra Dorros Chair in Cardiovascular Disease; Director, Wilf Family Cardiovascular Research Institute, Albert Einstein College of Medicine
10.	Javid Moslehi, MD	Consultant	William Grossman Distinguished Professor in Cardiology Section Chief, Cardio-Oncology & Immunology; Professor in Residence Cardiovascular Research Institute (CVRI) UCSF School of Medicine
11.	Carmen L. Falcon	CRO	Charles River



3805 Old Easton Road
Doylestown, PA 18902
215-489-4900 • blumberginstitute.org

Richard G. Pestell
AO., MB, BS, MD, PhD, FRACP (Australia), FACP (USA), FRS of Medicine, MBA., FRSB, FRCP (Ireland) FRCP (London).
President
Pennsylvania Cancer and Regenerative Medicine Center
3805 Old Easton Rd,
Doylestown, PA, 18902
T 215.503.5692 F 215.503.9334

Associate Professor Xuanmao Jiao
LightSeed LLC.,

Dear Jiao,

I am writing this letter to provide my most enthusiastic endorsement of your proposed grant application entitled "Improving Outcomes in Cancer Treatment-Related Cardiotoxicity".

Your grant application is designed to address the unmet need of ongoing anthracycline-related cardiotoxicity. Doxorubicin is one of the most widely used chemotherapies in the world, with the unpredictable consequences of cardiotoxicity and death. Your compelling pre-clinical studies using a widely accepted murine model show compelling evidence that the CCR5 inhibitor Maraviroc, used at bioequivalent doses approved by the FDA for HIV, abrogates Dox-induced cardiac dysfunction by echocardiogram, biochemical indices and histology.

Dox Alternative formulations have been, and are, actively under development in an attempt to mitigate this risk. The proposed studies will test several DOX reformulations under development ((PEGylated liposome (DoxilCaelix, JNS2022); non pegylated liposome (Myocet) PM: polymeric micelle (SP1049C, NK911); PNP: polymeric nanoparticle (LivaTag)) in combination with the CCR5i to identify the optimal DOX/CCR5i combination and determine the most effective sequencing of administration.

Your Milestones will quantitate: (1) the IC₅₀ for cardio protection in cells (canine myocytes, human cardiomyocyte cell lines) (2). the cardio protection provided by CCR5i to the alternate DOX reformulations in functional studies using the bioequivalent dose used in human in our murine model.

Meeting these milestones will enable LightSeed to partner with companies developing these technologies through exclusive or non-exclusive licensing agreements as the basis of a future clinical trial.

If there is any further information I can provide, please do not hesitate to contact me.

Yours sincerely,

A handwritten signature in black ink that reads 'Richard Pestell'.



LANKENAU INSTITUTE FOR MEDICAL RESEARCH

www.limr.org

Division of Cardiovascular Research | Room 128 | 100 E. Lancaster Ave | Wynnewood, PA 19096

Dr Xuanmao Jiao
Lightseed LLC,
Rm 234, Lankenau Institute for Medical Research
100 E Lancaster Ave
Wynnewood, PA 19096

Anthony W. Ashton, PhD
PHONE: (484) 476-2888
FAX: (215) 816-7597

Re: Letter of Support for SBIR Application

29th February, 2024

Dear Jiao,

I am pleased to continue to collaborate with you as a site-PI on the Phase 2 SBIR grant submission entitled "Improving Outcomes in Cancer Treatment-Related Cardiotoxicity". I will provide expertise and hands-on-work for echocardiography of mice treated with different Doxorubicin reformulations and CCR5i to establish the optimal DOX/CCR5i combination and therapeutic approach, assessment of DOX/CCR5i combination of myocytes *in vitro* and assessment of cardiac function/anatomy/histology in the TNBC models. My lab is in close proximity to your laboratory (the floor below). Having spent the two years studying this topic I am very familiar with the area and the importance of the "dual function" compounds we are seeking. I have the expertise and motivation to successfully assist you in the execution of all cardiac related aspects of this project.

I have training in mouse models of heart disease, molecular biology, and cancer biology. My expertise in molecular regulation of cardiovascular remodeling is well matched with the proposed grant. I have provided substantial preliminary data for the enclosed application as an example of my familiarity with the proposed work. Your proposal to search for cardioprotective anticancer (dual function) drugs is very important given this is a serious unmet need. I am looking forward to your successful grant application and contributing to this effort.

Best Regards

A handwritten signature of Anthony W. Ashton in black ink.

Anthony W. Ashton

Head, Cardiovascular Remodeling Program

Professor, LIMR

"In Medicine, Hope Springs From Research"



October 2, 2023

The National Institutes of Health

RE: Richard Pestell, MD, PhD - Grant: entitled *“Improving Outcomes in Cancer Treatment-Related Cardiotoxicity”*.

Dear Sir or Madam:

I am pleased to write this endorsement for the application submitted by Richard Pestell, M.D., Ph.D. for the NIH grant reapplication entitled entitled *“Improving Outcomes in Cancer Treatment-Related Cardiotoxicity”*. As the President of the Baruch S. Blumberg Institute, I am very excited about Dr. Pestell's work and have the utmost confidence in this application. Dr. Pestell has a distinguished career built on novel ideas which are being translated into the clinic. This is to reaffirm my enthusiasm and describe, in part, the institutional support from the Baruch S. Blumberg Institute for, and to, Richard Pestell, M.D., Ph.D., M.B.A.

Dr. Pestell joined the Baruch S. Blumberg Institute (the Blumberg/BSBI) in January 2017. In addition to his role leading the Blumberg's Pennsylvania Cancer and Regenerative Medicine Center, he is designated as “Institute Distinguished Professor.” This is a status that is also held by Michael Sofia, Ph.D. (2016 Lasker Prize winner) and Patrick Lam, Ph.D. (inventor: Chan-Lam reaction). This Board designation is intended to provide recognition of members of our faculty of extraordinary accomplishment. Queen Elizabeth II acknowledged Dr. Pestell's distinguished discoveries with the award of Order of Australia in 2019 for “distinguished service to medicine” in “endocrinology and oncology”.

Dr. Pestell receives substantial Institutional support. Since his arrival, the Blumberg has provided his laboratory with an excess of \$200,000 in research support, used for research at the Blumberg at his discretion. This level of support, we believe, is similar to support from an endowed chair and can be used for his salary (as he budgets), and support can be expected to continue, following the awarding of the proposal submitted herein.

Dr. Pestell's studies novel proposal addresses the significant challenge of reducing cardiotoxicity from DNA damage inducing chemotherapies, while maintaining – or in the current studies- enhancing the efficacy of chemotherapy. With cancer survivors at 19 million in the USA by 2025, Doxorubicin-cardiotoxicity is part of a very serious “cardio-oncology epidemic”.

Dr. Pestell has developed substantial pivotal preliminary data:

- (i). identified novel mechanisms governing cardioprotection from Doxorubicin.
- (ii). generated strong preliminary data that CCR5 inhibitors convey cardioprotection in a murine model (C57Bl/6j mice) of Dox-induced cardio toxicity (hazards ratio of >4),
- (iii). developed highly sensitive (Aqua-NLS), reporter breast cancer cell lines mesenchymal-like, TNBC PY8119 and epithelial-like Py230), that grow in this mouse model of Dox-induced cardiotoxicity (C57Bl/6j mice).

(iv). Shown that the Doxorubicin cardioprotectants actually enhances Doxorubicin induced cell killing of these breast cancer cell lines.

(v). Demonstrated the application of a novel technology to study heart damage induced inflammation. In this regard Dr Pestell has deployed *Digital spatial profiling (DSP)* (NanoString GeoMxTM) together with RNAscopeTM to study local myocardial inflammatory signaling pathways.

These studies are highly innovative and highly impactful as the compounds being used have been approved as safe by the FDA for use in other diseases (HIV), therefore the findings may be rapidly translated to the clinic.

Dr. Pestell has a track record of seminal discoveries in cancer with issued patents a substantial number of citations of his publications (>92,500), and an H-index of 154. He is clearly a researcher on the rise, with >21,000 citations in the last 5 years a number 1 ranking by Google Scholar, in the field of “cell-cycle” and top 10 in the world for “breast cancer” and “prostate cancer.” He published 46 papers since 2015. His ~500 publications and book chapters, and his papers have been published in high-profile journals include publications in top journals such as *Cell, Science, Nature Medicine, and Molecular Cell*.

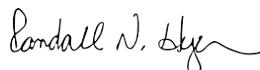
Interestingly, one of Dr. Pestell first publications was as a clinician on the use of desferroxamine to reduce cardiac toxicity from iron overload, demonstrated by serial echocardiograms. Furthermore, the team assembled in the proposed grant includes Professors Rick Kitsis and Anthony Ashton, who have co-authored papers together for more than 20 years.

Dr. Pestell has access to multiple institutes through formal appointments which we believe expands his research resources to provide a rich environment for the proposed studies. The institutions include the Lankenau Institute for Medical Research (LIMR) where Dr Pestell's laboratory is located together with Dr. Ashton (14 PI, 41 research personnel); the Baruch S. Blumberg Institute (15 PI, 26 faculty, 300+ researchers at the entire PA Biotechnology Center) where Dr. Pestell holds an appointment as Distinguished Professor; and the Wistar Institute where Dr. Pestell is an Adjunct Professor (29 PI, 276 researchers). For this reason, there are three distinct resources pages provided for this revised application, extending substantially the quality and repertoire of his research environment.

I have every confidence that the proposed project will make a major beneficial impact in advancing novel strategies for prostate cancer therapeutics. I believe that Dr. Pestell has made and will continue to make significant new discoveries in cancer research and I am looking forward to seeing the outcome of this new project.

I believe that Dr. Pestell will have everything he needs to complete the studies in this research proposal. Should you have any questions or require any additional information, please do not hesitate to contact me.

Sincerely,



Randall N. Hyer, MD, PhD, MPH
President, Baruch S. Blumberg Institute



LANKENAU INSTITUTE FOR MEDICAL RESEARCH

www.limr.org

100 E. Lancaster Avenue • Wynnewood, PA 19096 • p: 484.476.8475 • f: 484.476.8533

February 29th, 2024

RE: Letter of Institutional Support for PA-23-230
"Improving Outcomes in Cancer Treatment-Related Cardiotoxicity"

George C. Prendergast, PhD
President and Professor

Dear Madam/Sir:

I am pleased to provide this letter to confirm the full institutional resource and support of my faculty colleague Anthony Ashton, Ph.D. for his role in LightSeed's **NIH Small Business Innovation Research Grant** entitled "Improving Outcomes in Cancer Treatment-Related Cardiotoxicity".

Dr. Ashton has an appointment at the Lankenau Institute for Medical Research (LIMR) at the rank of Professor which includes ~1,000 sq. ft. of laboratory and office space for his research endeavors. Common use equipment and facilities including small animal imaging systems and animal housing in our AAALAC-approved vivarium are available for his use. Research Services to assist with grant, manuscript, and report preparation, as well as financial services, are also available.

In this application, Dr. Ashton intends to continue his study into interventions that salvage the adverse cardiac remodeling associated with chemotherapy in cancer patients. The success of many cancer therapies has resulted in cancer patients surviving their initial disease in greater numbers. However, for survivors the iatrogenic effects of cancer chemotherapy are becoming a significant source of mortality and health care burden. For instance, patients with triple negative breast cancer treated with anthracyclines show a dose-dependent decline in cardiac function: at recommended doses of <550 mg/m², more than 30% of patients receiving doxorubicin will develop severe cardiotoxicity each year.

With 19 million cancer survivors in the US by 2025, doxorubicin cardiotoxicity has been described by some as a burgeoning cardio-oncology epidemic. In the current proposal Dr. Ashton hopes to advance his studies of approved CCR5 inhibitors as a beneficial adjunct therapy to new formulations of doxorubicin that can maximize cancer killing without cardiotoxicity. I believe his work will lead to a breakthrough in its ability to safely promote the unrealized potential of this anti-tumor medication, the full use of which has been limited. Repurposing CCR5 inhibitors to widen the therapeutic window for use of doxorubicin and other anthracyclines will have an enormous positive impact with a short timeline to reshape patient management and improve cancer survivorship globally.

I am grateful for your consideration of the opportunity to host this high impact research.

Sincerely,

A handwritten signature in black ink that reads "George C. Prendergast". The signature is fluid and cursive, with "George" and "C." being more stylized and "Prendergast" being more clearly legible.

George C. Prendergast, Ph.D.
President and CEO
Lankenau Institute for Medical Research

"In Medicine, Hope Springs from Research"



March 7, 2024

Professor Richard G. Pestell AO
Pennsylvania Cancer and Regenerative Medicine Center
Baruch S. Blumberg Institute
Lankenau Institute for Medical Research

Re: Letter of Support for SBIR Phase II Application

Dear Professor Pestell:

I am pleased to provide this letter of collaboration with you and agree to serve as a consultant on your SBIR Phase II grant submission entitled "Improving Outcomes in Cancer Treatment-Related Cardiotoxicity".

My main research interests and expertise are in polymeric drug delivery systems for small molecules, nucleic acids, and proteins for treatment of cancer and CNS diseases. I have contributed work on block copolymer micelles, polyplexes, nanogels, macrophage-based drug and gene delivery carriers, and exosomes, which are now widely used in the nanomedicine and drug delivery field.

My efforts have contributed to translation, clinical evaluation, and approval of polymeric micelle-based delivery systems. Relevant to your proposed studies I co-developed a formulation of Doxorubicin in poloxamer polymeric micelles (SP1049C, now SKC1049) that showed increased efficacy against drug resistant cancers in preclinical studies, and both safety and clinical efficacy in a phase II study PMID: 20179989. This doxorubicin formulation obtained a SPA on a single approvable Phase 3 trial in refractory upper GI adenocarcinoma and has obtained an orphan drug designation in adenocarcinoma of the esophagus from US FDA.

With this background, I am pleased to provide consultative expertise and synthesize SP1049C for these proposed studies. I have discussed with you the recent studies and the Aims of the grant. I understand you will conduct studies in tissue culture and in mice to determine whether CCR5i enhance the cancer cell killing and reduce cardiotoxicity of bioequivalent doses of doxorubicin. I am familiar with your academic laboratory studies showing that CCR5i are dual function compounds in that they augment the ability of doxorubicin to treat cancer while protecting the heart from doxorubicin-induced damage.

By screening libraries of FDA approved drugs, your studies may fast track an approach to the clinic. Because the cardiotoxicity of doxorubicin is highly dose-dependent, being able to reduce the required dose of doxorubicin may also substantially reduce cardiotoxicity.

Your proposal to identify the optimal sequencing of a cardio protectant with DOX and to define the optimal combination of alternative reformulations of doxorubicin with the cardio protectants will inform clinical trial design. The team you have recruited for the GLP work is highly experienced. Your commercialization strategy, to work with companies developing reformulated doxorubicin products has substantial value for both the potential licensing companies and for patients. I look forward to your successful grant application and contributing to this effort.

Good luck with your proposal!

Best regards,



Alexander (Sasha) V. Kabanov, Ph.D., D.Sc. in Chemical Sciences,
M.A.E., FAAAS, FNAI, FAIMBE, FCRS, corr. member of RAS
Director, Center for Nanotechnology in Drug Delivery and Carolina Institute for Nanomedicine
Mescal S. Ferguson Distinguished Professor at the UNC Eshelman School of Pharmacy



DUKE UNIVERSITY MEDICAL CENTER

Dawn E. Bowles, Ph.D.
Assistant Professor
Department of Surgery
Division of Surgical Sciences
Co-director, Duke Human Heart Repository

September 28, 2023

Dear Richard,

It is my pleasure to write this letter of support and collaboration for your NIH grant entitled "*Improving Outcomes in Cancer Treatment-Related Cardiotoxicity*". The Duke Human Heart Repository is excited to collaborate with you to provide explanted cardiac tissue specimens for your exploration into the role, and therapeutic opportunities, around CCR5 antagonism in the prevention of anthracycline-induced cardiotoxicity.

I along with Dr. Carmelo Milano are currently the co-directors of the Duke Human Heart Repository and have considerable experience in the collection, storage, and usage of human myocardial tissues to address basic and translational research problems in human heart failure and disease. The Duke Human Heart Repository currently contains approximately 40,000 individual cardiac tissue specimens arising from nearly 1200 individuals. These specimens have been acquired from three sources: i) explanted diseased or failing human hearts from transplant procedures; ii) unused donor hearts from local organ procurement organizations; and iii) surgical excisions and other surgical protocols. The majority of samples are annotated with extensive demographic and clinical information adding considerable value to the tissue collection. Since the early 2000's the Duke Human Heart Repository has provided quality samples and clinical information to investigators both internal and external to Duke. This has enabled these investigators the opportunity to use the most relevant models to discover and test causes and treatments for human cardiovascular disease.

It is our vision to enable more investigators access to this rare and valuable resource. For your specific proposal, we can provide OCT embedded tissue for histological analysis and frozen tissues for transcriptomic and proteomic analysis. We have identified samples from 15 patients that meet your criteria. Below is a summary of these samples.

	Age (mean)	Race	Time post-AC	Gender (M:F)	Anthracycline	Cancer Types
DoxTox	54.8 years	Black 40%, White 46.7%, Other 13.3%	69.6 months	20%:80%	Doxorubicin 86.7%, Daunorubicin 13.3%	breast cancer, leukemia/lymphoma

We are enthusiastic about your research proposal and look forward to collaborating with you.

Sincerely,

A handwritten signature in black ink that reads "Dawn E. Bowles".

Dawn E. Bowles, PhD



Dr SEAN LAL

BMedSci (Hons), MBBS (Hons), MPhil, PhD, FRACP



Clinical Academic Cardiologist

Faculty of Medicine and Health, University of Sydney
Department of Cardiology, Royal Prince Alfred Hospital

Level 3 East | Charles Perkins Centre (D17) | School of Medical Sciences | University of Sydney | NSW 2006

Richard Pestell, A.O., M.D., Ph.D., MB., B.S., F.A.C.P., F.R.A.C.P., F.A.A.A.S., M.B.A.
President Pennsylvania Cancer and Regenerative Medicine Center
Institute Distinguished Professor
Baruch S. Blumberg Institute
3805 Old Easton Road
Doylestown, PA 18902

Re: Collaboration on NIH Proposal

28 March, 2024

Dear Richard,

I am writing to confirm my strong interest and enthusiasm to collaborate with you on your R01 grant application titled "*Improving Outcomes in Cancer Treatment-Related Cardiotoxicity*". We believe that your investigation into the pathogenic role for dysregulation of CCR5 and its ligands in the cardiotoxicity of DNA-damaging therapies is very exciting and timely. I am more than happy to share with you tissues from the Sydney Heart Bank for you to perform expression analysis for CCR5 and its ligands and to genotype the samples for CCR5- gene mutations as a predisposing factor to disease susceptibility. I would expect to ship tissues and sections to you within the month. Ethics is already in place as you know.

The Sydney Heart Bank is one of the largest human heart tissue banks in existence. Its mission is to provide high-quality human heart tissue for research into the molecular basis of human heart failure by working collaboratively with experts in this field. Collection has been ongoing for over 30 years and the bank currently contains over 17,000 myocardial samples. We can provide you with frozen biopsies (total 50 mg tissue each), histological sections and the associated clinical data for patients (in a de-identified manner) with confirmed anthracycline-induced cardiotoxicity and normal gender/age matched controls. Controls and heart failure samples are matched for anatomical region of relevance.

Director - Sydney Heart Bank | Head - Cardiac Research Laboratory | Faculty of Medicine and Health | University of Sydney

Consultant Cardiologist and Director - Acute Heart Failure Services | Royal Prince Alfred Hospital

Chair - Heart Failure Council | Cardiac Society of Australia and New Zealand



Dr SEAN LAL

BMedSci (Hons), MBBS (Hons), MPhil, PhD, FRACP

Level 3 East | Charles Perkins Centre (D17) | School of Medical Sciences | University of Sydney | NSW 2006
E sean.lal@sydney.edu.au



Clinical Academic Cardiologist

Faculty of Medicine and Health, University of Sydney
Department of Cardiology, Royal Prince Alfred Hospital

Samples available include:

	Number	Age (years)	Gender (%M/%F)	EF (%)
Normal Myocardium	8	30.5±18.5	69/31	62±7.6
Anthracycline DoxTox	8	26.7±8.7	85/15%	17±8.2

Completion of this project will provide important insights into developing novel adjunct therapies for patients with triple negative breast cancer and other cancers where anthracyclines and γ -radiation have cardiotoxic side effects that limit their clinical utility. I wish you success with your interesting and highly significant proposal. Look forward to continuing the fruitful collaboration with you.

Sincerely,

A handwritten signature in black ink, appearing to read "S. Lal".

Sean Lal

Director - Sydney Heart Bank | Head - Cardiac Research Laboratory | Faculty of Medicine and Health | University of Sydney

Consultant Cardiologist and Director - Acute Heart Failure Services | Royal Prince Alfred Hospital

Chair - Heart Failure Council | Cardiac Society of Australia and New Zealand



Department of Internal Medicine
College of Medicine
4301 W. Markham St., #832
Little Rock, AR 72205-7199
OFFICE: 501-686-7045
EYeh@UAMS.edu
medicine.UAMS.edu



Edward T. H. Yeh, M.D.
The Nolan Chair in Internal Medicine
Professor and Chairman

December 14, 2023

Professor Richard G. Pestell AO
Pennsylvania Cancer and Regenerative Medicine Center
Baruch S. Blumberg Institute
Lankenau Institute for Medical Research

Re: Letter of Support for SBIR Phase II Application

Dear Richard,

I am pleased to collaborate with you as consultant on your SBIR Phase II grant submission entitled "Improving Outcomes in Cancer Treatment-Related Cardiotoxicity". I am well qualified to serve in this role. My discovery of topoisomerase 2b as the molecular basis of anthracycline-induced cardiotoxicity has changed the old paradigm that reactive oxygen species generation from anthracycline is responsible for cardiotoxicity (Nature Medicine 18:1639, 2012). I have received multiple grants from the National Institute of Health and Cancer Prevention Research Institute of Texas on the subject of anthracycline-induced cardiotoxicity. I am a co-investigator of an active NIH-RO1 (HL151993) to study a novel strategy of using dexrazoxane to degrade topoisomerase 2b in order to protect the heart from doxorubicin-induced cardiotoxicity in breast cancer patients.

With this background I am pleased to provide consultive expertise for these proposed studies. I have discussed with you the recent studies and the Aims of the grant. I am very familiar with your academic laboratory studies showing that CCR5i are dual function compounds in that they augment the ability of doxorubicin to treat cancer while protecting the heart from doxorubicin-induced damage.

Identifying cardio protectants is very important. Doxorubicin is one of the most widely used chemotherapeutics and is employed in the treatment of a wide spectrum of pediatric and adult malignancies. The proposed cardiac studies are of broad importance with respect to many different types of malignancies. By screening libraries FDA approved drugs, your studies may fast track an approach to the clinic. Because the cardiotoxicity of doxorubicin is highly dose-dependent, being able to reduce the required dose of doxorubicin may also substantially reduce cardiotoxicity.

Your proposal to identify the optimal sequencing of cardio-protectant with DOX and to define the optimal combination of alternative reformulations of DOX with the cardio protectants will inform clinical trial design. The team you have recruited for the GLP work are highly experienced. Your commercialization strategy, to work with

companies developing reformulated DOX products has substantial value for both the potential licensing companies and for patients. I look forward to your successful grant application and contributing to this effort.

Sincerely,

A handwritten signature in dark ink, appearing to read "Edward T.H. Yeh".

Edward T.H. Yeh, M.D.
Nolan Family Distinguished Chair in Internal Medicine
Professor of Medicine
Chair, Department of Internal Medicine College of Medicine
University of Arkansas for Medical Science



Albert Einstein College of Medicine

Montefiore

Richard N. Kitsis, M.D.

Professor of Medicine and Cell Biology

The Dr. Gerald and Myra Dorros Chair in Cardiovascular Disease

Director, Wilf Family Cardiovascular Research Institute

Albert Einstein College of Medicine

1300 Morris Park Avenue

Forchheimer G46

Bronx, New York 10461

Telephone 718 430 2609

FAX 718 430 8989

Cell 917 538 0528

December 13, 2023

Professor Richard G. Pestell AO
Pennsylvania Cancer and Regenerative Medicine Center
Baruch S. Blumberg Institute
Lankenau Institute for Medical Research

Re: Letter of Support for SBIR Application

I am pleased to continue to collaborate with you as consultant on your SBIR Phase II grant submission entitled "Improving Outcomes in Cancer Treatment-Related Cardiotoxicity". I previously served as the Chief of Cardiology at Albert Einstein College of Medicine and Montefiore Medical Center and am currently director of the Einstein-Wilf Family Cardiovascular Research Institute. Over the past 30 years, my lab has studied fundamental mechanisms of cell death and roles of cell death in a variety of human diseases, in particular heart disease. Doxorubicin-induced cardiotoxicity has been a major focus of my work. Accordingly, I have the expertise to provide scientific advice on this topic.

I currently serve as the head of the clinical scientific advisory board of LightSeed and will provide consultive expertise for these proposed studies. We have collaborated in the academic sphere on several prior publications. I am very familiar with your academic laboratory studies showing that CCR5i are dual function compounds in that they augment the ability of doxorubicin to treat cancer while protecting the heart from doxorubicin-induced damage.

Your Identification of "dual function" compounds is important for two reasons. Firstly, because doxorubicin is one of the most widely used chemotherapeutics and is employed in the treatment of a wide spectrum of cancer types, the proposed cardiac studies are of broad importance with respect to many different types of malignancies. Secondly, identifying drugs that are FDA approved and that improve the efficacy of doxorubicin-induced cancer cell killing may allow a reduction in the dose of doxorubicin used in treating cancer. Because the cardiotoxicity of doxorubicin is highly dose-dependent, being able to reduce the required dose of doxorubicin may also substantially reduce cardiotoxicity.

This proposal to now define the optimal combination of alternative reformulations of DOX with the cardio protectants and to identify the optimal sequencing of cardioprotectant with DOX is essential to inform clinical trial design. The team recruited for the GLP are highly experienced increasing the likelihood of achieving the milestones proposed. As the patent application covers all reformulations of DOX the commercialization path forward appears feasible, contingent upon the successful outcome of these studies. I look forward to your successful grant application and contributing to this effort.

Sincerely,

A handwritten signature in black ink that reads "Richard N. Kitsis".

Richard N. Kitsis, M.D.

University of California San Francisco

BERKELEY • DAVIS • IRVINE • LOS ANGELES • RIVERSIDE • SAN DIEGO • SAN FRANCISCO • SANTA BARBARA



SANTA CRUZ • MERCED

JAVID MOSLEHI, MD

WILLIAM GROSSMAN DISTINGUISHED PROFESSOR OF MEDICINE
CHIEF, SECTION OF CARDIO-ONCOLOGY AND IMMUNOLOGY
PROFESSOR IN RESIDENCE, UCSF

MAIN OFFICE

Smith Cardiovascular Research Institute
555 Mission Bay Blvd. South,
Mail Code 3118
San Francisco, CA 94143

PHONE: (415) 502-3119 • FAX: (415) 476-9802

Richard Pestell, A.O., M.D., Ph.D., MB., B.S., F.A.C.P., F.R.A.C.P., F.A.A.A.S., M.B.A.
President Pennsylvania Cancer and Regenerative Medicine Center
Institute Distinguished Professor
Baruch S. Blumberg Institute
3805 Old Easton Road Doylestown, PA 18902
Re: Collaboration on Grant Proposal

December 13, 2023

Dear Richard,

I am pleased to continue to collaborate with you as consultant on your SBIR Phase II grant submission entitled "Improving Outcomes in Cancer Treatment-Related Cardiotoxicity". As a cardio oncologist my research program is actively investigating the mechanisms governing cardio toxicity consequent upon oncology therapeutics. Your proposal to study new compounds that have undergone prior FDA safety approval and repurpose as cardio protectants is novel and timely given the current cardio oncology epidemic recognized by NIH.

Your studies as proposed have additional broad significance to the field. Firstly, because doxorubicin is so widely used for many different types of cancer, cardiotoxicity remains a significant problem well beyond breast cancer. The preclinical data you have developed therefore has implications for cardiotoxicity writ large. Secondly, the three fluorophore mouse model you have developed, to study the immune response in both the heart and the tumor may be of real value to investigators studying other types of cardiotoxicity in which the immune response remains a mechanistic driver. Thirdly, your prior studies of over 2,000 breast cancers showing CCR5 is enriched in triple negative breast cancer, and a variety of other types of cancers, suggests this "dual function" model may be relevant to improving the efficacy of other DNA damage-based therapeutics. The specific Aims of the Phase II will be of substantial clinical relevance to fine tune the optimal clinical trial. Your testing of the new formulations of DOX and determining the optimal sequencing of cardioprotectant to the DOX reformulations in Aim 1 will be key to providing the optimal clinical trial design. Confirming the anti-tumor effect of the agents you have identified in Aim 2 will be of importance. Your prior published and preliminary data demonstrating the inhibition of tumor expansion by two of these agents strongly supports the feasibilities of the studies proposed in Aim 2. The GLP and toxicology studies in Aim 3 you are conducting with a very experienced team increasing the probability of a successful clinical translation of your studies. Please let me know how to help further and I look forward to this collaboration.

I wish you every success with this highly significant grant application.

A handwritten signature in black ink, appearing to read "Javid Moslehi, M.D.".

William Grossman Distinguished Professor in Cardiology
Section Chief, Cardio-Oncology & Immunology
Professor in Residence
Cardiovascular Research Institute (CVRI)
UCSF School of Medicine



January 3rd, 2024

Richard Pestell, MD, Ph.D
CEO
LightSeed, Inc.
2845 NE 9th Street, Apt. 604
Fort Lauderdale, FL 3334-3650
Telephone: 267-402-0545

Re: Letter of Commitment for grant application entitled: "**PHS 2023-2 Omnibus Solicitation of the NIH, CDC and FDA for Small Business Innovation Research Grant Applications (Parent SBIR [R43/R44] Clinical Trial Not Allowed).**" FOA Notice No. PA-23-230

Dear Dr. Richard Pestell:

Charles River Laboratories, Inc., together with its affiliates ("Charles River") is pleased to offer our experience, expertise, and professional resources in support of the LightSeed, Inc. ("LightSeed")'s above referenced grant proposal to the National Institute of Health (NIH), Small Business Innovations Research (SBIR) grants programs. Specifically, this confirms the intent of Charles River to serve as LightSeed's vendor to perform safety assessment and toxicology services on a Firm Fixed Price basis in support of this effort.

This Letter outlines in broad terms the relationship between Charles River and LightSeed that will be entered into once a grant has been awarded to LightSeed.

Once the grant is awarded, Charles River and LightSeed will execute a definitive contract including fixed prices for specific services, if one is not already in place at that time. Charles River will work closely with LightSeed to carry out its responsibilities of performance under the contract. All study information, publications rights, and patents related to the sponsor's compound are handled in accordance with NIH policy or specified in a master service agreement.

Charles River's expertise and staff depth will help to ensure that the program's requirements (time allotment and required facilities) are met in a timely manner, and our work on this program will be prioritized to allow us to meet all agreed upon timelines. Leveraging a global network of AAALAC-accredited preclinical facilities, we design and perform *in vitro* and *in vivo* safety programs to best characterize potential human drug toxicity. Charles River currently operates 110+ safety assessment facilities in 20+ countries in the US, Canada, UK, and Europe. Charles River provided support for the development of ~80% of the drugs approved by the FDA in the past 5 years.

Charles River represents that the services and/or products provided under this Letter of Commitment qualify as "commercial items and services" as defined in FAR 2.101.

Charles River welcomes the opportunity to support LightSeed and your efforts in this important program. If you have any questions or require additional information, please do not hesitate to contact us.

Best Regards,

Carmen L. Falcon

Carmen L. Falcon, Proposal Specialist
Proposal Management Team | Government Contracts | Business Relations
Mobile: 301-401-2613

RESOURCE SHARING PLAN

LightSeed and its collaborators at Baruch S. Blumberg Institute will adhere to the NIH Grants Policy on Sharing of Unique Research Resources including the “Sharing of Biomedical Research Resources: Principles and Guidelines for Recipients of NIH Grants and Contracts”. We will make the results and accomplishments of this research available to the research community and to the public at large by the timely release and sharing of data. As a means of sharing knowledge, the investigators supported by this grant will seek to publish the original research in primary scientific journals. For each publication that results from the grant-supported research, we will include an acknowledgment of NIH grant support and follow guidelines regarding free access to published materials. Information on each publication resulting from work performed under the NIH grant-supported project will be included in the annual and/or final progress report submitted to the NIH awarding office. We will work with other investigators to respond to requests for data for reanalysis or assistance replicating the research, and all reasonable requests will be accommodated given a sound scientific rationale and purpose, appropriate data and privacy protections, feasibility of complying with the request, and compliance with the policies of all participating institutions and organizations.

LightSeed will follow on Dr. Pestell’s model on resource sharing. Reagents generated in his laboratory have been shared widely with the research community. More than 300 national and international laboratories have received DNA clones, transgenic lines, and inducible mice from his laboratory since 10/2006. Any reagent generated with funds from this grant will be freely distributed upon request to qualified academic investigators for non-commercial research upon signing of an MTA. Material transfers would be made with no more restrictive terms than in the Simple Letter Agreement or the UBMTA and without reach through requirements.

INTELLECTUAL PROPERTY RIGHTS

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

NIH Generated message:

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

AUTHENTICATION OF KEY BIOLOGICAL AND/OR CHEMICAL RESOURCES

Overview:

- Our laboratory follows NIH basic best practices for data storage, basic experimental design and statistical tests. All cell lines, antibodies, chemicals and animal usage are fully authenticated.

1. Cell Lines

- Cell lines were either obtained from ATCC or developed in my laboratory. Cell lines authenticated from ATCC (Py230, Py8119) (AC16, H9C2) and PDX (WU-BC3, WU-BC4, WHIM3, BCM-4913, gifts from and authenticated by Siteman Cancer Center Breast Cancer Program, Washington University in St. Louis), will also be authenticated by examination for morphology by microscope and analysis of doubling times. If there are any concerns regarding authenticity the cells will be tested by ATCC using their complete cell line authentication service utilizing Short Tandem Repeat (STR) profiling. The authentication of cell lines includes Western blot analysis for the oncogene used to transform the cells. Cells are used from the original freeze of the lines, which are stored in liquid N2. The cells are free of pathogens, oncogene expression is validated by Western blot and aliquots of cells are frozen at the time of generation to ensure their authenticity. Mycoplasma testing is conducted semiannually or upon any suspicion of contamination using the Universal Mycoplasma Detection Kit" from ATCC. (catalog 30-1012K). For mycoplasma treatment, we use "Plasmocin" from Invivogen and the Catalog number is "ant-mpt".

2. Antibodies

- Antibodies utilized in the studies have been purchased by reputable vendors and remain in a centralized stock and not aliquoted, lot number and catalogue number for each antibody will be quoted in manuscript text. Antibodies are authenticated using positive control cell lines from the literature, with analysis of the target protein verified by molecular weight on Western blot and/or epitope tagged proteins and or by the behavior of the protein in response to a known stimulus or gene knockdown. Cardiac troponin T (cTnT; MBS262307, MyBioSource), and brain natriuretic protein (BNP; LSF25183-1, LSBio). Antibodies (CCL3 (MBS7049468), CCL4, CCL5 (MBS9706754) and CD31 (MBS2518602) were purchased from MyBiosource. APC conjugated mouse anti-human/mouse/rat CCR5 antibody (FAB1802A) (R&D Systems (Minneapolis, MN). For TnT (ab45932), CD31 (ab28364), mTOR^{Ser2448P} (ab109268) (Abcam (Waltham, MA). For p70S6K (#9202), p70S6K^{Thr389P} (#9205), mTOR (#2983), γH2A.X (#80312), H2A (#3636) or activated Caspase 3 (#9661) (Cell Signaling (Danvers, MA). Antibodies against Bcl2 (SC7382) and Lamin B1 (SC-377000) (Santa Cruz Biotechnology (Dallas, TX). Antibodies against the apoptosis inhibitor ARC (RA15042) (Neuromics (Edina, MN) and Top2B antibody (PA527750) (Thermo Fisher Scientific (Waltham, MA))

3. Specialty Chemicals

- Antagonists, agonists and other drugs used in the study will be obtained from a reputable source and purchased as pharmaceutical grade. siRNA obtained efficacy will be assessed through western blot of the target molecule. New batch testing of maraviroc, virciviroc and dexamzoxane (Selleck Chemicals, (Houston, TX), is conducted using ligand calcium flux assays for CCR5i and batch quality control from the vendor. DOX was obtained from Millipore Sigma (St. Louis, MO). Reformulated DOX will be provided by individual vendors who provide QC authentication as described in the application text.

4. Purified Proteins

- N/A

5. Vertebrate Animals

- Female C57BL/6J mice were obtained from and authenticated by Charles River through SNP analysis to validate strain background of mice. (PCR was used to confirm Nnt mutation). FVB and Nude mice (BALB/c and NCr-nu/nu) 6-8 weeks will be obtained from the National Cancer Institute. PDX will be grown in hNSG (humanized nonobese diabetic/severe combined immunodeficiency IL2R^{Ynull} (hNSG) mice will be obtained from and authenticated by Jackson laboratories.

6. Plasmids

- All plasmids to be used are sequence validated in our laboratory.

7. Nucleic acids (e.g. siRNA, shRNA, gRNA, etc.)

- CCR5 siRNA/shRNA knockdown will be conducted using multiple RNAs and scrambled negative controls with efficacy validated immunoblot and RT-PCR.