PI: **Liu, George Y**	Title: Interrogating human anti-staphylococcal antibody responses for S. aureus vaccine insights	
Received: 04/10/2023	Opportunity: PA-20-185	Council: 10/2023
Competition ID: FORMS-H	FOA Title: NIH Research Project Grant (Parent R01 Clinical Trial Not Allowed)	
1R01AI181321-01	Dual:	Accession Number: 4826613
IPF: 577507	Organization: UNIVERSITY OF CALIFORNIA, SAN DIEGO	
Former Number:	Department: Pediatrics	
IRG/SRG: IHD	AIDS: N	Expedited: N
Subtotal Direct Costs (excludes consortium F&A) Year 1: [redacted] Year 2: [redacted] Year 3: [redacted] Year 4: [redacted] Year 5: [redacted]	Animals: Y Humans: Y Clinical Trial: N Current HS Code: 30 HESC: N HFT: N Special Topics: Data Management Sharing	New Investigator: N Early Stage Investigator: N

Senior/Key Personnel:	*Organization:*	*Role Category:*
George Liu Ph.D	The Regents of the Univ. of Calif., U.C. San Diego	PD/PI
James Slaughter	Vanderbilt University	Co-Investigator
Chih-Ming Tsai	The Regents of the Univ. of Calif., U.C. San Diego	Co-Investigator
Isaac Thomsen	Vanderbilt University	Consultant
C. Creech M.D.	Vanderbilt University Medical Center	MPI

OMB Number: 4040-0001
Expiration Date: 11/30/2025

APPLICATION FOR FEDERAL ASSISTANCE
SF 424 (R&R)

3. DATE RECEIVED BY STATE	State Application Identifier

1. TYPE OF SUBMISSION*

❍ Pre-application ● Application ❍ Changed/Corrected Application

4.a. Federal Identifier

b. Agency Routing Number

2. DATE SUBMITTED

Application Identifier
50259

c. Previous Grants.gov Tracking Number

5. APPLICANT INFORMATION

UEI*: [redacted]

Legal Name*: The Regents of the Univ. of Calif., U.C. San Diego
Department: Health Sciences SPO
Division: School of Medicine
Street1*: [redacted]
Street2: [redacted]
City*: [redacted]
County:
State*: CA: California
Province:
Country*: USA: UNITED STATES
ZIP / Postal Code*: [redacted]

Person to be contacted on matters involving this application

Prefix: First Name*: Angela Middle Name: Last Name*: Vivola Suffix:

Position/Title: Senior Grant Analyst
Street1*: [redacted]
Street2: [redacted]
City*: [redacted]
County:
State*: CA: California
Province:
Country*: USA: UNITED STATES
ZIP / Postal Code*: [redacted]
Phone Number*: [redacted] Fax Number: Email: [redacted]

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

7. TYPE OF APPLICANT* H: Public/State Controlled Institution of Higher Education

Other (Specify):

Small Business Organization Type ❍ Women Owned ❍ Socially and Economically Disadvantaged

8. TYPE OF APPLICATION*

● New ❍ Resubmission
❍ Renewal ❍ Continuation ❍ Revision

If Revision, mark appropriate box(es).

❍ A. Increase Award ❍ B. Decrease Award ❍ C. Increase Duration
❍ D. Decrease Duration ❍ E. Other *(specify)* :

Is this application being submitted to other agencies?* ❍Yes ●No What other Agencies?

9. NAME OF FEDERAL AGENCY*
National Institutes of Health

10. CATALOG OF FEDERAL DOMESTIC ASSISTANCE NUMBER
TITLE:

11. DESCRIPTIVE TITLE OF APPLICANT'S PROJECT*
Interrogating human anti-staphylococcal antibody responses for S. aureus vaccine insights

12. PROPOSED PROJECT

Start Date* Ending Date*
09/01/2023 08/31/2028

13. CONGRESSIONAL DISTRICTS OF APPLICANT
CA-050

SF 424 (R&R) APPLICATION FOR FEDERAL ASSISTANCE

14. PROJECT DIRECTOR/PRINCIPAL INVESTIGATOR CONTACT INFORMATION

Prefix: First Name*: George Middle Name: Last Name*: Liu Suffix: Ph.D
Position/Title: Professor and Chief
Organization Name*: The Regents of the Univ. of Calif., U.C. San Diego
Department: Pediatrics
Division: Infectious Diseases
Street1*: [redacted]
Street2: [redacted]
City*: [redacted]
County:
State*: CA: California
Province:
Country*: USA: UNITED STATES
ZIP / Postal Code*: [redacted]
Phone Number*: [redacted] Fax Number: Email*: [redacted]

15. ESTIMATED PROJECT FUNDING

a. Total Federal Funds Requested* $[redacted]
b. Total Non-Federal Funds* $[redacted]
c. Total Federal & Non-Federal Funds* $[redacted]
d. Estimated Program Income* $[redacted]

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*: Angela Middle Name: Last Name*: Vivola Suffix:
Position/Title*: Senior Grant Analyst
Organization Name*: The Regents of the Univ. of Calif., U.C. San Diego.
Department: Health Sciences SPO
Division: School of Medicine
Street1*: [redacted]
Street2: [redacted]
City*: [redacted]
County:
State*: CA: California
Province:
Country*: USA: UNITED STATES
ZIP / Postal Code*: [redacted]
Phone Number*: [redacted] Fax Number: Email*: [redacted]

Signature of Authorized Representative*
Angela Vivola

Date Signed*
04/10/2023

20. PRE-APPLICATION File Name:

21. COVER LETTER ATTACHMENT File Name:Liu_Creech_R01_Cover_letter.pdf

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 ... 5
Project Summary/Abstract(Description) ... 6
Project Narrative ... 7
Facilities & Other Resources ... 8
Equipment ... 12
Research & Related Senior/Key Person ... 14
Research & Related Budget Year - 1 ... 37
Research & Related Budget Year - 2 ... 40
Research & Related Budget Year - 3 ... 43
Research & Related Budget Year - 4 ... 46
Research & Related Budget Year - 5 ... 49
Budget Justification ... 52
Research & Related Cumulative Budget ... 54
Research & Related Budget - Consortium Budget (Subaward 1) ... 56
Total Direct Costs Less Consortium F&A ... 75
PHS398 Cover Page Supplement ... 76
PHS 398 Research Plan ... 78
Specific Aims ... 79
Research Strategy ... 80
PHS Human Subjects and Clinical Trials Information ... 92
Study 1: Interrogating human anti-staphylococcal antibody responses for staphylococcal vaccine insights ... 94
Inclusion Enrollment Reports ... 100
Vertebrate Animals ... 109
Select Agent Research ... 110
Multiple PD/PI Leadership Plan ... 111
Bibliography & References Cited ... 112
Consortium/Contractual Arrangements ... 116
Letters of Support ... 117
Resource Sharing Plan(s) ... 118
Other Plan(s) ... 119
Authentication of Key Biological and/or Chemical Resources ... 120

OMB Number: 4040-0010
Expiration Date: 11/30/2025

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: The Regents of the Univ. of Calif., U.C. San Diego.
UEI: [redacted]
Street1*: [redacted]
Street2: [redacted]
City*: [redacted]
County: San Diego
State*: CA: California
Province:
Country*: USA: UNITED STATES
Zip / Postal Code*: [redacted]
Project/Performance Site Congressional District*: [redacted]

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: Vanderbilt University Medical Center
UEI: [redacted]
Street1*: [redacted]
Street2:
City*: [redacted]
County:
State*: TN: Tennessee
Province:
Country*: USA: UNITED STATES
Zip / Postal Code*: [redacted]
Project/Performance Site Congressional District*: [redacted]

Additional Location(s) File Name:

Funding Opportunity Number: PA-20-185. Received Date: 2023-04-10T16:10:08.000-04:00

OMB Number: 4040-0001
Expiration Date: 11/30/2025

RESEARCH & RELATED Other Project Information

1. Are Human Subjects Involved?* ● **Yes** ❍ **No**

1.a. If YES to Human Subjects

Is the Project Exempt from Federal regulations? ❍ Yes ● No

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

If NO, is the IRB review Pending? ● **Yes** ❍ **No**

IRB Approval Date:

Human Subject Assurance Number [redacted]

2. Are Vertebrate Animals Used?* ● **Yes** ❍ **No**

2.a. If YES to Vertebrate Animals

Is the IACUC review Pending? ● Yes ❍ No

IACUC Approval Date:

Animal Welfare Assurance Number [redacted]

3. Is proprietary/privileged information included in the application?* ❍ **Yes** ● **No**

4.a. Does this project have an actual or potential impact - positive or negative - on the environment?* ❍ **Yes** ● **No**

4.b. If yes, please explain:

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

4.d. If yes, please explain:

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

5.a. If yes, please explain:

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

6.a. If yes, identify countries:

6.b. Optional Explanation:

	Filename
7. Project Summary/Abstract*	Lui_Creech_Summary_Abstract.pdf
8. Project Narrative*	Liu_Creech_Narrative.pdf
9. Bibliography & References Cited	References_-_final.pdf
10.Facilities & Other Resources	Combined_Facilities_and_Resources.pdf
11.Equipment	Liu_Creech_R01_Combined_Equipment.pdf

Funding Opportunity Number: PA-20-185. Received Date: 2023-04-10T16:10:08.000-04:00

Abstract

Staphylococcus aureus is a leading cause of infection worldwide and a major driver of antibiotic resistance. Although many experimental staphylococcal vaccines have been reported, all vaccines tested to date in human trials have failed for unclear reasons. Unlike mice, humans are exposed to *S. aureus* beginning early in life, leading to generation of antibodies to *S. aureus* antigens. In preliminary experiments, we have shown that select human anti-*S. aureus* antibodies are protective, but many are not. In mice exposed to *S. aureus*, vaccination against protective antigens leads to immunity against *S. aureus* whereas vaccination against non-protective antigens induced recall of non-protective immunity which further interferes with protective antibodies by direct competition. Based on these findings, we generated a model of how pre-existing antibodies shape vaccine responses and how this predicts novel ways to develop effective vaccines against *S. aureus*. To query the validity of our model, in Aim 1, we will recruit children and old adults with invasive *S. aureus* infections, survey the anti-*S. aureus* antibody profile and define functionally protective antibody responses to *S. aureus* antigens. In Aim 2, we will identify and characterize protective and non-protective anti-*S. aureus* antibodies from candidate samples acquired in Aim 1, and assess structural and functional features of the specific antibodies that confer protection or non-protection. In Aim 3, we will study these antibodies and their target in the context of naïve mice and mice previously exposed to *S. aureus*. We will evaluate mechanisms whereby non-protective memory shapes vaccine efficacy and test strategies that circumvent interference. Overall, using the novel model systems, we aim to develop a more predictive framework for explaining staphylococcal vaccine failures and developing novel strategies for effective vaccination against *S. aureus*.

Narrative

Based on preliminary studies of *S. aureus* vaccine failures, we have generated a model of how pre-existing anti-staphylococcal humoral response shapes protective and non-protective immune responses to *S. aureus*. We will leverage new in vivo model systems to interrogate our hypothesis using protective and non-protective antibodies isolated from children and elderly subjects with invasive *S. aureus* disease.

FACILITIES AND RESOURCES: UCSD

Laboratory:
Dr. Liu is assigned ~ 1,000 square feet of space including 2 research bays (4 full 8-foot benches and full desks per bay = 8 workstations) and a shared tissue culture room and procedure room within a large open floor design in the [redacted] on the [redacted] of the state-of-the-art, [redacted] building in the heart of the UCSD Health Sciences Campus. The Liu laboratory has all facilities and equipment required for modern molecular microbiology and immunity research. The lab routinely studies a number of bacterial pathogens, including *S. aureus, S. pyogenes and S. agalactiae*, under biosafety level II protocols approved by the UCSD Biosafety Committee, with every lab member trained to handle BSL II pathogens. Core facilities for DNA and protein sequencing, electron and fluorescent de-convolution microscopy, microarray, genomics and proteomics are located in the same or adjacent buildings. Members of the Liu lab participate in joint research forums with several investigators in microbiology/immunology and therefore all trainees have diverse opportunities for critical insight and feedback on all aspects of their ongoing work. The number of high-profile seminar series in biomedical sciences, often featuring outside speakers of international renown, occur in the auditoriums of [redacted], and the adjacent [redacted] several times each week throughout the academic calendar and lab attendance is encouraged.

Office:
Dr. Liu has a private office and a 50% dedicated administrative assistant who can help him in grant administration and manuscript submissions. Dr. Liu's floor has a large conference room with videoconferencing capability that is shared with Immunity, Infection & Inflammation investigators and the adjacent UCSD Glycobiology Research and Training Center. The Liu Lab has continuous footprint with benches and offices adjacent to the Victor Nizet laboratory group focused on Gram-positive infection, the Bernhard Palsson laboratory group focused on microbial systems biology relevant to human infectious disease, the Lance Prince Laboratory working on pulmonary inflammation, the Hal Hoffman Laboratory studying inflammasome function in innate immunity, and the Investigators of the UCSD Glycobiology Research and Training Center, including Ajit Varki (sialic acids) and Jeff Esko (glycosaminoglycans).

Animal Facility:
UCSD is fully accredited by the AAALAC. There are currently over 102,000 sq. ft. of animal facilities at [redacted] locations. Veterinary care for all University research animals is under the supervision of the campus office of Veterinary services on a 24 hour basis, including holidays and weekends, in accordance with the PHS, U.S. Department of Agriculture and AAALAC requirements. The Liu lab uses mice under the approved IACUC protocol S18200.

Computers:
The Liu laboratory proper has 4 PCs, color inkjet and laser printers, high resolution scanners and digital cameras, an LCD projector, high speed internet access and extensive software for DNA sequence analysis, molecular biology, statistics, literature search and referencing, graphics, etc. All trainees have personal laptop computers with Ethernet access at 8 individual desk stations.

Others:
Other resources that are accessible on the 4th floor next to the lab include a Kodak digital camera/documentation system and transilluminator, flow cytometer, a Molecular Biology Assistant desktop spectrophotometer, a Bioscreen-C Automated Growth Curve Analysis System, a nanodrop spectrophotometer, a Enspire high performance ultrasensitive multiplate reader (fluorescence, luminescence, absorbance) with automated stacker, a Bravo Liquid Handling Robotic Platform with 394 well capability for High throughput screening, a hybridization oven, two tube spectrophotometers, a UV crosslinker, and Nikon standard and inverted microscopes with fluorescence capability and video monitor attachments, acquired a Riveal High-Contrast Microscopy System with Two-Fluorescence and live imaging capabilities. The laboratory complex also includes a darkroom with developer, a walk-in cold room and an autoclave/glasswash facility. Please see the Equipment section for additional details. Please see the Equipment section for additional details.
CFAR animal facility at UCLA will be the vendor source of humanized BLT mice. HDX-MS sequencing, BCR sequencing, generation of B cell hybridomas and determination of antibody glycosylation will be outsourced (Creative Biolabs, NY, www.creative-biolabs.com).

FACILITIES AND RESOURCES: VANDERBILT

Vanderbilt University Medical Center has a rich history of scientific discovery and collaborative research. To ensure this legacy continues, the institution has invested considerably in its research mission in terms of physical space, centralized research support services, recruitment and retention of established and early stage investigators, and educational programs in research. The result is an outstanding and highly collaborative scientific environment that is second to none in terms of institutional support, physical and educational resources, faculty development, and intellectual camaraderie. A detailed description of selected resources and their relevance to the proposed research program are provided in the paragraphs below.

Laboratory Facilities

The Vanderbilt Vaccine Research Program (VVRP). Dr. Creech serves as the director of the VVRP Laboratory, and all equipment and facilities contained within this ample laboratory space is available for use in this proposal.

The VVRP exists as four separate rooms in a shared hallway within the [redacted] space in [redacted], each with dedicated uses and with ample desk and bench space. In total, the Laboratory consists of 1477 square feet of research space, and the rooms are allocated for: molecular epidemiology and serology (D7215), microbiology (D7214), cell culture and sample processing (D7209), and immunology (D7201). The laboratory has sufficient capital equipment, biologicals, tissue culture hoods, freezers, refrigerators, incubators, and supplies for all proposed microbiologic and immunologic work (*See: Equipment Attachment*). Within the Pediatric ID Research space on the same floor, Dr. Creech also has full access to the shared equipment room, which includes an additional microplate reader, an infrared imager, two ultracentrifuges, quantitative PCR apparatus (2), Nucleic Acid Extraction System, and a walk-in cold room for overflow cold storage.

Biosafety. The Creech laboratory is designated as a Biohazard Safety Level 2 facility, meaning that all equipment and resources are in place to work with the biohazardous agents described in this application (primarily *Staphylococcus aureus*). Furthermore, all members of the Creech laboratory undergo extensive training prior to working with biohazardous material. We have worked closely with the Institutional Biosafety Officers at Vanderbilt to ensure that BSL-2 practices are maintained during all facets of this proposal. No select agents will be employed within the proposal.

Computers. The Creech Research Team uses primarily Apple operating systems and hardware for collection and analysis of data. Each of the 3 iMac workstations and 3 MacBook Pro devices are protected through appropriate antiviral and VPN software, and run both Apple OS Sierra and Windows 10 via VMware. Statistical analysis software (Stata), reference software (EndNote), Adobe Professional Suite, and appropriate productivity software suites are provided by the Department of Pediatrics to these workstations. All computers in the Creech lab are linked to a shared server that can be accessed from each computer ensuring that all data are backed up on tape. A networked, color laser printer is housed in the Creech laboratory space.

Office Space and Administrative Support. Dr. Creech has 122 sq. ft. of private office within the Vanderbilt Vaccine Research Program. This office is equipped with a 21.5” iMac Desktop Computer, 22” Dell auxiliary widescreen display monitor, Dell 2300d Laser Printer, phone, and ample file and shelf space. Dr. Creech’s office is adjacent to a central administrative office with a copier, fax machine, scanner, and office supplies.

Cell-Sorting Core Lab: The Creech lab has access to the Vanderbilt Flow Cytometry and Cell Sorting Core Laboratory, an institutional facility, which will be used for the cell sorting and flow cytometry experiments. The core has 3 dedicated operators and a Managing Director, 2 custom LSRII analyzers, a Fortessa five-laser cytometer, three custom FACSAria III sorters, all with aerosol containment for protection of operators during sorting of unfixed materials.

VANTAGE (Vanderbilt Technologies for Advanced Genomics, vantage.vanderbilt.edu) provides Vanderbilt researchers with guided access to a diverse and comprehensive set of genomics workflows, including next generation sequencing, Sanger sequencing, high- mid- and low-throughput genotyping, expression microarray,

flow cytometry, and bio-banking. These services are important to the proposed work in this application. The VANTAGE facility occupies 12,505 square feet of newly renovated lab space, supported by over $[redacted] of federal and institutional funding. The 28 staff and managers of VANTAGE have contributed data and analysis to hundreds of successful genomics experiments, manuscripts, and grant applications. Sequence analysis experiments will be conducted in VANTAGE.

Vanderbilt University Medical Center (VUMC). VUMC has built a strong national reputation as a leader in medical education, research, and patient care over the course of its 136-year history. The Medical Center is driven by discovery and the incorporation of new knowledge into patient care, education, and research.

The Vanderbilt campus is comprised of 300 acres and was designated as a national arboretum in 1988. Buildings on the original campus date to 1873, the year Vanderbilt was founded, and the Peabody section of campus has been a registered historic landmark since 1966. VUMC is located between the undergraduate and Peabody campuses and has approximately 7.2 million square feet of building space. The close proximity of Vanderbilt University and Vanderbilt Medical Center promotes interactions, sharing of resources, and collaboration. VUMC has greatly expanded its physical plant beyond Medical Center North (1925) through construction of Rudolph Light Hall (1977), Vanderbilt University Hospital (1980), the Vanderbilt Clinic (1988), the Robinson Research Building (1989), Medical Center East (1990), the Village at Vanderbilt (1990), the Eskind Biomedical Library (1994), and the Monroe Carell Jr. Children's Hospital (2004). To stimulate and support its unparalleled growth in biomedical research, Vanderbilt has aggressively expanded research space over the last two decades, creating the Preston Research Building (1995; 205,000 sq. ft.), the Vanderbilt-Ingram Cancer Center (2001; 54,000 sq. ft.), Medical Research Building III (2002; 289,643 sq. ft.), the Medical Center East South Tower (2005; 300,000 sq. ft.), and Medical Research Building IV (2008, 290,820 sq. ft.).

The Medical School's reputation for outstanding research is reflected in the amount of federal and private support it receives, with the School of Medicine now ranking No. 8 in the nation for NIH funding, as of federal fiscal year 2016. Paralleling the growth in research has been a clear commitment to the training and mentorship of physician investigators in basic, translational, clinical and population-based science. This commitment includes 91 NIH career development (K) awards, 65 T32 grants, and 5 NIH K12/KL2 awards. As a result of these training opportunities, approximately 75% of trainees across the institution remain in academic medicine.

The Monroe Carell Jr. Children's Hospital at Vanderbilt and the Department of Pediatrics. The Monroe Carell Jr. Children's Hospital at Vanderbilt, a 276-bed tertiary care teaching hospital occupying over 650,000 square feet of physical space, is the only tertiary care pediatric health center in Middle Tennessee. Consistently ranked as one of the nation's best children's hospitals by *Parents* magazine and *U.S. News and World Report* magazine, the hospital has over 300,000 pediatric visits per year, including over 235,000 clinic visits, 52,000 visits to the Pediatric Emergency Department and 13,800 inpatient encounters. Constructed in 2004, the freestanding children's hospital is filled with state-of-the-art equipment and information systems to provide the best treatment for children, and it offers a variety of accommodations for family-centered care. As a nonprofit teaching and research hospital, the hospital serves patients from diverse socioeconomic backgrounds. The Children's Hospital is also the institutional base for the Department of Pediatrics at Vanderbilt, training over 200 medical students, residents, and fellows each year.

The Department of Pediatrics at Vanderbilt has 450 full-time faculty and 114 residents in training across 13 subspecialties. Nearly 50% of the faculty holds federally funded grants, and many serve on NIH study sections and advisory panels. **In 2016, the Department of Pediatrics received NIH funding greater than $[redacted] ranking 4th in total NIH funding to pediatrics departments at US medical schools with total grant funding exceeding $[redacted]** Currently 38 active faculty are members of the Society for Pediatric Research, 6 are members of the American Society for Clinical Investigation, 16 are members of the American Pediatric Society, 4 are members of the Institute of Medicine, and 4 have received the E. Mead Johnson Award.

Division of Pediatric Infectious Diseases. The Department of Pediatrics' Division of Pediatric Infectious Diseases, established in 1974 and directed by Mark Denison, MD, is comprised of 20 full-time faculty and is one of the largest in the country. In addition to providing inpatient and outpatient clinical care of children with infectious diseases and immunodeficiencies, division faculty are heavily engaged in research, medical education, and quality improvement. The division consistently receives the largest amount of research funding

in the Vanderbilt Department of Pediatrics, with over $[redacted] in annual research funding for the past several years. The Division enjoys investigative strengths in microbial pathogenesis, human immunology, vaccine science, and public health.

Department of Biostatistics. The School of Medicine at Vanderbilt created the Department of Biostatistics in September 2003 with a major funding commitment to build a world-class department. Chaired by Yu Shyr, the department offers a full array of biostatistical support with an emphasis on establishing long-term collaborative relationships with investigators. The Department currently has 45 faculty biostatisticians and 60 staff Masters-level biostatisticians. The department operates the Biostatistics Shared Resource for the Vanderbilt Ingram Cancer Center, the VICTR Design, Biostatistics, and Research Ethics Core, the Statistics and Methodology Core for the Vanderbilt Kennedy Center for Research on Human Development, and the Biostatistics Collaboration Center (BCC). The department also holds a walk-in Biostatistics Clinic each day. These clinics are designed for Vanderbilt and Meharry Medical College community members to receive assistance from experienced biostatisticians in study design, measurement refinement, statistical analysis, and statistical interpretation of their results. The Biostatistics Clinic staff also assists investigators in navigating all the quantitative resources at Vanderbilt. Dr. Creech will continue to take full advantage of the Department's expertise and programs (led by Key Study Personnel Dr. Christopher Slaughter).

Scientific Environment and Institutional Resources

Vanderbilt Institute for Clinical and Translational Research (VICTR). Vanderbilt University (VU) was fortunate to be chosen early (2007) to receive one of approximately 60 Clinical and Translational Science Awards (CTSA). Through these awards, National Center for Research Resources (NCRR), a part of the National Institutes of Health (NIH), supports a national consortium of medical research institutions working together to improve the way biomedical research is conducted across the country, and Vanderbilt serves as the coordinating center for the CTSAs. The CTSA, along with the Vanderbilt Office of Research, sponsors the Vanderbilt Institute for Clinical and Translational Research (VICTR), an extraordinarily rich enterprise designed to transform the way ideas and research discoveries make their way from origin to patient care. This is accomplished using a multi-faceted approach: through collaboration with a wide variety of research partners; by training, nurturing and rewarding participating researchers; by providing research funding and clinical resources; by developing new and innovative ways to involve the community in research; by developing new informatics and biostatistical systems; and by making available the latest technologies and sound research results affecting patient care. There are many resources available to investigators through VICTR and the CTSA). The VICTR Program provides an environment highly suited to Dr. Creech's success as a translational scientist conducting high-quality patient-oriented research, and resources particularly pertinent to the current proposal are outlined below:

- **Research Electronic Data Capture (REDCap)**: REDCap is a secure, web-based application developed at Vanderbilt and designed to support traditional case report form data capture for research studies. REDCap is widely used in the Vanderbilt research enterprise and across a large consortium of institutional partners. Investigators can access this intuitive interface for data entry, data validation, audit trails, export procedures, seamless downloads to statistical packages and more. Research Support Services staff are available to guide investigators in the use of this product. Another product is REDCap Survey: a powerful tool for building and managing online surveys with data that is easily exported to Microsoft Excel or other statistical analysis packages. The Creech Laboratory team has extensive experience with REDCap will use it for efficient and secure data management in the proposed studies.

- **StarBRITE Web Portal**: StarBRITE is an interactive system that provides a single portal for researchers to identify resources, obtain regulatory support, access templates for research preparation and study conduct, obtain database development software, learn about educational requirements and opportunities, find research volunteers, and more. StarBRITE also provides institutional application and research approval process support. Unique features of the site includes a Customized Action Plan, which guides researchers through the regulatory process via a comprehensive list of applications tailored to each investigator's individual study, and My Research, which allows investigators to view applications and see status from departments such as IRB, Grants and Contracts, funding support requests, web-based data resources for individual studies and more.

EQUIPMENT

All major equipment required for the proposed work at the UCSD site are available in the **Liu Laboratory** and in the adjacent core space. Available to Liu lab members are: -80, -20, and 4°C freezer/refrigeration units, two 5 cubic ft, water-jacketed $C0_2$ tissue culture incubators, two 5 cubic foot standard incubators, 2 Forma shaking incubators, two Eppendorf desktop centrifuges, six microfuges, two thermal cyclers, one real-time RT-PCR system, numerous DNA and protein gel apparatuses with power supplies, an electroporation apparatus, several shakers, a Kodak digital camera/documentation system and transilluminator, a Molecular Biology Assistant desktop spectrophotometer, a Bioscreen-C Automated Growth Curve Analysis System, a nanodrop spectrophotometer, a Enspire high performance ultrasensitive multiplate reader (fluorescence, luminescence, absorbance) with automated stacker, a Bravo Liquid Handling Robotic Platform with 394 well capability for high throughput screening, a hybridization oven, two tube spectrophotometers, a UV crosslinker, a Nikon standard and inverted microscopes with fluorescence capability and video monitor attachments, a Riveal High-Contrast Microscopy System with two-fluorescence and live imaging capabilities, a BD FACSCanto II and a BD FACSCalibur. Additional equipment to which there is ready access include refrigerated superspeed centrifuges, ultracentrifuges, liquid nitrogen storage, SpeedEvac, a Beckman DU 640 UV spectrophotometer, top-loading and balances. The laboratory complex also includes a darkroom with developer, a walk-in cold room and an autoclave/glasswash facility.

For the components of the proposal to be conducted at the VUMC site, the **Creech Laboratory** exists as four separate rooms in a shared hallway within the Pediatric Infectious Diseases space at Vanderbilt, each with dedicated uses and with ample desk and bench space. In total, the Laboratory consists of 1477 square feet of research space, which are fully functioning BSL-2 level facilities, with cell culture and infectious agent equipment.

The equipment contained in the dedicated microbiology laboratory (D7214) for use in this proposal includes:

- Innova 40 (37°C) and Innova 40R (Refrigerated) shaking incubator for bacteria
- 35°C incubator for bacteria with CO_2 input
- Grumbach Egg Incubator
- Ambient air bacterial incubator
- Forma 1200 BioSafety Cabinet/laminar flow hood
- Bench top microcentrifuges [2]
- Heat block
- NanoDrop One Spectrophotometer
- Vortexers [3]
- Microscope with 4x,10x,40x, and Immersion Oil lens
- Miltenyi Biotec AutoMACS Pro cell separator
- Cold Storage: -20°C chest freezer; 4°C refrigerator

The equipment contained in the dedicated molecular epidemiology and serology laboratory (D7215) for use in this proposal includes:

- Eppendorf Thermocyclers [2]
- Power supplies for electrophoresis [3]
- Bio-Rad SDS PAGE system
- Invitrogen iBright FL1000 multi mode gel reader
- BioTek 405LS plate washer
- BioTek MultiFlow dispenser
- BioTek Synergy HT plate reader and Synergy neo2 multi mode plate reader
- Simplicity benchtop water purifier
- Applied Biosystems Quant Studio 3 for qPCR
- Plate spinner
- Ismatec variable speed peristaltic pump
- Titer plate shaker

- Cold Storage: -80°C tall freezer; -20°C tall freezer; 4°C tall refrigerator

The equipment contained in the dedicated immunology laboratory (D7201) for use in this proposal includes:

- Luminex MAGPIX
- Luminex Flexmap 3D
- Beckman Coulter Allegra X-14R Bench top Centrifuge
- Branson sonicator
- Accuspin Micro R microcentrifuge
- Biotek Plate Washer and Molecular Devices Plate Reader
- Aqua Solutions water purifier
- Water Bath
- Nexcelom cellometer Auto 2000
- SterilGard III and Thermo 1300 Biosafety cabinet/laminar flow hoods.
- Cold Storage: -80°C tall freezer; -20°C freezer; 4°C tall refrigerator

The equipment contained in the dedicated cell culture and sample processing laboratory (D7209) for use in this proposal includes:

- Labconco BioSafety Cabinet/laminar flow hood
- Heracell CO_2 Copper lined cell incubators [2]
- Dual water bath
- Inverted Microscope
- AKTA go protein purification system
- ThermoScientific pH meter
- Ohaus Voyager Pro analytical scale
- Maxwell (Promega) RSC for nucleic acids extractions
- Molecular Devices SpectraMax M5 plate reader
- Beckman Coulter Allegra X-14R Bench top Centrifuge
- Hettich Refrigerated Centrifuge
- Cold Storage: Liquid Nitrogen; CryoMed Controlled Rate Freezer ;-80°C freezer [2]; Small -20°C Freezer; Small 4°C Refrigerator
- Mobile View temperature monitoring system for Creech Laboratory -80° freezers

Flow Cytometry Core. Within the Cell Sorting / Flow Cytometry Core, Dr. Creech has access to seven cytometers: BD FACSAria II and BD FACSAria III sorters capable single-cell, four-way, or bulk sorting based on simultaneous detection of 9 fluorochromes; a five-laser custom BD Fortessa capable of detecting a large panel of existing or potential fluorochromes and equipped with a 96-well microplate loading device for high throughput cell analysis and cytokine bead arrays; a five laser custom BD LSRII (including UV) with very broad capabilities for multicolor analysis and 96-well loading device; a custom four laser BD LSRII setup for 17-color analysis including use of quantum dot detection; a three laser LSRII available for unassisted use; and a Guava EasyCyte system for cell counts, viability testing, and apoptosis assays.

OMB Number: 4040-0001
Expiration Date: 11/30/2025

RESEARCH & RELATED Senior/Key Person Profile (Expanded)

PROFILE - Project Director/Principal Investigator

Prefix: First Name*: George Middle Name Last Name*: Liu Suffix: Ph.D

Position/Title*: Professor and Chief
Organization Name*: The Regents of the Univ. of Calif., U.C. San Diego
Department: Pediatrics
Division: Infectious Diseases
Street1*: [redacted]
Street2: [redacted]
City*: [redacted]
County:
State*: CA: California
Province:
Country*: USA: UNITED STATES
Zip / Postal Code*: [redacted]

Phone Number*: [redacted] Fax Number:

E-Mail*: [redacted]

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

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

Degree Type: MD,PHD,PHD,BS Degree Year: 1998,1996,1995,1990

Attach Biographical Sketch*: File Name: Biosketch_Liu.pdf

Attach Current & Pending Support: File Name:

Funding Opportunity Number: PA-20-185 . Received Date: 2023-04-10T16:10:08.000-04:00

PROFILE - Senior/Key Person

Prefix: First Name*: C. Middle Name Buddy Last Name*: Creech Suffix: M.D.

Position/Title*: Chair and Professor
Organization Name*: Vanderbilt University Medical Center
Department: Pediatrics
Division: Pediatric Infectious Diseases
Street1*: [redacted]
Street2:
City*: [redacted]
County:
State*: TN: Tennessee
Province:
Country*: USA: UNITED STATES
Zip / Postal Code*: [redacted]

Phone Number*: [redacted] Fax Number: [redacted]

E-Mail*: [redacted]

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

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

Degree Type: MD,MPH,BS Degree Year: 1999,2006,1995

Attach Biographical Sketch*: File Name: bc_Creech_Biosketch_March_202320.pdf

Attach Current & Pending Support: File Name:

PROFILE - Senior/Key Person

Prefix: First Name*: James Middle Name Christopher Last Name*: Slaughter Suffix:

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

Phone Number*: [redacted] Fax Number: [redacted]

E-Mail*: [redacted]

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

Project Role*: Co-Investigator Other Project Role Category:

Degree Type: DRPH,MS Degree Year: 2007,2000

Attach Biographical Sketch*: File Name: Biosketch_Slaughter.pdf

Attach Current & Pending Support: File Name:

PROFILE - Senior/Key Person

Prefix: First Name*: Isaac Middle Name P Last Name*: Thomsen Suffix:

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

Phone Number*: [redacted] Fax Number: [redacted]

E-Mail*: [redacted]

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

Project Role*: Consultant Other Project Role Category:

Degree Type: MD,MS,BS Degree Year: 2004,2013,2000

Attach Biographical Sketch*: File Name: Thomsen_Biosketch.pdf

Attach Current & Pending Support: File Name:

PROFILE - Senior/Key Person

Prefix: First Name*: Chih-Ming Middle Name Last Name*: Tsai Suffix:

Position/Title*: Assistant Project Scientist
Organization Name*: The Regents of the Univ. of Calif., U.C. San Diego
Department: Pediatrics
Division: Infectious Diseases
Street1*: [redacted]
Street2:
City*: [redacted]
County:
State*: CA: California
Province:
Country*: USA: UNITED STATES
Zip / Postal Code*: [redacted]

Phone Number*: [redacted] Fax Number:

E-Mail*: [redacted]

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

Project Role*: Co-Investigator Other Project Role Category:

Degree Type: PHD Degree Year: 2011

Attach Biographical Sketch*: File Name: Biosketch_Tsai.pdf

Attach Current & Pending Support: File Name:

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

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: George Y. Liu, M.D., Ph.D.

eRA COMMONS USER NAME: [redacted]

POSITION TITLE: Professor and Chief of Infectious Diseases

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
California Institute of Technology, Pasadena, CA	B.S.	1990	Biology
University of Cambridge, England	Ph.D.	1996	Immunology
Univ. of California, San Diego, La Jolla, CA	M.D.	1998	Medicine
Univ. of California, Davis, Sacramento, CA	Residency	2001	Pediatrics
Univ. of California, San Diego, La Jolla, CA	Fellowship	2005	Infectious Diseases

A. Personal Statement

The major goal of our (current) research program is to determine why we do not have a working *S. aureus* vaccine after thirty vaccine trials. We have made significant advances by demonstrating that the naive mouse is not a good model for the study of *S. aureus* vaccines because mice are rarely exposed to human *S. aureus*, unlike humans. Exposure to the pathogen leads to the generation of non-protective anti-*S. aureus* antibody imprints that, we propose, are preferentially recalled, leading to vaccine failures. To broadly examine if these principles could be used to explain the failure of other *S. aureus* vaccines and identify human-relevant vaccine strategies, we have previously partnered with Dr. Isaac Thomsen at Vanderbilt with whom we developed a murine adoptive transfer model to better evaluate the protective effect of human anti-*S. aureus* antibodies. Dr. Thomsen recently accepted a position at Pfizer to continue *S. aureus* vaccine research. In his place, we are partnering with his longtime Vanderbilt colleague and former mentor, Dr. Buddy Creech, who has agreed to assume the position of co-PI. Dr. Creech is a leading *S. aureus* clinical researcher and vaccine expert, and has all the expertise required for execution of this proposal. He and Dr. Liu have known each other for more than 5 years as a result of shared interest. To make transition seamless, Dr. Thomsen has agreed to remain on as consultant for at least the first 9 months of the project.

Publications directly relevant to this proposal:

1. Tsai CM, Hajam IA, Caldera JR, Liu G.Y. (2022) Integrating complex host-pathogen immune environments into S. aureus vaccine studies. ***Cell Chem Biol.*** 29(5):730-740. PMID: 35594849.

2. Tsai CM, Caldera JR, Hajam IA. … Liu, GY. (2022) Non-protective immune imprint underlies failure of *S. aureus* IsdB vaccine. ***Cell Host Microbe*** 10; 30(8):1163-1172. PMID: 35803276.
3. Tsai CM, Soper N, Bennett M, Fallon JK, Michell AR, Alter G, **Liu GY**, Thomsen I. (2021) Adoptive Transfer of Serum Samples From Children With Invasive Staphylococcal Infection and Protection Against Staphylococcus aureus Sepsis. **J Infect Dis** 223(7):1222-1231. PMID: 32990305.

B. Positions, Scientific Appointments, and Honors

Positions and Scientific Appointments

1998-2001	Residency in Pediatrics, University of California, Davis Medical School
2001-2004	Fellowship in Pediatric Infectious Diseases, University of California, San Diego
2004-2006	Post-Doctoral Fellowship, University of California, San Diego
2006-2010	Assistant professor of Pediatrics, Cedars-Sinai Medical Center / UCLA
2010-	Associate professor of Pediatrics, in residence, UCLA
2012-	Associate director of the Infectious and Immunologic Disease Research Center at Cedars-Sinai
2016-	Professor of Pediatrics, in residence, UCLA
2019-	Professor and Chief, Pediatric Infectious Diseases, UCSD

Honors

2004	Burroughs-Wellcome Career Award in Biomedical Sciences
2008	Pediatric Infectious Disease Society 2008 Young Investigator Award
2008-	Ad Hoc Member of NIAID F13, F15, R21 and R01 HIBP study sections
2009	ASM Merck Irving S. Sigal Memorial Young Investigator Award
2010	Aspire Young Investigator Award – Pfizer
2017-	American Society for Clinical Investigation – Elected member
2019-	Editorial Board, Infection and Immunity
2020-	Editorial Board, Pathogens.
2020-24	HIBP study section – Member

C. Contribution to Science

1. Group B *Streptococcus* virulence functions: GBS hemolysin/cytolysin is a major virulence that contributes to significant tissue destruction in GBS diseases. We provided important insight on how the hemolysin induces damage to host tissues. More recently, we demonstrated that GBS hyaluronidase is an immune evasion tool that facilitates spread of GBS while minimizing detection by host DAMPs and PAMPs.

    a. Liu G.Y., Doran K., Lawrence T., Turkson N., Puliti M., Tissi L., Nizet V. (2004). Sword and Shield: Linked Group B *Streptococcal* βHemolysin/ Cytolysin and Carotenoid Pigment Function to Subvert Phagocytic Defense. ***Proc Natl Acad Sci USA*** 101:14491-14496. PMC521972.
    b. Doran K., Liu G.Y., Nizet V. (2003). Group B Streptococcal ß-Hemolysin/Cytolysin Activates Neutrophil Signaling Pathways in Brain Endothelium and Contributes to Development of Meningitis. ***J Clin Invest*** 112:736-744. PMC182187.

c. Bebien M, Hensler ME, Hsu LC, Karin M, Park JM, Alexopoulou L, Liu G.Y., Nizet V, Lawrence T. (2012) The pore-forming ß-hemolysin/cytolysin of group B *Streptococcus* activates p38 MAPK-dependent IL-10 production in macrophages and inhibits innate immunity. ***PLoS Pathog*** 8:e1002812. PMC3400567
d. Kolar SL, Kyme P, Tseng CW, Soliman A, Kaplan A, Liang J, Nizet V, Jiang D, Murali R, Arditi M, Underhill DM, Liu GY. (2015) Group B Streptococcus Evades Host Immunity by Degrading Hyaluronan. ***Cell Host Microbe*** 18:694-704. PMC4683412.

2. GBS and *S. aureus* both express an orange pigment on its surface. We demonstrated that both pigments play an important role in immune evasion via their anti-oxidant function. For *S. aureus*, we further demonstrated that a cholesterol inhibitor that interferes with *S. aureus* pigment biosynthesis augments host killing of the pathogen.

a. Liu C-I*, Liu G.Y.* shared first authorship, Song Y, Yin F, Hensler ME, Nizet V, Wang AH, Oldfield E. (2008) A cholesterol lowering drug inhibits *S. aureus* virulence. ***Science*** 319:391-394. PMC2747771
b. Liu G.Y., Essex A, Buchanan JT, Datta V, Fierer J, Nizet V. (2005) *Staphylococcus aureus* golden pigment impairs neutrophil killing and promotes virulence through its antioxidant activity. ***J Exp Med*** 202: 209-215. PMC2213009.
c. Liu G.Y., Doran K., Lawrence T., Turkson N., Puliti M., Tissi L., Nizet V. (2004). Sword and Shield: Linked Group B *Streptococcal* βHemolysin/ Cytolysin and Carotenoid Pigment Function to Subvert Phagocytic Defense. ***Proc Natl Acad Sci USA*** 101:14491-14496. PMC521972.

3. The emergence of community-associated MRSA has led to increased incidence of abscesses, necrotizing pneumonia, and severe diseases. Panton Valentine Leukocidin is a toxin expressed by most isolates of CA-MRSA. However its role in pathogenesis has been controversial. We have provided evidence in human biopsy as well as animals that PVL contributes to immunopathology. Most recently we have validated the use of a humanized mouse model to study the role of PVL and other potential virulence factors in human infections.

a. Lehman D, Tseng,CW, Eells S, Miller LG, Fan X, Beenhouwer D, Liu G.Y. (2010) *S. aureus* Panton-Valentine Leukocidin Targets Muscle Tissue in a Child with Myositis and Necrotizing Fasciitis. ***Clin Infect Dis*** 50:69-72.
b. Diep B, Chan L, Tattevin P, Kajikawa O, Martin T, Li Basuino , Mai T, Braughton K, Whitney A, Gardner D, Fan X, Tseng C, Liu G.Y., Badiou C, Etienne J, Lina G, Matthay M, DeLeo F, Chambers H. (2010) Polymorphonuclear Leukocytes Mediate Staphylococcus aureus Panton-Valentine Leukocidin-Induced Lung Inflammation and Injury ***Proc Natl Acad Sci USA*** 107(12):5587-92. PMC2851770
c. Tseng CW, Biancotti JC, Berg BL, Gate D, Kolar SL, Müller S, Rodriguez MD, Rezai-Zadeh K, Fan X, Beenhouwer DO, Town T, Liu G.Y. (2015) Increased Susceptibility of Humanized NSG Mice to Panton-Valentine Leukocidin and Staphylococcus aureus Skin Infection. ***PLoS Pathog*** 11:e1005292. PMC4664407.
d. Thomsen IP, Liu G.Y. (2018) Targeting fundamental pathways to disrupt *Staphylococcus aureus* survival: clinical implications of recent discoveries. ***JCI Insight*** 3(5). PMC5922289.

4. C/EBP epsilon deficiency is responsible for the human condition specific granule deficiency. We demonstrated that high dose nicotinamide induces expression of C/EBP epsilon in mature neutrophils and augments expression of C/EBP epsilon regulated genes, lactoferrin and cathelicidin. Administration of nicotinamide boosts clearance of MRSA and other pathogens. Based on our study, nicotinamide is now undergoing clinical trial for treatment of bronchiectasis.

In addition to C/EBP epsilon, we have recently identified angiotensin converting enzyme as another host factor that promotes neutrophil killing of MRSA by augmenting ROS.

a. Kyme P, Thoennissen NH, Tseng CW, Iwanski GB, Shimada K, Krug UO, Lee K, Hardy WD, Gombart AF, Koeffler HP, Liu G.Y. (2012) CCAAT/enhancer binding protein *epsilon* mediates nicotinamide-enhanced clearance of *Staphylococcus aureus* infection. ***J Clin Invest*** 122:3316-29. PMC3428083.
b. Khan Z, Shen XZ, Bernstein EA, Giani JF, Eriguchi M, Zhao TV, Gonzalez-Villalobos RA, Fuchs S, Liu G.Y., Bernstein KE. Angiotensin converting enzyme enhances the oxidative response and bactericidal activity of neutrophils. (2017) ***Blood*** 130:328-339. PMC5520468.

5. The failure of all staphylococcal vaccine trials has been a conundrum but could be related to pathogen's immune evasion mechanisms. We have shown that *S. aureus* circumvents host adaptive immune protection by OatA mediated peptidoglycan modification, which blunts Th17 anti-*S. aureus* immunity. We suggest and show that the non-protective imprints left after *S. aureus* infection or colonization are preferentially recalled by *S. aureus* vaccines leading to vaccine failures.

a. Shimada T, Park B, Goodridge HS, Wolf AJ, Becker CA, Reyes CN, Miao EA, Aderem A, Götz F, Liu G.Y., Underhill DM. (2010) Lysozyme-based digestion of *S. aureus* peptidoglycan in macrophage phagosomes activates Nalp3 inflammasomes. ***Cell Host Microbe*** 7:38-49. PMC2818986.
b. Sabrina Müller, Wolf A.J., Iliev I.D., Berg B.L., Underhill D.M., and Liu G.Y. (2015) Poorly cross-linked peptidoglycan formed in MRSA due to mecA induction strongly activates the NLRP3 inflammasome and exacerbates immunopathology. ***Cell Host Microbe*** 18(5):604-12. PMC4648675.
c. Sanchez M, Kolar SL, Muller S, Reyes CN, Wolf AJ, Ogawa C, deCarvalho D, Arditi M, Underhill DM, Martins GA, Liu G.Y. (2017) O-acetylation of peptidoglycan limits helper T cell priming and permits Staphylococcus aureus reinfection. ***Cell Host Microbe*** 22:543-551. PMC5679255.
d. Tsai CM, Hajam IA, Caldera JR, Liu G.Y. Integrating complex host-pathogen immune environments into S. aureus vaccine studies. ***Cell Chem Biol.*** 2022 29(5):730-740. PMID: 35594849.
e. Tsai CM, Caldera JR, Hajam IA. … Liu G.Y. Non-protective immune imprint underlies failure of S. aureus IsdB vaccine. ***Cell Host Microbe*** 2022 10; 30(8):1163-1172. PMID: 35803276.

Complete List of Published Work in MyBibliography:

http://www.ncbi.nlm.nih.gov/sites/myncbi/george.liu.1/bibliography/47993492/public/?sort=date&direction=descending

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

BIOGRAPHICAL SKETCH

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

NAME: **C. Buddy Creech, MD, MPH**

eRA COMMONS USER NAME (credential, e.g., agency login): [redacted]

POSITION TITLE: **Professor, Pediatric Infectious Diseases; Director, Vanderbilt Vaccine Research Program**

EDUCATION/TRAINING

INSTITUTION AND LOCATION	DEGREE	DATE	FIELD OF STUDY
Vanderbilt University, Nashville, Tennessee	BS	05/1995	Biology
University of Tennessee College of Medicine, Memphis	MD	06/1999	Medicine
Vanderbilt University Medical Center, Nashville, TN	Residency	06/2002	Pediatrics
Vanderbilt University Medical Center, Nashville, TN	Chief Residency	06/2003	Pediatrics
Vanderbilt University Medical Center, Nashville, TN	Fellowship	06/2006	Pediatric Infectious Diseases
Vanderbilt University School of Medicine, Nashville, TN	MPH	05/2006	Public Health

A. Personal Statement

I currently serve as Professor and Edie Carell Johnson Chair in Pediatric Infectious Diseases and Director of the Vanderbilt Vaccine Research Program (VVRP). The VVRP is home to one of 10 NIAID-funded Vaccine and Treatment Evaluation Units (VTEU) and is the coordinating center for the CDC-funded Clinical Immunization Safety Assessment Network (CISA). I have conducted clinical and translational research for nearly 20 years, focusing first on the clinical and molecular epidemiology of *S. aureus* colonization and disease and then on the evaluation of new vaccines and therapeutics targeting influenza, malaria, pertussis, and other pathogens. Over the past several years, we have leveraged the robust laboratory and bioinformatics tools at Vanderbilt to conduct comprehensive analyses of the response to influenza and pertussis vaccines, taking a systems vaccinology approach to evaluate transcriptomic, proteomic, and metabolomic signatures following vaccination. We are using similar approaches to characterize transcriptional profiles that may identify children with bacterial pneumonia.

In my role as PI of the Vanderbilt VTEU, I have led Phase I-IV clinical trials of vaccines and therapeutics in infants, children, adults, pregnant women, and elderly individuals. These studies have evaluated strategies for improving immunogenicity of influenza vaccine in young infants; the safety and immunogenicity of novel A/H1N1, A/H5N1, and A/H7N9 influenza vaccines, with the recent application of systems biology tools to define better immune correlates of protection; the safety and immunogenicity of adjuvanted seasonal influenza vaccines; the safety of an adenoviral vectored malaria vaccine (Phase I); trials of candidate staphylococcal vaccines and immunotherapeutics; and comparator studies of Tdap vaccines in adolescents. I also direct studies in the VVRP Laboratory, with expertise in basic microbiology and immunology techniques; clinical trial sample processing; PBMC storage; human immune cell sorting into specific immune cell compartments (e.g., isolation and purification of human T-cells, B-cells, monocytes, and neutrophils by MACS and FACS); and functional immune assays. Our team also has an active research program in population pharmacokinetic (popPK) studies in special populations. I currently serve as the multicenter PI for DMID 16-0078, a popPK study of beta-lactams in children and adults with cystic fibrosis, and as site PI for DMID 16-0077, a similar study in critically ill adults. Finally, I lead efforts through the Pediatric Infectious Diseases Society (where I serve as President) to improve education of healthcare providers through the CoVER initiative (Collaboration for Vaccine Education and Research), using innovative educational modules to combat vaccine hesitancy and reduce healthcare utilization disparities.

For this project, I will add specific clinical trial expertise and facilitate obtaining clinical samples for subsequent laboratory analysis.

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

UM1-AI-149452
Creech (PI)
12/2019 – 11/2026
NIAID Vaccine and Treatment Evaluation Unit (VTEU)
The VTEU is a network of 10 academic centers whose work focuses on the evaluation of new vaccines and new therapeutics for infectious diseases.

UM1-AI-149452-02S2_NCE
Creech (PI)
03/2020 – 11/2022
Clinical Trials for COVID-19 Vaccines (Moderna Phase 3 and Janssen Phase 3 studies)

UM1-AI-149452-03S1
Creech (PI)
08/2021-11/2023
Clinical Trials for COVID-19 Vaccines – DMID 21-0004 - Observational, Prospective Cohort Study of the Immunogenicity and Safety of SARS-CoV-2 Vaccines Administered during Pregnancy or Postpartum and Evaluation of Antibody Transfer and Durability in Infants

UM1-AI-149452-03S2
Creech (PI)
07/2021-11/2026
Clinical Trials for COVID-19 Vaccines – DMID 21-0012 - A Phase 1/2 Study of Delayed Heterologous SARS-CoV-2 Vaccine Dosing (Boost) after Receipt of EUA Vaccines.

UM1-AI-149452-03S3
Creech (PI)
05/2021-11/2024
Clinical Trials for COVID-19 Vaccines - Phase 2/3, Two-Part, Open-Label, Dose-Escalation, Age De-escalation and Randomized, Observer-Blind, Placebo-Controlled Expansion Study to Evaluate the Safety, Tolerability, Reactogenicity, and Effectiveness of mRNA-1273 SARS-CoV-2 Vaccine in Healthy Children 6 Months to Less Than 12 Years of Age

CISA Contract ##200-2012-50430
Edwards (PI), Role: Co-PI
09/2020 – 09/2022
CDC Clinical Immunization Safety Assessment Network (CISA) – COVID-19 Clinical Lead Evaluation Site

CISA Contract ##200-2012-50430
Edwards (PI), Role: Co-PI
08/2013 – 09/2022
CDC Clinical Immunization Safety Assessment Network (CISA) – Coordinating Center

B. Positions, Scientific Appointments, and Honors

Positions and Scientific Appointments

2021	Professor of Pediatrics, Division of Pediatric Infectious Diseases, Vanderbilt University School of Medicine
2020	President, Pediatric Infectious Diseases Society
2016	Secretary/Treasurer, Pediatric Infectious Diseases Society
2015	NIH/NICHD Pediatric Infectious Diseases Terminology Working Group
2015 – present	Director, Vanderbilt Vaccine Research Program
2014 – 2021	Associate Professor of Pediatrics, Division of Pediatric Infectious Diseases, Vanderbilt University School of Medicine
2014	Fellow, Pediatric Infectious Diseases Society
2013 – 2014	Chair, Senate Affairs Committee, Vanderbilt University Faculty Senate
2013 – 2016	Member, Board of Directors, Pediatric Infectious Diseases Society

2013	National Vaccine Program Office (NVPO) Working Group on Adult Immunization
2010 – 2015	Associate Director, Vanderbilt Vaccine Research Program
2009 – 2018	Program Director, Pediatric Infectious Diseases Fellowship
2008	Centers for Disease Control and Prevention Staphylococcal Decolonization Advisory Board
2006 – 2014	Assistant Professor of Pediatrics, Division of Pediatric Infectious Diseases, Vanderbilt University School of Medicine
2006	Fellow, American Academy of Pediatrics
2003 – present	Member, Pediatric Infectious Diseases Society
2003 – present	Member, Infectious Diseases Society of America

Honors

2021	Physician of the Year, Nashville Business Journal
2020	Election to Academic Pediatric Society
2012	Young Investigator Award, Pediatric Infectious Diseases Society
2009	Election to Society for Pediatric Research
2009	Advanced Vaccinology Course (ADVAC) Travel Award, National Foundation for Infectious Diseases
2007	Young Investigator Award in MRSA Research, Infectious Diseases Society of America
2004	National Institutes of Health Loan Repayment Program
2004	Pediatric Infectious Diseases Society Fellowship Award
2000	Robert C. Boerth Intern of the Year Award, Monroe Carell, Jr. Children's Hospital at Vanderbilt
1999	Outstanding Graduating Medical Student in Pediatrics, LeBonheur Children's Hospital
1998	Alpha Omega Alpha Medical Honor Society, University of Tennessee, Memphis

C. Contributions to Science

1. Our research team continues to seek a greater understanding of the rules of immunity and correlates of durable protection for a variety of pathogens, principally *S. aureus,* influenza, and pertussis. Our team has optimized clinical trials approaches that provide excellent source material for our own research laboratory and those of our colleagues, including high-yield/high-viability peripheral blood mononuclear cells, highly enriched/purified immune cell compartments (e.g., T-cells, B-cells, monocytes, dendritic cells, and NK cells), and tissue-based assessments of immune responses (e.g., draining lymph nodes and bone marrow).
    a. Thornburg NJ,* Zhang H, Bangaru S, Sapparapu G, Kose N, Lampley RM, Bombardi RG, Yu Y, Graham S, Branchizio A, Yoder SM, Rock MT, **Creech CB**, Edwards KM, Lee D, Li S, Wilson IA, García-Sastre A, Albrecht RA, Crowe JE Jr. H7N9 influenza virus neutralizing antibodies that possess few somatic mutations. J Clin Invest. 2016 Apr 1;126(4):1482-94. PMC4811156
    b. Howard LM*, Hoek KL, Goll JB, Samir P, Galassie A, Allos TM, Niu X, Gordy LE, **Creech CB**, Prasad N, Jensen TL, Hill H, Levy SE, Joyce S, Link AJ, Edwards KM. Cell-Based Systems Biology Analysis of Human AS03-Adjuvanted H5N1 Avian Influenza Vaccine Responses: A Phase I Randomized Controlled Trial. PLoS One. 2017 Jan 18; 12(1) PMC5242433
    c. Galassie AC*, Goll JB, Samir P, Jensen TL, Hoek KL, Howard LM, Allos TM, Niu X, Gordy LE, **Creech CB**, Hill H, Joyce S, Edwards KM, Link AJ. Proteomics show antigen presentation processes in human immune cells after AS03-H5N1 vaccination. Proteomics. 2017 Jun;17(12). PMC5736144
    d. Howard LM*, Goll JB, Jensen TL, Hoek KL, Prasad N, Gelber CE, Levy SE, Joyce S, Link AJ, **Creech CB**, Edwards KM. AS03-Adjuvanted H5N1 Avian Influenza Vaccine Modulates Early Innate Immune Signatures in Human Peripheral Blood Mononuclear Cells. J Infect Dis. 2018 Dec 19. PMID: 30566602

2. The VVRP is experienced at evaluating new vaccines at various places along the product development pipeline; these include early first-in-human studies, as well as evaluation of new vaccine constructs in special populations (e.g., children and pregnant women). The trials listed below highlight the types of influenza vaccine candidates that have been evaluated at Vanderbilt.
    a. Hoft DF, Babusis E, Worku S, Spencer CT, Lottenbach K, Truscott SM, Abate G, Sakala IG, Edwards KM, **Creech CB**, Gerber MA, Bernstein DI, Newman F, Graham I, Anderson EL, Belshe RB. Live and inactivated influenza vaccines induce similar humoral responses, but only

live vaccines induce diverse T-cell responses in young children. J Infect Dis. 2011 Sep 15;204(6):845-53. PMC3156924

b. Jackson LA, Patel SM, Swamy GK, Frey SE, **Creech CB**, Munoz FM, Artal R, Keitel WA, Noah DL, Petrie CR, Wolff M, Edwards KM.Immunogenicity of an inactivated monovalent 2009 H1N1 influenza vaccine in pregnant women. J Infect Dis. 2011 Sep 15;204(6):854-63. PMC3156926

c. Hoft DF, Lottenbach K, Goll JB, Hill H, Winokur PL, Patel SM, Brady RC, Chen WH, Edwards K, **Creech CB**, Frey SE, Blevins TP, Salomon R, Belshe RB. Priming Vaccination with Influenza Virus H5 Hemagglutinin Antigen Significantly Increases the Duration of T cell Responses Induced by a Heterologous H5 Booster Vaccination. J Infect Dis. 2016 Oct 1;214(7):1020-9. PMC5021235

d. Houser KV, Yamshchikov GV, Bellamy AR, May J, Enama ME, Sarwar U, Larkin B, Bailer RT, Koup R, Paskel M, Subbarao K, Anderson E, Bernstein DI, **Creech CB**, Keyserling H, Spearman P, Wright PF, Graham BS, Ledgerwood JE; VRC 702 study team. DNA vaccine priming for seasonal influenza vaccine in children and adolescents 6 to 17 years of age: A phase 1 randomized clinical trial. PLoS One. 2018 Nov 2;13(11):e0206837. PMC6214651.

3. I have a longstanding interest in the optimal use of antimicrobials for the treatment of pediatric and adult infections. This work has primarily focused on the use of vancomycin for *S. aureus* infections, risk factors for vancomycin toxicity, comparative effectiveness of a variety of antimicrobials for staphylococcal skin and soft tissue infections, and the evaluation of novel antimicrobials.
    a. Jimenez-Truque N*, Thomsen I, Saye E, **Creech CB**. Should higher vancomycin trough levels be targeted for invasive community-acquired methicillin-resistant Staphylococcus aureus infections in children? Pediatr Infect Dis J. 2010 Apr;29(4):368-70. PMC3848324
    b. Van Driest SL*, McGregor TL, Velez Edwards DR, Saville BR, Kitchner TE, Hebbring SJ, Brilliant M, Jouni H, Kullo IJ, **Creech CB**, Kannankeril PJ, Vear SI, Brothers KB, Bowton EA, Shaffer CM, Patel N, Delaney JT, Bradford Y, Wilson S, Olson LM, Crawford DC, Potts AL, Ho RH, Roden DM, Denny JC. Genome-Wide Association Study of Serum Creatinine Levels during Vancomycin Therapy. PLoS One. 2015 Jun 1;10(6):e0127791. PMC4452656
    c. Miller LG, Daum RS, **Creech CB**, Young D, Downing MD, Eells SJ, Pettibone S, Hoagland RJ, Chambers HF; DMID 07-0051 Team. Clindamycin versus trimethoprim-sulfamethoxazole for uncomplicated skin infections. N Engl J Med. 2015 Mar 19;372(12):1093-103. PMC4547538
    d. Daum RS, Miller LG, Immergluck L, Fritz S, **Creech CB**, Young D, Kumar N, Downing M, Pettibone S, Hoagland R, Eells SJ, Boyle MG, Parker TC, Chambers HF; DMID 07-0051 Team. A Placebo-Controlled Trial of Antibiotics for Smaller Skin Abscesses. N Engl J Med. 2017 Jun 29;376(26):2545-2555. PMID: 28657870

4. We continue to lead an active program in *S. aureus* molecular epidemiology, staphylococcal colonization, staphylococcal vaccine development, and immunopathogenesis, much of which is conducted through the Vanderbilt Vaccine Research Program Laboratory, directed by my former trainee and current colleague, Dr. Isaac Thomsen.
    a. Mohamed N, Timofeyeva Y, Jamrozy D, Rojas E, Hao L, Silmon de Monerri NC, Hawkins J, Singh G, Cai B, Liberator P, Sebastian S, Donald RGK, Scully IL, Jones CH, **Creech CB**, Thomsen I, Parkhill J, Peacock SJ, Jansen KU, Holden MTG, Anderson AS. Molecular epidemiology and expression of capsular polysaccharides in Staphylococcus aureus clinical isolates in the United States. PLoS One. 2019 Jan 14;14(1) PMID: 30641545.
    b. Kotloff KL, Shirley DT, **Creech CB**, Frey SE, Harrison CJ, Staat M, Anderson EJ, Dulkerian S, Thomsen IP, Al-Hosni M, Pahud BA, Bernstein DI, Yi J, Petrikin JE, Haberman B, Stephens K, Stephens I, Oler RE Jr, Conrad TM. Mupirocin for Staphylococcus aureus Decolonization of Infants in Neonatal Intensive Care Units. Pediatrics. 2019 Jan;143(1). pii: e20181565. doi: 10.1542/peds.2018-1565. PMC6317770.
    c. Thomsen IP*, Sapparapu G, James DBA, Cassat JE, Nagarsheth M, Kose N, Putnam N, Boguslawski KM, Jones LS, Wood JB, **Creech CB**, Torres VJ, Crowe JE Jr. Monoclonal Antibodies Against the Staphylococcus aureus Bicomponent Leukotoxin AB Isolated Following Invasive Human Infection Reveal Diverse Binding and Modes of Action. J Infect Dis. 2017 Apr 1;215(7):1124-1131. PMC5426380.

d. **Creech CB**, Frenck RW Jr, Sheldon EA, Seiden DJ, Kankam MK, Zito ET, Girgenti D, Severs JM, Immermann FW, McNeil LK, Cooper D, Jansen KU, Gruber W, Eiden J, Anderson AS, Baber J. Safety, tolerability, and immunogenicity of a single dose 4-antigen or 3-antigen Staphylococcus aureus vaccine in healthy older adults: Results of a randomised trial. Vaccine. 2017 Jan 5;35(2):385-394. PMID:27866765.

5. Since early 2020, we have been singularly focused on the SARS-CoV-2 pandemic. We have led protocol development and execution of both treatment studies and prevention strategies for SARS-CoV-2. We have brought together a remarkable team of basic scientists with over 30 years' experience in coronavirus biology and a clinical team capable of recruiting rapidly for Phase 2-3 clinical trials. Work accomplished during this time include the first randomized, placebo-controlled clinical trial of remdesivir in hospitalized adults and evaluation of one of the first SARS-CoV-2 vaccines to receive approval for use in the US.
    a. Baden LR, El Sahly HM, Essink B, Kotloff K, Frey S, Novak R, Diemert D, Spector SA, Rouphael N, **Creech CB,** McGettigan J, Kehtan S, Segall N, Solis J, Brosz A, Fierro C, Schwartz H, Neuzil K, Corey L, Gilbert P, Janes H, Follmann D, Marovich M, Mascola J, Polakowski L, Ledgerwood J, Graham BS, Bennett H, Pajon R, Knightly C, Leav B, Deng W, Zhou H, Han S, Ivarsson M, Miller J, Zaks T; COVE Study Group. Efficacy and Safety of the mRNA-1273 SARS-CoV-2 Vaccine. N Engl J Med. 2020 Dec 30: NEJMoa2035389. doi: 10.1056/NEJMoa2035389. Online ahead of print. PMID: 33378609
    b. Anderson EJ, Campbell JD, **Creech CB**, Frenck R, Kamidani S, Munoz FM, Nachman S, Spearman P. Warp Speed for COVID-19 Vaccines: Why are Children Stuck in Neutral? Clin Infect Dis. 2020 Sep 18:ciaa1425. doi: 10.1093/cid/ciaa1425. Online ahead of print.PMID: 32945335
    c. **Creech CB**. It's True Even in a Pandemic: Children Are Not Merely Little Adults. Clin Infect Dis. 2020 Dec 3;71(9):2480-2481. doi: 10.1093/cid/ciaa680. PMID: 32472937
    d. Beigel JH, Tomashek KM, Dodd LE, Mehta AK, Zingman BS, Kalil AC, Hohmann E, Chu HY, Luetkemeyer A, Kline S, Lopez de Castilla D, Finberg RW, Dierberg K, Tapson V, Hsieh L, Patterson TF, Paredes R, Sweeney DA, Short WR, Touloumi G, Lye DC, Ohmagari N, Oh MD, Ruiz-Palacios GM, Benfield T, Fätkenheuer G, Kortepeter MG, Atmar RL, **Creech CB,** Lundgren J, Babiker AG, Pett S, Neaton JD, Burgess TH, Bonnett T, Green M, Makowski M, Osinusi A, Nayak S, Lane HC; ACTT-1 Study Group Members. Remdesivir for the Treatment of Covid-19 - Final Report. N Engl J Med. 2020 Nov 5;383(19):1813-1826. doi: 10.1056/NEJMoa2007764. Epub 2020 Oct 8. PMID: 32445440
    e. Kalil AC, Patterson TF, Mehta AK, Tomashek KM, Wolfe CR, Ghazaryan V….. Deye GA, Dempsey W, Nayak SU, Dodd LE, Beigel JH; **ACTT-2 Study Group.** Baricitinib plus Remdesivir for Hospitalized Adults with Covid-19. N Engl J Med. 2020 Dec 11:NEJMoa2031994. doi: 10.1056/NEJMoa2031994. Epub ahead of print. PMID: 33306283; PMCID: PMC7745180.

Complete List of Published Work in MyBibliopgraphy:
https://pubmed.ncbi.nlm.nih.gov/?term=creech+cb+%5Bau%5D

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

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: Slaughter, James

eRA COMMONS USER NAME (credential, e.g., agency login): [redacted]

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
Tulane University, New Orleans, LA	BS	05/1998	Mathematics
Tulane University, New Orleans, LA	BS	05/1998	Cell and Molec Bio
University of Washington, Seattle, WA	MS	08/2000	Biostatistics
University of North Carolina at Chapel Hill	DrPH	08/2007	Biostatistics

A. Personal Statement

I am a Biostatistician and have collaborated with investigators in the Department of Pediatrics and the Vanderbilt Vaccine Center since 2007. The department conducts both clinical and basic science research, and I have been an active collaborator in both areas. Out of 202 total peer reviewed publications, I have been a coauthor on 98 such publications within Pediatrics and seven within the Vaccine Center. I have ample experience teaching and implementing the analysis approaches that will be used in this grant. For the proposed grant, I will develop analysis plans and provide expertise on data quality and collection to ensure that all analyses are technically sounds, are completely reproducible, and utilize modern applied statistical methodology. Dr. Creech and I have developed an excellent research rapport, with a bidirectional exchange of knowledge and novel ideas. I anticipate that this will continue to grow as we work together in the future. I have enjoyed participating in this application and look forward to the to the interesting questions we will answer.

B. Positions, Scientific Appointments, and Honors

Positions and Scientific Appointments

2015 - Associate Professor, Vanderbilt University Medical Center, Department of Biostatistics, Nashville, TN
2007 - 2015 Assistant Professor, Vanderbilt University Medical Center, Department of Biostatistics, Nashville, TN
2002 - 2007 Trainee, UNC Training Grant in Environmental Epidemiology, Chapel Hill, NC
2000 - 2002 Research Scientist, UW-EPA Northwest Research Center for Particulate Matter and Health, Seattle, WA

2005 - Member, American Statistical Association
2004 - Member, International Biometrics Society

Honors

2007 Distinguished Student Paper Award, International Biometric Society, ENAR
2006 Professional Development Award, Summer Institute in Reproductive and Perinatal Epidemiology, NICHD
2002 Scholars for Tomorrow Scholarship, University of North Carolina Graduate School

C. Contributions to Science

1. My methodological research focuses on flexible Bayesian regression models for epidemiologic data. Data analysts often use overly simplistic statistical methods, which can lead to results that are reproducible. For example, it is common practice to reduce a complex health outcome to a simple 0/1 indicator of disease status and then apply a logistic regression model. While such an analysis is easy to conduct and interpret for clinicians unfamiliar with statistics, it is inefficient because it discards data and requires the analyst to choose arbitrary cut-points to define disease. These limitations can lead to misinterpretations of the exposure-disease relationship. A Bayesian latent variable model can flexibly model the exposure-disease relationship without relying on arbitrary cutoffs. It can also naturally handle multivariate responses that are collected longitudinally or cross-sectionally. I have two key papers in this area. The first proposed a Bayesian multistate growth model with unknown initiation time that we applied to first trimester fetal growth. In a growth model, individuals move progressively through a series of states in which each state is indicative of developmental status. Interest lies in estimating the rate of change through each state while incorporating covariates that might impact the rate of change through each state. We developed a Bayesian discrete time multistate growth model to link developmental progress to an underlying latent growth variable that can affect state transition rates. In addition to the methodological contribution, our model found evidence in favor of a previously hypothesized but unproven link between slow growth very early in pregnancy and increased risk of future spontaneous abortion. In a second paper we used latent variable models to estimate the association between fetal growth during pregnancy and birth outcomes. Our procedure was more flexible than typical latent variable approaches in that we relax the Normality assumption by allowing the latent factors to follow finite mixture distributions. We were able to jointly model birth weight and gestational age at delivery using a latent variable mixture distribution, which allowed for making inference about the important tails of the distribution. Adverse health outcomes are more likely to occur at early gestational age and low birth weight, and the model can be used to draw conclusions about factors that are associated with being in the lower tail.

   a. Slaughter JC, Herring AH, Hartmann KE. Bayesian modeling of embryonic growth using latent variables. *Biostatistics*. 2008 Apr;9(2):373-89. PMID: 18056115.
   b. Slaughter JC, Herring AH, Thorp JM. A Bayesian latent variable mixture model for longitudinal fetal growth. *Biometrics*. 2009 Dec;65(4):1233-42. PMCID: PMC3717393.

2. I have worked with investigators in Pediatrics since I arrived to Vanderbilt in 2007. The division engages in clinical and basic science research, and I have participated in writing grants and manuscripts with researchers in both areas. I also mentor fellows and meet with presenters before journal clubs to discuss statistical questions. Within Neonatology, there are two main research areas in which I focus. First, I am co-investigator on a grant to find early biomarkers of bronchopulmonary dysplasia (BPD). BPD is a complex outcome involving several clinical indicators that historically has been simplified to a 0/1 outcome. We are considering BPD as and ordinal outcome reflecting disease severity as well as applying latent variable methods for even more flexible models. I also have a special interest in longitudinal data analysis, and have been able to apply this to studies of developmental progress of children who were born preterm. In these studies, we track language, motor and cognitive development over time using traditional functional testing (e.g. Bayley scores) as well as novel approaches that directly measure brain responses (e.g. ERP).

   a. Jaser SS, Bergner EM, Hamburger ER, Bhatia S, Lyttle M, Bell GE, Slaughter JC, Malow BA, Simmons JH. Pilot Trial of a Sleep-Promoting Intervention for Children With Type 1 Diabetes. J Pediatr Psychol. 2021 Mar 18;46(3):304-313. doi: 10.1093/jpepsy/jsaa105. PMID: 33180913; PMCID: PMC8679215.
   b. Jaser SS, Hamburger ER, Bergner EM, Williams R, Slaughter JC, Simmons JH, Malow BA. Sleep coach intervention for teens with type 1 diabetes: Randomized pilot study. Pediatr Diabetes. 2020 May;21(3):473-478. doi: 10.1111/pedi.12991. Epub 2020 Feb 11. PMID: 32003520; PMCID: PMC7670490.
   c. Gregory JM, Slaughter JC, Duffus SH, Smith TJ, LeStourgeon LM, Jaser SS, McCoy AB, Luther JM, Giovannetti ER, Boeder S, Pettus JH, Moore DJ. COVID-19 Severity Is Tripled in the Diabetes Community: A Prospective Analysis of the Pandemic's Impact in Type 1 and Type 2 Diabetes. Diabetes Care. 2020 Dec 2:dc202260. doi: 10.2337/dc20-2260. Online ahead of print. PMID: 33268335
   d. Abdullahi SU, Jibir BW, Bello-Manga H, Gambo S, Inuwa H, Tijjani AG, Idris N, Galadanci A, Hikima MS, Galadanci N, Borodo A, Tabari AM, Haliru L, Suleiman A, Ibrahim J, Greene BC, Ghafuri DL, Rodeghier M, Slaughter JC, Kirkham FJ, Neville K, Kassim A, Trevathan E, Jordan LC, Aliyu MH, DeBaun MR. Hydroxyurea for primary stroke prevention in children with sickle cell anaemia in Nigeria

(SPRING): a double-blind, multicentre, randomised, phase 3 trial. Lancet Haematol. 2022 Jan;9(1):e26-e37. doi: 10.1016/S2352-3026(21)00368-9. PMID: 34971579.

3. I collaborate with several basic science investigators in neonatology and vaccine research. Basic science research historically has received less statistical attention than clinical research. I work closely with these investigators to ensure they are applying the appropriate, modern statistical methodology in their data analyses.

   a. Bates JT, Keefer CJ, Slaughter JC, Kulp DW, Schief WR, et al. Escape from neutralization by the respiratory syncytial virus-specific neutralizing monoclonal antibody palivizumab is driven by changes in on-rate of binding to the fusion protein. *Virology*. 2014 Apr;454-455:139-44. PMCID: PMC4004766.
   b. Flyak AI, Ilinykh PA, Murin CD, Garron T, Shen X, Fusco ML, Hashiguchi T, Bornholdt ZA, Slaughter JC, Sapparapu G, Klages C, Ksiazek TG, Ward AB, Saphire EO, Bukreyev A, Crowe JE Jr. Mechanism of human antibody-mediated neutralization of marburg virus. *Cell.* 2015 Feb 26;160(5):893-903. PMCID: PMC4344968.
   c. Bangaru S, Nieusma T, Kose N, Thornburg NJ, Finn JA, Kaplan BS, King HG, Singh V, Lampley RM, Sapparapu G, Cisneros A 3rd, Edwards KM, Slaughter JC, Edupuganti S, Lai L, Richt JA, Webby RJ, Ward AB, Crowe JE Jr. Recognition of influenza H3N2 variant virus by human neutralizing antibodies. *JCI Insight.* 2016 Jul 7;1(10). PMCID: PMC4962875.
   d. Gilchuk I, Gilchuk P, Sapparapu G, Lampley R, Singh V, Kose N, Blum DL, Hughes LJ, Satheshkumar PS, Townsend MB, Kondas AV, Reed Z, Weiner Z, Olson VA, Hammarlund E, Raue HP, Slifka MK, Slaughter JC, Graham BS, Edwards KM, Eisenberg RJ, Cohen GH, Joyce S, Crowe JE Jr. Cross-Neutralizing and Protective Human Antibody Specificities to Poxvirus Infections. *Cell.* 2016 Oct 20;167(3):684-694.e9. PMCID: PMC5093772.

4. I have worked with investigators in the division of Gastroenterology since I arrived at Vanderbilt. The majority of my time with GI is spent on clinical research projects and industry-sponsored grant submissions. Although I support the entire division, more than half of my research is focused on understanding gastroesophageal reflux disease (GERD) with Michael Vaezi, MD PhD. My methodological interest with Dr Vaezi includes new statistical approaches for defining reflux disease and symptom-associated reflux. The clinical goal is to improve the identification of individuals who have symptom-related reflux and suggest the appropriate treatment (e.g. PPIs or surgery) for alleviation of symptoms. Current research is hampered by simplistic definitions of reflux based on the total percent time spent below pH 4. Not only is a pH of 4 an arbitrary cutoff, but subjects are further defined as being pH positive if the percent of time is above another cutoff, 4.2. We have published a manuscript and presented our results at a national GI meeting (DDW) describing how current approaches are leading to wrong clinical decisions. We also have another manuscript under review describing a new approach. I am developing latent time series models to flexibly model pH levels over time in an effort to better understand the relationship between symptoms and reflux.

   a. Vaezi MF, Hagaman DD, Slaughter JC, Tanner SB, Duncavage JA, et al. Proton pump inhibitor therapy improves symptoms in postnasal drainage. *Gastroenterology*. 2010 Dec;139(6):1887-1893.e1; quiz e11. PMID: 20801120.
   b. Slaughter JC, Goutte M, Rymer JA, Oranu AC, Schneider JA, Vaezi MF. Caution about overinterpretation of symptom indexes in reflux monitoring for refractory gastroesophageal reflux disease. *Clin Gastroenterol Hepatol.* 2011 Oct;9(10):868-74. PMID: 21782769.
   c. Kavitt RT, Higginbotham T, Slaughter JC, Patel D, Yuksel ES, Vaezi MF. Symptom reports are not reliable during ambulatory reflux monitoring. *Am J Gastroenterol.* 2012 Dec;107(12):1826-32. PMID: 23090349.
   d. Ates F, Yuksel ES, Higginbotham T, Slaughter JC, Mabary J, Vaezi MF. Mucosal Impedance Discriminates GERD From Non-GERD Conditions. *Gastroenterology.* 2015 Feb;148(2):334-43. PMID: 25448923.

Complete List of Published Work in MyBibliography:
http://www.ncbi.nlm.nih.gov/myncbi/james.slaughter.1/bibliography/47395835/public/?sort=date&direction=ascending

OMB No. 0925-0001 and 0925-0002 (Rev. 10/21 Approved Through 09/30/2024)

BIOGRAPHICAL SKETCH

NAME: Thomsen, Isaac P

eRA COMMONS USER NAME: [redacted]

POSITION TITLE: Associate Professor, Pediatric Infectious Diseases

EDUCATION/TRAINING

INSTITUTION AND LOCATION	DEGREE	DATE COMPLETED	FIELD OF STUDY
Rhodes College, Memphis, TN	BS	05/2000	Biochemistry
Univ. of Arkansas for Medical Sciences, Little Rock, AR	MD	05/2004	Medicine
Vanderbilt University, Nashville, TN	Resident	06/2008	Internal Medicine and Pediatrics
Vanderbilt University, Nashville, TN	Resident	06/2009	Chief Resident in Internal Medicine
Vanderbilt University, Nashville, TN	Fellow	06/2012	Pediatric Infectious Diseases
Vanderbilt University, Nashville, TN	MSCI	06/2013	Clinical Investigation

A. Personal Statement

I have conducted patient-oriented infectious diseases research since 2006, when I began to investigate the molecular epidemiology and pathogenesis of *Staphylococcus aureus* infections in children. Trained in both Internal Medicine and Pediatrics, with subspecialty training in Pediatric Infectious Diseases, my primary research is focused on the host response to invasive staphylococcal infections in children. Our team is conducting a series of studies in adults and children to decipher the humoral immune response to invasive staphylococcal disease and to investigate active and passive immunization strategies to combat staphylococcal infections. I have received advanced training in patient-oriented research and biostatistics through the Masters of Science in Clinical Investigation (MSCI) Program, and I currently serve as director of the Vanderbilt Vaccine Research Program (VVRP) Laboratory. I have also served as an Investigator on numerous NIH-funded clinical trials over the past 10 years and as a CDC Clinical Immunization Safety Assessment (CISA) Network Investigator for the past 8 years. I am delighted to serve as a consultant for this grant, which proposes to leverage a well established infrastructure for the rapid enrollment of children with invasive *S. aureus* infections. Dr. Liu and his team at UCSD, in collaboration with Dr. Creech and his team at VUMC, are well positioned to identify key mediators of adaptive host defense against invasive *S. aureus*, which will directly inform rational vaccine design against this major human pathogen.

Ongoing and recently completed projects include:

R01 AI139172 **Thomsen (PI)** **01/2019 – 12/2023**
NIH/NIAID
Functional Antibody Repertoire Against S. aureus Leukocidins after Invasive Human Infection
The overall goal of this proposal is to define key components of the human immune response against an important group of toxins secreted by S. aureus, the leukocidins, known to be produced during invasive disease in children.
Role: Principal Investigator

Vaccine and Treatment Evaluation Unit (VTEU) **12/2019 – 11/2026**
NIH / NIAID / UM1 AI48452-01 (Creech, Vanderbilt PI)
The goal of this network is to develop new and improved vaccines and therapies against infectious diseases and to conduct clinical trials of vaccines and treatments.
Role: Co-Investigator and Laboratory Director

[redacted] [redacted] **06/2020 – 05/2021**

[redacted]

Symptom Severity and Functional Correlation of SARS-CoV-2 IgG Following Human Disease
The primary goal of this project is to develop a novel liquid bead-array assay for the detection of SARS-CoV-2 antibodies and to investigate the hypothesis that severity of COVID-19 disease directly correlates with anti-virus antibody titers.
Role: Principal Investigator

UL1TR002243-04S4 **Bernard (PI)** **09/2020 – 03/2021**
NIH/NCATS
Identifying correlates of functional immunity in SARS-CoV-2 convalescent plasma
The overall goal of this proposal is to assess SARS-CoV-2 antibody responses in sera from subjects recovered from COVID-19 across a large number of platforms, including both binding and functional assays. This will allow correlation of binding measurements / titers with functional neutralization of the virus.
Role: Co-Investigator

K23-AI113150 **Thomsen (PI)** **08/2014 – 12/2019**
NIH/NIAID
Evaluating the Functional Antibody Response to Pediatric *S. aureus* Infections
The major goals of the project are to evaluate the host response to S. aureus disease via cohorts of children with specific phenotypes of infection, and to define the mechanism of the cytoprotective effects of antibodies targeting specific staphylococcal exotoxins.
Role: Principal Investigator

Citations:

a. Tsai CM, Soper N, Bennett M, Fallon J, Michell A, Alter G, Liu G*, **Thomsen I*** [*co-corresponding]. *Adoptive Transfer of Serum from Children with Invasive Staphylococcal Infection and Protection Against* S. aureus *Sepsis*. **Journal of Infectious Diseases**. 2020 Aug 06. **PMID: 32990305**

b. **Thomsen I**, Liu G. *Targeting fundamental pathways to disrupt* Staphylococcus aureus *survival: clinical implications of recent discoveries*. **Journal of Clinical Investigation: Insight**. 2018 Mar 8;3(5). **PMID: 29515041. PMCID: PMC5922289**

B. Positions, Scientific Appointments, and Honors

Positions and Scientific Appointments

2020-present	Associate Professor, Pediatric Infectious Diseases, Vanderbilt University School of Medicine, Nashville, TN
2020-present	Program Director, Pediatric Infectious Diseases Clinical Fellowship, Vanderbilt University Medical Center, Nashville, TN
2018-present	Associate Program Director, Pediatric Infectious Diseases Clinical Fellowship, Vanderbilt University Medical Center, Nashville, TN
2015-present	Director, Vanderbilt Vaccine Research Program Laboratory, Vanderbilt University Medical Center, Nashville, TN
2013-2020	Assitant Professor, Pediatric Infectious Diseases, Vanderbilt University School of Medicine, Nashville, TN
2012 - 2013	Instructor, Pediatric Infectious Diseases, Vanderbilt University School of Medicine, Nashville, TN
2008 - 2009	Chief Resident, Internal Medicine, Vanderbilt University Medical Center, Nashville, TN

2002-present	Member, Alpha Omega Alpha Honor Society
2004-present	Member, American Academy of Pediatrics
2007-present	Member, American College of Physicians
2009-present	Member, Pediatric Infectious Diseases Society

2009-present	Member, Infectious Diseases Society of America
2014-2017	Pediatric Infectious Diseases Society (PIDS) National Vaccine Advocacy Committee
2017-present	Vanderbilt Program in Molecular Medicine (VPMM) Bench to Bedside Committee
2019-present	Pediatric Infectious Diseases Society (PIDS) National Finance Committee

Honors

1996	Morse Fellowship, Full Tuition Scholarship for 4 years of Undergraduate Training, Rhodes College
2000	Frank Williams Memorial Scholarship, University of Arkansas for Medical Sciences (UAMS)
2002	Parents Club Scholarship, UAMS, "for the student who displays the greatest of compassion and empathy for patients and their families"
2004	Richard V. Ebert Award for Excellence in Internal Medicine, UAMS
2004	Joseph Thomas Roberts Scholarship, UAMS, awarded to the graduating senior with the highest overall GPA
2004	Dr. and Mrs. Charles Moon Scholarship, UAMS, for completing medical school with a 4.0 GPA
2004	Senior Buchanan Key, UAMS, elected by vote of the class "for the student who most exemplifies the ideal qualities of a physician"
2008	First Place Award, National Clinical Vignette Presentation, American College of Physicians National Meeting, Washington, D.C.
2010	Department of Pediatric Fellow Teaching Award, Monroe Carell, Jr. Children's Hospital at Vanderbilt
2011	Edwards Fellowship Award, Pediatric Infectious Diseases, Vanderbilt University Medical Center
2011	Fellows Travel Award, Infectious Diseases Society of America
2011	Thomas A. Hazinski, M.D. Scholarship, Vanderbilt University MSCI Program
2012	Elected as Class Representative, Vanderbilt MSCI Class of 2013
2013	David T. Karzon Fellowship Award in Pediatric Infectious Diseases
2014	Attendee, Advanced Course of Vaccinology (ADVAC), Annecy, France
2017	Caroline B. Hall Clinically Innovative Research Award, Pediatric Infectious Diseases Society
2018	Selected for Membership, Society for Pediatric Research (SPR)

C. Contribution to Science

c. Our laboratory is focused on exploring critical host-pathogen interactions when *Staphylococcus aureus* causes invasive disease in humans, with the goal of identifying novel targets of intervention. We propose that critical aspects of the host response to invasive *S. aureus* infection in humans will be determined by defining the host response during natural human disease. Our contributions in this area include discoveries that the cytotoxin LukAB was ubiquitously present in a large series of clinical isolates causing invasive disease in children, that LukAB is produced and recognized by the host during invasive human disease, and that neutralization of the toxin is achieved by the host response via diverse mechanisms. We continue to investigate this and other vaccine-relevant antigens of *Staphylococcus aureus*.

   a. **Thomsen I**, Dumont AL, James DB, Yoong P, Saville BR, Soper N, Torres VJ, Creech CB. Children with invasive Staphylococcus aureus disease exhibit a potently neutralizing antibody response to the cytotoxin LukAB. **Infection and Immunity**. 2014 Mar;82(3):1234-42. **PMCID: PMC3957992.**

   b. **Thomsen I**, Sapparapu G, James DBA, Cassat JE, Nagarsheth M, Kose N, Putnam N, Boguslawski K, Jones LS, Wood JB, Creech CB, Torres VJ*, Crowe JE* [*co-corresponding}. *Monoclonal antibodies against the* Staphylococcus aureus *bicomponent leukotoxin AB (LukAB) isolated following invasive human infection reveal diverse binding and modes of action*. **Journal of Infectious Diseases**. 2017 Feb 10. **PMCID: PMC5426380**

   c. Wood JB, Jones LS, Soper N, Nagarsheth M, Creech CB, **Thomsen I**. *Commercial Intravenous Immunoglobulin (IVIg) Preparations Contain Functional Neutralizing Antibodies Against the*

Staphylococcus aureus *Leukocidin LukAB (LukGH)*. **Antimicrobial Agents and Chemotherapeutics.** 2017 Sep 5. **PMCID: PMC5655085**

d. Tsai CM, Soper N, Bennett M, Fallon J, Michell A, Alter G, Liu G*, **Thomsen I*** [*co-corresponding]. *Adoptive Transfer of Serum from Children with Invasive Staphylococcal Infection and Protection Against* S. aureus *Sepsis*. **Journal of Infectious Diseases**. 2020 Aug 06. **PMID: 32990305**

d. I have a particular interest in the pathogenesis, diagnosis, and optimal care of children with musculoskeletal infections, particularly hematogenous osteomyelitis and septic arthritis. *Staphylococcus aureus* remains the leading cause of these invasive infections, and our group has actively enrolled all children admitted to the Monroe Carell, Jr. Children's Hospital at Vanderbilt for the past several years, for the purpose of studying both MSKI in general and *S. aureus* in particular. We have contributed to this field both by working to characterize current epidemiology in this area, as well as to assess the impact of the rapidly changing molecular epidemiology of *S. aureus* as it relates to pediatric MSKI.

a. **Thomsen I,** Creech CB. Advances in the diagnosis and management of pediatric osteomyelitis. **Curr Infect Dis Rep**. 2011 Oct;13(5):451-60. **PMID**: **21789499.**

b. Benvenuti M, An TJ, Mignemi M, Martus J, **Thomsen I**, Schoenecker JG. *Effects of Antibiotic Timing on Culture Results and Clinical Outcomes in Pediatric Musculoskeletal Infection*. **Journal of Pediatric Orthopedics**. 2016 Sep 22 **PMID: 27662389**

c. Wood JB, Jones LJ, Soper NS, Xu M, Torres VJ, Creech CB, **Thomsen I.** *Serologic Detection of Antibodies Targeting the Leukocidin LukAB Strongly Predicts Staphylococcus aureus in Children with Musculoskeletal Infections.* **Journal of the Pediatric Infectious Diseases Society**. 2018 Mar 10. **PMID: 29538707. PMCID: PMC6510946**

d. Yi J, Wood JB, Creech CB, Williams DJ, Jiminez-Truque N, Yildirim I, Sederdahl B, Daugherty M, Hussaini L, Munye M, Tomashek K, Focht C, Watson N, Anderson EJ*, **Thomsen I*** [* = co-corresponding]. *Clinical Epidemiology and Outcomes of Pediatric Musculoskeletal Infections*. **The Journal of Pediatrics**. 2021 Jul; 234:236-244. **PMID: 33771580**

e. During my post-doctoral training period, I began to examine the molecular characteristics of clinical isolates of methicillin-resistant S. aureus (MRSA). With financial support from the NIH-funded Vaccine and Treatment Evaluation Unit, we characterized MRSA isolates that colonized the nares of healthy adults and children and compared their molecular characteristics with disease-causing isolates, and we found that colonization and disease-causing isolates were distinct. We have since investigated the molecular epidemiology of MRSA in a variety of populations.

a. **Thomsen I,** McKenna BD, Saye EJ, Jimenez N, Edwards KM, Creech CB. Molecular distinctions exist between community-associated methicillin-resistant Staphylococcus aureus colonization and disease-associated isolates in children. **Pediatr Infect Dis J**. 2011 May;30(5):418-21. **PMID: 21263373; PMCID: PMC3077447.**

b. Jimenez-Truque N, Tedeschi S, Saye EJ, McKenna BD, Langdon W, Wright JP, Alsentzer A, Arnold S, Saville BR, Wang W, **Thomsen I**, Creech CB. Relationship between maternal and neonatal Staphylococcus aureus colonization. **Pediatrics**. 2012 May;129(5):e1252-9. **PMID**: **22473373; PMCID: PMC3340589.**

c. Johnson JG, Saye EJ, Jimenez-Truque N, Soper N, **Thomsen I**, Talbot TR, Creech CB. Frequency of disinfectant resistance genes in pediatric strains of methicillin-resistant Staphylococcus aureus. **Infect Control Hosp Epidemiol**. 2013 Dec;34(12):1326-7. **PMID: 24225622; PMCID**: **PMC3965576.**

d. **Thomsen I**, Kadari P, Soper NR, Riddell S, Kiska D, Creech CB, Shaw J. Molecular Epidemiology of Invasive Staphylococcus aureus Infections and Concordance with Colonization Isolates. **Journal of Pediatrics.** 2019 July; 210:173-177. **PMID**: **30961989 PMCID**: **PMC6592716**

f. I have a strong interest in host-pathogen interactions, including those that may represent potential opportunities for intervention against important pathogens such as *S. aureus*, as well as instances where the host response is deficient due to primary immunodefiency syndromes. We have published several reports and discussions defining these interactions and leveraging this understanding to achieve optimal care of children and adults with these infections, which has led to productive relationships with colleagues in the fields of clinical infectious diseases and immunology.

a. **Thomsen I**, Liu G. *Targeting fundamental pathways to disrupt* Staphylococcus aureus *survival: clinical implications of recent discoveries*. **Journal of Clinical Investigation: Insight**. 2018 Mar 8;3(5). **PMID: 29515041. PMCID: PMC5922289**

b. Slack MA, **Thomsen I**. *The Prevention of Infectious Complications in Chronic Granulomatous Disease*. **Journal of the Pediatric Infectious Diseases Society**. 2018 May 9;7(suppl_1):S25-S30. **PMID: 29746681**. **PMCID: PMC5946879**

c. **Thomsen I**. *Antibody-Based Intervention Against the Pore-Forming Toxins of* Staphylococcus aureus. **Virulence**. 2018 Mar; 9(1)645-647. **PMID: 29405823. PMCID**: **PMC5955459**

d. **Thomsen I**. *The Concern for Vancomycin Failure in the Treatment of Pediatric Staphylococcus aureus Disease.* **Clinical Infectious Diseases**. Epub, 2018 Jun. **PMID**: **29893812**. **PMCID PMC6336910.**

Complete List of Published Work in MyBibliography:
http://www.ncbi.nlm.nih.gov/myncbi/browse/collection/48350599/?sort=date&direction=descending

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

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: Chih-Ming Tsai, Ph.D.

eRA COMMONS USER NAME: [redacted]

POSITION TITLE: assistant project scientist

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

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	Completion Date MM/YYYY	FIELD OF STUDY
National Chiayi University, Taiwan	B.S.	2004	Biology
National Yang-Ming University, Taiwan	M.S.	2006	Immunology
National Yang-Ming University, Taiwan	Ph.D.	2011	Immunology/Glyco-biology
Academia Sinica, Taiwan	Postdoc fellowship	20014	Immunology/Glyco-biology
Univ. of California, San Diego, La Jolla, CA	Postdoc	2017	Immunology/Glyco-biology
Cedars-Sinai Medical Center, Los Angeles, CA	Postdoc	2018	Immunology/Glyco-biology
Univ. of California, San Diego, La Jolla, CA	Project Scientist	2019	Immunology/Glyco-biology

A. Personal Statement

Our research focuses on understanding the B cell responses to *S. aureus* infection including plasma cell development, antibody glycosylation and epitope selection to improve the S. aureus vaccine development. We aim to elucidate the suppression mechanisms of *S. aureus* infection that underly the failure of *S. aureus* vaccine in human clinical trials. Though understanding of these mechanisms, we aim to discover novel approaches to developing effective *S. aureus* vaccines.

Publications directly relevant to this proposal:

1. **Tsai CM**, Hajam IA, Caldera JR, Liu GY. Integrating complex host-pathogen immune environments into S. aureus vaccine studies. ***Cell Chem Biol.*** 2022 29(5):730-740. PMID: 35594849.
2. **Tsai CM**, Caldera JR, Hajam IA. … George Y. Liu. Non-protective immune imprint underlies failure of *S. aureus* IsdB vaccine. ***Cell Host Microbe*** 2022 10; 30(8):1163-1172. PMID: 35803276.
3. **Tsai CM**, Nicole Soper, Monique Bennett, Jonathan K Fallon, Ashlin R Michell, Galit Alter, George Y Liu, Isaac Thomsen, 2021, “Adoptive Transfer of Serum Samples From Children With Invasive Staphylococcal Infection and Protection Against Staphylococcus aureus Sepsis”, ***The Journal of Infectious Diseases***, 223(7): 1222-1231

B. Positions and Honors

Academic Positions

2011-2014 Postdoctoral Fellow**,** Academia Sinica, Taiwan
2014-2017 Postdoctoral Fellow, University of California, San Diego, CA
2017-2018 Postdoctoral Fellow, Cedars-Sinai Medical Center, Los Angeles, CA
2018-2019 Project Scientist, Cedars-Sinai Medical Center, Los Angeles, CA
2019-Current Project Scientist, University of California, San Diego, CA

Honors and Awards

2010 Travel award from National Science Council, Taiwan to attend the 14th International Congress of Immunology, Kobe, Japan.
2012 Travel award for the 8th International Symposium on Glycosyltransferases to attend GlycoT 2012, Hannover, Germany
2012-2014 Postdoctoral Fellowship at Academia Sinica, Taiwan

C. Contribution to Science

1. The role of galectins in plasma cell differentiation. Galectins play important roles in regulating immune cell homeostasis and host-pathogen interactions. We provided important insight on how galectins promote plasma cell differentiation and regulates B cell receptor signaling. We also identified different glycan structure expression on mature B cells and plasma cells that correlated to the function of B cells and can be used as the therapeutic targets by using synthesis glycans.

    a. **Tsai CM**, Y. K. Chiu, T. L. Hsu, I. Y. Lin, S. L. Hsieh and K. I Lin, 2008, “Galectin-1 promotes immunoglobulin production during plasma cell differentiation”, ***Journal of Immunology***, 181, 4570-4579.
    b. **Tsai CM**, Chin-Huey Guan, Hsiao-Wu Hsieh, Tsui-Ling Hsu, Zhijay Tu, Kuan-Jung Wu, Chun-Hung Lin*, and Kuo-I Lin, 2011, “Galectin-1 and galectin-8 have redundant roles in promoting plasma cell formation”, ***Journal of Immunology***, 187 (4),1643-1652
    c. Shui-Hua Wang, **Tsai CM**, Kuo-I Lin, Kay-Hooi Khoo, 2013, “Advanced Mass Spectrometry and Chemical Analyses Reveal the Presence of Terminal Disialyl Motif on mouse B cell Glycoproteins”, ***Glycobiology***, 23(6):677-89. (co-author)
    d. **Tsai CM** et al., 2014, “Phosphoproteomic analyses reveal that galectin-1 augments the dynamics of B-cell receptor signaling”, ***Journal of Proteomics***, 103C:241-253. (co-author)

2. The GBS and Trichomonas vaginalis surface capsule containing sialic acid influence innate and adaptive immune responses through siglecs in inflammasome activation in macrophages. We demonstrated that Siglec 14 has tonic effects of inflammasome activation in macrophages in GBS infection. The expression level of Siglec14 and the polymorphic paried Siglec 5 can regulate NLRP3 inflammasome activation. Moreover, we found the Siglec 7 has negative regulation of inflammasome activation in NK cells in GBS infection.

    a. **Tsai CM**, Angelica M. Riestra, Syed Raza Ali, Jerry J. Fong, Janet Z. Liu, Ajit Varki, Victor Nizet, 2020, “Siglec-14 Enhances NLRP3-Inflammasome Activation in Macrophages”, ***Journal of innate Immunity***, 2020;12(4):333-343.

b. Fong JJ, **Tsai CM**, Saha S., Nizet V, Varki A, Nizet V., 2018, “Direct Functional Interaction Between Human Bacterial Pathogen and Natural Killer Cells: Silencing the Sentinels”, ***Proc Natl Acad Sci U S A**,* 115(41): 10410-10415
c. Angelica Montenegro Riestra, J. Andrés Valderrama, Kathryn A. Patras, Sharon Booth, Xing Quek, **Tsai CM**, Victor Nizet, 2018, “Trichomonas vaginalis Induces NLRP3 Inflammasome Activation and Pyroptosis in Human Macrophages”, ***Journal of Innate Immunity**,* 2019;11(1):86-98.

3. The non-protective immune imprint of *S. aureus* failure staphylococcal vaccines. We found that *S. aureus* infection can induce non-protective immune imprint on B cells and generate non-neutralizing antibodies by limiting B cell clone generation and antibody glycosylation. The suppression of vaccine efficacy can be transferred by B cells from *S. aureus*-exposed mice. We found a potential vaccine candidate targeting NEAT2 domain of IsdB which had untouched epitopes and can be able to overcome immune imprint of *S. aureus* to clear the infection. We proposed potential staphylococcal vaccine approaches by targeting untouched or subdominant region of staphylococcal antigens.

a. **Tsai CM**, Hajam IA, Caldera JR, Liu GY. Integrating complex host-pathogen immune environments into S. aureus vaccine studies. ***Cell Chem Biol.*** 2022 29(5):730-740. PMID: 35594849.
b. **Tsai CM**, Caldera JR, Hajam IA. … George Y. Liu. Non-protective immune imprint underlies failure of S. aureus IsdB vaccine. ***Cell Host Microbe*** 2022 10; 30(8):1163-1172. PMID: 35803276.
c. **Tsai CM**, Nicole Soper, Monique Bennett, Jonathan K Fallon, Ashlin R Michell, Galit Alter, George Y Liu, Isaac Thomsen, 2021, “Adoptive Transfer of Serum Samples From Children With Invasive Staphylococcal Infection and Protection Against Staphylococcus aureus Sepsis”, ***The Journal of Infectious Diseases***, 223(7): 1222-1231

Complete List of Published Work in MyBibliography:

https://www.ncbi.nlm.nih.gov/myncbi/chih-ming.tsai.1/bibliography/public/

OMB Number: 4040-0001
Expiration Date: 11/30/2025

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

UEI*: [redacted]

Budget Type*: ● Project ❍ Subaward/Consortium

Enter name of Organization: The Regents of the Univ. of Calif., U.C. San Diego

Start Date*: 09-01-2023 **End Date*:** 08-31-2024 **Budget Period: 1**

A. Senior/Key Person

	Prefix	First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base Salary ($)	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits ($)*	Funds Requested ($)*
1 .	Dr.	George		Liu	Ph.D	PD/PI	[redacted]	2.4			[redacted]	[redacted]	[redacted]
2 .		Chih-Ming		Tsai		Co-Investigator	[redacted]	6.0			[redacted]	[redacted]	[redacted]

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

Additional Senior Key Persons: File Name:

Total Senior/Key Person [redacted]

B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits*	Funds Requested ($)*
1	Post Doctoral Associates	12.0			[redacted]	[redacted]	[redacted]
	Graduate Students						
	Undergraduate Students						
	Secretarial/Clerical						
1	**Total Number Other Personnel**					**Total Other Personnel**	[redacted]
						Total Salary, Wages and Fringe Benefits (A+B)	[redacted]

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

Funding Opportunity Number: PA-20-185 . Received Date: 2023-04-10T16:10:08.000-04:00

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

UEI*: [redacted]

Budget Type*: ● Project ❍ Subaward/Consortium

Organization: The Regents of the Univ. of Calif., U.C. San Diego

Start Date*: 09-01-2023 **End Date*:** 08-31-2024 **Budget Period: 1**

C. Equipment Description	
List items and dollar amount for each item exceeding $[redacted]	
Equipment Item	**Funds Requested ($)***
Total funds requested for all equipment listed in the attached file	
Total Equipment	**0.00**

Additional Equipment: File Name:

D. Travel	**Funds Requested ($)***
1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)	
2. Foreign Travel Costs	
Total Travel Cost	**0.00**

E. Participant/Trainee Support Costs		**Funds Requested ($)***
1. Tuition/Fees/Health Insurance		
2. Stipends		
3. Travel		
4. Subsistence		
5. Other:		
Number of Participants/Trainees	**Total Participant Trainee Support Costs**	**0.00**

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

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

UEI*: [redacted]

Budget Type*: ● Project ❍ Subaward/Consortium

Organization: The Regents of the Univ. of Calif., U.C. San Diego

Start Date*: 09-01-2023 **End Date*:** 08-31-2024 **Budget Period: 1**

F. Other Direct Costs	Funds Requested ($)*
1. Materials and Supplies	[redacted]
2. Publication Costs	
3. Consultant Services	
4. ADP/Computer Services	
5. Subawards/Consortium/Contractual Costs	[redacted]
6. Equipment or Facility Rental/User Fees	
7. Alterations and Renovations	
8. Animal Costs	[redacted]
9. NGN Charge (Next Generation Network)	[redacted]
10. HS-TSC (Health Sciences Technology Services Charge)	[redacted]
11. Data Management and Sharing Costs	[redacted]
Total Other Direct Costs	[redacted]

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

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)*
1 . MTDC	58.0	[redacted]	[redacted]
		Total Indirect Costs	[redacted]

Cognizant Federal Agency (Agency Name, POC Name, and POC Phone Number): DHHS Office of Inspector General, Arif Karim, (415) 437-7859

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

J. Fee	Funds Requested ($)*

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

L. Budget Justification*	File Name: Budget_Justification_Liu_-_2023.pdf

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

OMB Number: 4040-0001
Expiration Date: 11/30/2025

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

UEI*: [redacted]

Budget Type*: ● Project ○ Subaward/Consortium

Enter name of Organization: The Regents of the Univ. of Calif., U.C. San Diego

Start Date*: 09-01-2024 **End Date*:** 08-31-2025 **Budget Period: 2**

A. Senior/Key Person

	Prefix	First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base Salary ($)	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits ($)*	Funds Requested ($)*
1 .	Dr.	George		Liu	Ph.D	PD/PI	[redacted]	2.4			[redacted]	[redacted]	[redacted]
2 .		Chih-Ming		Tsai		Co-Investigator	[redacted]	6.0			[redacted]	[redacted]	[redacted]

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

Additional Senior Key Persons: File Name:

Total Senior/Key Person [redacted]

B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits*	Funds Requested ($)*
1	Post Doctoral Associates	12.0			[redacted]	[redacted]	[redacted]
	Graduate Students						
	Undergraduate Students						
	Secretarial/Clerical						
1	**Total Number Other Personnel**					**Total Other Personnel**	[redacted]
						Total Salary, Wages and Fringe Benefits (A+B)	[redacted]

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

Funding Opportunity Number: PA-20-185 . Received Date: 2023-04-10T16:10:08.000-04:00

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

UEI*: [redacted]

Budget Type*: ● Project ❍ Subaward/Consortium

Organization: The Regents of the Univ. of Calif., U.C. San Diego

Start Date*: 09-01-2024 **End Date*:** 08-31-2025 **Budget Period: 2**

C. Equipment Description	
List items and dollar amount for each item exceeding $[redacted]	
Equipment Item	**Funds Requested ($)***
Total funds requested for all equipment listed in the attached file	
Total Equipment	**0.00**

Additional Equipment: File Name:

D. Travel	**Funds Requested ($)***
1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)	
2. Foreign Travel Costs	
Total Travel Cost	**0.00**

E. Participant/Trainee Support Costs		**Funds Requested ($)***
1. Tuition/Fees/Health Insurance		
2. Stipends		
3. Travel		
4. Subsistence		
5. Other:		
Number of Participants/Trainees	**Total Participant Trainee Support Costs**	**0.00**

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

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

UEI*: [redacted]

Budget Type*: ● Project ❍ Subaward/Consortium

Organization: The Regents of the Univ. of Calif., U.C. San Diego

Start Date*: 09-01-2024 **End Date*:** 08-31-2025 **Budget Period: 2**

F. Other Direct Costs	Funds Requested ($)*
1. Materials and Supplies	[redacted]
2. Publication Costs	
3. Consultant Services	
4. ADP/Computer Services	
5. Subawards/Consortium/Contractual Costs	[redacted]
6. Equipment or Facility Rental/User Fees	
7. Alterations and Renovations	
8. Animal Costs	[redacted]
9. NGN Charge (Next Generation Network)	[redacted]
10. HS-TSC (Health Sciences Technology Services Charge)	[redacted]
11. Data Management and Sharing Costs	[redacted]
Total Other Direct Costs	[redacted]

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

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)*
1 . MTDC	58.0	[redacted]	[redacted]
		Total Indirect Costs	[redacted]

Cognizant Federal Agency (Agency Name, POC Name, and POC Phone Number): DHHS Office of Inspector General, Arif Karim, (415) 437-7859

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

J. Fee	Funds Requested ($)*

K. Total Costs and Fee	Funds Requested ($)*
	817,237.00

L. Budget Justification*	File Name: Budget_Justification_Liu_-_2023.pdf

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

OMB Number: 4040-0001
Expiration Date: 11/30/2025

RESEARCH & RELATED BUDGET - SECTION A & B, Budget Period 3

UEI*: [redacted]

Budget Type*: ● Project ❍ Subaward/Consortium

Enter name of Organization: The Regents of the Univ. of Calif., U.C. San Diego

Start Date*: 09-01-2025 **End Date*:** 08-31-2026 **Budget Period: 3**

A. Senior/Key Person

	Prefix	First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base Salary ($)	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits ($)*	Funds Requested ($)*
1 .	Dr.	George		Liu	Ph.D	PD/PI	[redacted]	2.4			[redacted]	[redacted]	[redacted]
2 .		Chih-Ming		Tsai		Co-Investigator	[redacted]	6.0			[redacted]	[redacted]	[redacted]

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

Additional Senior Key Persons: File Name:

Total Senior/Key Person [redacted]

B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits*	Funds Requested ($)*
1	Post Doctoral Associates	12.0			[redacted]	[redacted]	[redacted]
	Graduate Students						
	Undergraduate Students						
	Secretarial/Clerical						
1	**Total Number Other Personnel**					**Total Other Personnel**	[redacted]
						Total Salary, Wages and Fringe Benefits (A+B)	[redacted]

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

Funding Opportunity Number: PA-20-185 . Received Date: 2023-04-10T16:10:08.000-04:00

RESEARCH & RELATED BUDGET - SECTION C, D, & E, Budget Period 3

UEI*: [redacted]

Budget Type*: ● Project ❍ Subaward/Consortium

Organization: The Regents of the Univ. of Calif., U.C. San Diego

Start Date*: 09-01-2025 **End Date*:** 08-31-2026 **Budget Period: 3**

C. Equipment Description

List items and dollar amount for each item exceeding $[redacted]

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 3

UEI*: [redacted]

Budget Type*: ● Project ❍ Subaward/Consortium

Organization: The Regents of the Univ. of Calif., U.C. San Diego

Start Date*: 09-01-2025 **End Date*:** 08-31-2026 **Budget Period: 3**

F. Other Direct Costs	Funds Requested ($)*
1. Materials and Supplies	[redacted]
2. Publication Costs	
3. Consultant Services	
4. ADP/Computer Services	
5. Subawards/Consortium/Contractual Costs	[redacted]
6. Equipment or Facility Rental/User Fees	
7. Alterations and Renovations	
8. Animal Costs	[redacted]
9. NGN Charge (Next Generation Network)	[redacted]
10. HS-TSC (Health Sciences Technology Services Charge)	[redacted]
11. Data Management and Sharing Costs	[redacted]
Total Other Direct Costs	[redacted]

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

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)*
1 . MTDC	58.0	337,728.00	[redacted]
		Total Indirect Costs	[redacted]

Cognizant Federal Agency (Agency Name, POC Name, and POC Phone Number): DHHS Office of Inspector General, Arif Karim, (415) 437-7859

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

J. Fee	Funds Requested ($)*

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

L. Budget Justification*	File Name: Budget_Justification_Liu_-_2023.pdf

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

OMB Number: 4040-0001
Expiration Date: 11/30/2025

RESEARCH & RELATED BUDGET - SECTION A & B, Budget Period 4

UEI*: [redacted]

Budget Type*: ● Project ❍ Subaward/Consortium

Enter name of Organization: The Regents of the Univ. of Calif., U.C. San Diego

Start Date*: 09-01-2026 **End Date*:** 08-31-2027 **Budget Period: 4**

A. Senior/Key Person

	Prefix	First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base Salary ($)	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits ($)*	Funds Requested ($)*
1 .	Dr.	George		Liu	Ph.D	PD/PI	[redacted]	2.4			[redacted]	[redacted]	[redacted]
2 .		Chih-Ming		Tsai		Co-Investigator	[redacted]	6.0			[redacted]	[redacted]	[redacted]

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

Additional Senior Key Persons: File Name:

Total Senior/Key Person [redacted]

B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits*	Funds Requested ($)*
1	Post Doctoral Associates	12.0			[redacted]	[redacted]	[redacted]
	Graduate Students						
	Undergraduate Students						
	Secretarial/Clerical						
1	**Total Number Other Personnel**					**Total Other Personnel**	[redacted]
						Total Salary, Wages and Fringe Benefits (A+B)	[redacted]

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

Funding Opportunity Number: PA-20-185 . Received Date: 2023-04-10T16:10:08.000-04:00

RESEARCH & RELATED BUDGET - SECTION C, D, & E, Budget Period 4

UEI*: [redacted]

Budget Type*: ● Project ❍ Subaward/Consortium

Organization: The Regents of the Univ. of Calif., U.C. San Diego

Start Date*: 09-01-2026 **End Date*:** 08-31-2027 **Budget Period: 4**

C. Equipment Description	
List items and dollar amount for each item exceeding $[redacted]	
Equipment Item	**Funds Requested ($)***
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 4

UEI*: [redacted]

Budget Type*: ● Project ❍ Subaward/Consortium

Organization: The Regents of the Univ. of Calif., U.C. San Diego

Start Date*: 09-01-2026 **End Date*:** 08-31-2027 **Budget Period: 4**

F. Other Direct Costs	Funds Requested ($)*
1. Materials and Supplies	[redacted]
2. Publication Costs	
3. Consultant Services	
4. ADP/Computer Services	
5. Subawards/Consortium/Contractual Costs	[redacted]
6. Equipment or Facility Rental/User Fees	
7. Alterations and Renovations	
8. Animal Costs	[redacted]
9. NGN Charge (Next Generation Network)	[redacted]
10. HS-TSC (Health Sciences Technology Services Charge)	[redacted]
11. Data Management and Sharing Costs	[redacted]
Total Other Direct Costs	[redacted]

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

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)*
1 . MTDC	58.0	[redacted]	[redacted]
		Total Indirect Costs	[redacted]

Cognizant Federal Agency (Agency Name, POC Name, and POC Phone Number): DHHS Office of Inspector General, Arif Karim, (415) 437-7859

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

J. Fee	Funds Requested ($)*

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

L. Budget Justification* File Name: Budget_Justification_Liu_-_2023.pdf

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

OMB Number: 4040-0001
Expiration Date: 11/30/2025

RESEARCH & RELATED BUDGET - SECTION A & B, Budget Period 5

UEI*: [redacted]

Budget Type*: ● Project ❍ Subaward/Consortium

Enter name of Organization: The Regents of the Univ. of Calif., U.C. San Diego

Start Date*: 09-01-2027 **End Date*:** 08-31-2028 **Budget Period: 5**

A. Senior/Key Person

	Prefix	First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base Salary ($)	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits ($)*	Funds Requested ($)*
1 .	Dr.	George		Liu	Ph.D	PD/PI	[redacted]	2.4			[redacted]	[redacted]	[redacted]
2 .		Chih-Ming		Tsai		Co-Investigator	[redacted]	6.0			[redacted]	[redacted]	[redacted]

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

Additional Senior Key Persons: File Name:

Total Senior/Key Person [redacted]

B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits*	Funds Requested ($)*
1	Post Doctoral Associates	12.0			[redacted]	[redacted]	[redacted]
	Graduate Students						
	Undergraduate Students						
	Secretarial/Clerical						
1	**Total Number Other Personnel**					**Total Other Personnel**	[redacted]
						Total Salary, Wages and Fringe Benefits (A+B)	[redacted]

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

Funding Opportunity Number: PA-20-185 . Received Date: 2023-04-10T16:10:08.000-04:00

RESEARCH & RELATED BUDGET - SECTION C, D, & E, Budget Period 5

UEI*: [redacted]

Budget Type*: ● Project ❍ Subaward/Consortium

Organization: The Regents of the Univ. of Calif., U.C. San Diego

Start Date*: 09-01-2027 **End Date*:** 08-31-2028 **Budget Period: 5**

C. Equipment Description	
List items and dollar amount for each item exceeding $[redacted]	
Equipment Item	**Funds Requested ($)***
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 5

UEI*: [redacted]

Budget Type*: ● Project ❍ Subaward/Consortium

Organization: The Regents of the Univ. of Calif., U.C. San Diego

Start Date*: 09-01-2027 **End Date*:** 08-31-2028 **Budget Period: 5**

F. Other Direct Costs	Funds Requested ($)*
1. Materials and Supplies	[redacted]
2. Publication Costs	
3. Consultant Services	
4. ADP/Computer Services	
5. Subawards/Consortium/Contractual Costs	[redacted]
6. Equipment or Facility Rental/User Fees	
7. Alterations and Renovations	
8. Animal Costs	[redacted]
9. NGN Charge (Next Generation Network)	[redacted]
10. HS-TSC (Health Sciences Technology Services Charge)	[redacted]
11. Data Management and Sharing Costs	[redacted]
Total Other Direct Costs	[redacted]

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

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)*
1 . MTDC	58.0	[redacted]	[redacted]
		Total Indirect Costs	[redacted]

Cognizant Federal Agency (Agency Name, POC Name, and POC Phone Number): DHHS Office of Inspector General, Arif Karim, (415) 437-7859

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

J. Fee	Funds Requested ($)*

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

L. Budget Justification* File Name: Budget_Justification_Liu_-_2023.pdf

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

BUDGET JUSTIFICATION

PERSONNEL (UCSD) DIRECT COST YEARS 1-5

Key Personnel

George Liu, M.D., Ph.D. (Principal Investigator, UCSD, will devote 2.4 calendar months for years 1-5): Dr. Liu is professor and chief of pediatric infectious diseases division at the University of California, San Diego. Dr. Liu will be responsible for supervision of all aspects of Aim 1 and 2. He will work closely with Dr. Creech's team, and both Dr. Tsai and the postdoctoral fellow on experimental design, data analysis, personnel management, research presentation and manuscript preparation. He will devote 20% effort to the project; however, he is committed to spending whatever time is required to successfully complete all Aims of the proposal.

Chih Ming Tsai, PhD. (Research scientist, UCSD, will devote 6 calendar months for years 1-5): Dr. Tsai spearheaded the immune imprinting project (Tsai et al. Cell Host Microbe 2022) and will once again devote fifty percent effort on the current project. He, along with Dr. Liu, will oversee Aim 2 and 3 of the proposal. In addition, Dr. Tsai will work with postdoctoral fellow 1 to complete all experiments outlined in the Aims.

Postdoctoral fellow TBD. (Postdoctoral fellow, UCSD, will devote 12 calendar months for years 1-5): A postdoctoral fellow (Ph.D.) with experience in microbiology, B cell immunology and mouse modeling will be recruited. He/she will be responsible for performing many of the experiments outlined in Aim 2 and 3 and will work closely with Dr. Tsai and Dr. Liu on analysis of the data.

- All salaries are adjusted +3% yearly up to NIH cap

OTHER DIRECT COSTS YEARS 1-5

A. Materials and supplies:
Cell culture and immunologic supplies (Antibodies, ELISAs, cell assay kits, glycosylation studies) = $[redacted]/year

Reagents & general laboratory supplies (molecular biology reagents for generating recombinant proteins, protein purification supply, adjuvant, custom probes/primers, Taq polymerase) = $[redacted] year 2, $[redacted] years 1,3,4,5

B. Animal cost:
- purchase cost including shipping and species surcharge fees
  C57BL6 mice from Jackson = 928/year x $[redacted]/mouse = $[redacted] / year
  Humanized NSG mice from UCLA CFA core = 250/year x $[redacted]/mouse = [redacted] year
- animal facility cost = 1178 mice x cage cost in infection facility ($[redacted]/3 mice/day) x average duration (28 days) = $[redacted]
- Total animal cost =$[redacted]/year

Total non-personnel direct cost per year for years 1-3 = $[redacted]/year

Fringe Benefits

Fringe benefits are calculated at rates currently in effect for the University of California

Subcontract
Vanderbilt University ($[redacted] Year 1, $[redacted] total)

Next Generation Network Charge (NGN)
UC San Diego Information and Technology Services (ITS) charges a flat per month fee for services to provide state-of-the-art technology infrastructure and services to the campus community. These charges are directly attributable and proportionally applied for the individual(s) included in the proposed budget on the project. These costs are not included in the campus' Facilities & Administration (F&A) rate as an indirect cost. UC San Diego auditors have determined that it is both equitable and consistent with the OMB Circular 2 CFR 200 provisions on cost allocability that the costs be assigned to FTE on grant and contract funds. Accordingly, an allocable portion of these NGN costs are included in this budget as direct project costs.

Health Sciences Technology Service Charge (HS-TSC)
UC San Diego Health Information Services costs have been included to reflect costs associated with the increased level of security required for all personnel within UC San Diego Health, which includes UC San Diego School of Medicine, Skaggs School of Pharmacy and Pharmaceutical Sciences, and the hospitals and clinics. These charges are directly attributable and proportionally applied for the individual(s) included in the proposed budget on the project.

Indirect Costs
UC San Diego's indirect costs are calculated based on Modified Total Direct Costs (MTDC) as defined in 2 CFR Part 200.68 using Facilities and Administration (F&A) rates approved by the U.S. Department of Health and Human Services (DHHS).

Rates established by UC San Diego's F&A rate agreement dated May 23, 2018 are as follows:

- July 1, 2017, to June 30, 2018: 56.0%
- July 1, 2018, to June 30, 2019: 57.0%
- July 1, 2019, to June 30, 2020: 57.5%
- July 1, 2020, to June 30, 2021: 57.5%
- July 1, 2021, until amended: 58.0%

RESEARCH & RELATED BUDGET - Cumulative Budget

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

Funding Opportunity Number: PA-20-185 . Received Date: 2023-04-10T16:10:08.000-04:00

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

OMB Number: 4040-0001
Expiration Date: 11/30/2025

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

UEI*: [redacted]

Budget Type*: ❍ Project ● Subaward/Consortium

Enter name of Organization: Vanderbilt University Medical Center

Start Date*: 09-01-2023 **End Date*:** 08-31-2024 **Budget Period: 1**

A. Senior/Key Person

	Prefix	First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base Salary ($)	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits ($)*	Funds Requested ($)*
1 .	Dr.	C.	Buddy	Creech	M.D.	Consortium PD/ PI	[redacted]	2.4			[redacted]	[redacted]	[redacted]
2 .	Dr.	James	Christopher	Slaughter	Ph.D	Consortium Co-Investigator	[redacted]	0.6			[redacted]	[redacted]	[redacted]

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

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

B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits*	Funds Requested ($)*
	Post Doctoral Associates						
	Graduate Students						
	Undergraduate Students						
	Secretarial/Clerical						
1	Senior Research Assistant	2.4			[redacted]	[redacted]	[redacted]
1	Research Assistant	2.4			[redacted]	[redacted]	[redacted]
2	**Total Number Other Personnel**					**Total Other Personnel**	[redacted]
						Total Salary, Wages and Fringe Benefits (A+B)	[redacted]

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

Funding Opportunity Number: PA-20-185 . Received Date: 2023-04-10T16:10:08.000-04:00

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

UEI*: [redacted]

Budget Type*: ❍ Project ● Subaward/Consortium

Organization: Vanderbilt University Medical Center

Start Date*: 09-01-2023 **End Date*:** 08-31-2024 **Budget Period: 1**

C. Equipment Description	
List items and dollar amount for each item exceeding $[redacted]	
Equipment Item	**Funds Requested ($)***
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)	[redacted]
2. Foreign Travel Costs	
Total Travel Cost	[redacted]

E. Participant/Trainee Support Costs		**Funds Requested ($)***
1. Tuition/Fees/Health Insurance		
2. Stipends		
3. Travel		
4. Subsistence		
5. Other:		
Number of Participants/Trainees	**Total Participant Trainee Support Costs**	**0.00**

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

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

UEI*: [redacted]

Budget Type*: ❍ Project ● Subaward/Consortium

Organization: Vanderbilt University Medical Center

Start Date*: 09-01-2023 **End Date*:** 08-31-2024 **Budget Period: 1**

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

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

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)*
1 . MTDC	75.0	[redacted]	[redacted]
		Total Indirect Costs	[redacted]

Cognizant Federal Agency (Agency Name, POC Name, and POC Phone Number) DHHS, Region 4, 404-562-7888

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

J. Fee	Funds Requested ($)*

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

L. Budget Justification* File Name: VUMC_Budget_Just.pdf

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

OMB Number: 4040-0001
Expiration Date: 11/30/2025

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

UEI*: [redacted]

Budget Type*: ❍ Project ● Subaward/Consortium

Enter name of Organization: Vanderbilt University Medical Center

Start Date*: 09-01-2024 **End Date*:** 08-31-2025 **Budget Period: 2**

A. Senior/Key Person

	Prefix	First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base Salary ($)	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits ($)*	Funds Requested ($)*
1 .	Dr.	C.	Buddy	Creech	M.D.	Consortium PD/ PI	[redacted]	2.4			[redacted]	[redacted]	[redacted]
2 .	Dr.	James	Christopher	Slaughter	Ph.D	Consortium Co-Investigator	[redacted]	0.6			[redacted]	[redacted]	[redacted]

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

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

B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits*	Funds Requested ($)*
	Post Doctoral Associates						
	Graduate Students						
	Undergraduate Students						
	Secretarial/Clerical						
1	Senior Research Assistant	2.4			[redacted]	[redacted]	[redacted]
1	Research Assistant	2.4			[redacted]	[redacted]	[redacted]
2	**Total Number Other Personnel**					**Total Other Personnel**	[redacted]
						Total Salary, Wages and Fringe Benefits (A+B)	[redacted]

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

Funding Opportunity Number: PA-20-185 . Received Date: 2023-04-10T16:10:08.000-04:00

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

UEI*: [redacted]

Budget Type*: ❍ Project ● Subaward/Consortium

Organization: Vanderbilt University Medical Center

Start Date*: 09-01-2024 **End Date*:** 08-31-2025 **Budget Period: 2**

C. Equipment Description

List items and dollar amount for each item exceeding $[redacted]

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)	[redacted]
2. Foreign Travel Costs	
Total Travel Cost	[redacted]

E. Participant/Trainee Support Costs		**Funds Requested ($)***
1. Tuition/Fees/Health Insurance		
2. Stipends		
3. Travel		
4. Subsistence		
5. Other:		
Number of Participants/Trainees	**Total Participant Trainee Support Costs**	**0.00**

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

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

UEI*: [redacted]

Budget Type*: ❍ Project ● Subaward/Consortium

Organization: Vanderbilt University Medical Center

Start Date*: 09-01-2024 **End Date*:** 08-31-2025 **Budget Period: 2**

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

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

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)*
1 . MTDC	75.0	[redacted]	[redacted]
		Total Indirect Costs	[redacted]

Cognizant Federal Agency (Agency Name, POC Name, and POC Phone Number) DHHS, Region 4, 404-562-7888

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

J. Fee	Funds Requested ($)*

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

L. Budget Justification* File Name: VUMC_Budget_Just.pdf

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

OMB Number: 4040-0001
Expiration Date: 11/30/2025

RESEARCH & RELATED BUDGET - SECTION A & B, Budget Period 3

UEI*: [redacted]

Budget Type*: ❍ Project ● Subaward/Consortium

Enter name of Organization: Vanderbilt University Medical Center

Start Date*: 09-01-2025 **End Date*:** 08-31-2026 **Budget Period: 3**

A. Senior/Key Person

	Prefix	First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base Salary ($)	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits ($)*	Funds Requested ($)*
1.	Dr.	C.	Buddy	Creech	M.D.	Consortium PD/ PI	[redacted]	2.4			[redacted]	[redacted]	[redacted]
2.	Dr.	James	Christopher	Slaughter	Ph.D	Consortium Co-Investigator	[redacted]	0.6			[redacted]	[redacted]	[redacted]

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

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

B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits*	Funds Requested ($)*
	Post Doctoral Associates						
	Graduate Students						
	Undergraduate Students						
	Secretarial/Clerical						
1	Senior Research Assistant	2.4			[redacted]	[redacted]	[redacted]
1	Research Assistant	2.4			[redacted]	[redacted]	[redacted]
2	**Total Number Other Personnel**					**Total Other Personnel**	[redacted]
						Total Salary, Wages and Fringe Benefits (A+B)	[redacted]

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

Funding Opportunity Number: PA-20-185 . Received Date: 2023-04-10T16:10:08.000-04:00

RESEARCH & RELATED BUDGET - SECTION C, D, & E, Budget Period 3

UEI*: [redacted]

Budget Type*: ❍ Project ● Subaward/Consortium

Organization: Vanderbilt University Medical Center

Start Date*: 09-01-2025 **End Date*:** 08-31-2026 **Budget Period: 3**

C. Equipment Description

List items and dollar amount for each item exceeding $[redacted]

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)	[redacted]
2. Foreign Travel Costs	
Total Travel Cost	[redacted]

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 3

UEI*: [redacted]

Budget Type*: ❍ Project ● Subaward/Consortium

Organization: Vanderbilt University Medical Center

Start Date*: 09-01-2025 **End Date*:** 08-31-2026 **Budget Period: 3**

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

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

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)*
1 . MTDC	75.0	[redacted]	[redacted]
		Total Indirect Costs	[redacted]

Cognizant Federal Agency (Agency Name, POC Name, and POC Phone Number): DHHS, Region 4, 404-562-7888

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

J. Fee	Funds Requested ($)*

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

L. Budget Justification* File Name: VUMC_Budget_Just.pdf

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

OMB Number: 4040-0001
Expiration Date: 11/30/2025

RESEARCH & RELATED BUDGET - SECTION A & B, Budget Period 4

UEI*: [redacted]

Budget Type*: ❍ Project ● Subaward/Consortium

Enter name of Organization: Vanderbilt University Medical Center

Start Date*: 09-01-2026 **End Date*:** 08-31-2027 **Budget Period: 4**

A. Senior/Key Person

	Prefix	First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base Salary ($)	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits ($)*	Funds Requested ($)*
1.	Dr.	C.	Buddy	Creech	M.D.	Consortium PD/ PI	[redacted]	2.4			[redacted]	[redacted]	[redacted]
2.	Dr.	James	Christopher	Slaughter	Ph.D	Consortium Co-Investigator	[redacted]	0.6			[redacted]	[redacted]	[redacted]

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

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

B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits*	Funds Requested ($)*
	Post Doctoral Associates						
	Graduate Students						
	Undergraduate Students						
	Secretarial/Clerical						
1	Senior Research Assistant	2.4			[redacted]	[redacted]	[redacted]
1	Research Assistant	2.4			[redacted]	[redacted]	[redacted]
2	**Total Number Other Personnel**					**Total Other Personnel**	[redacted]
						Total Salary, Wages and Fringe Benefits (A+B)	[redacted]

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

Funding Opportunity Number: PA-20-185 . Received Date: 2023-04-10T16:10:08.000-04:00

RESEARCH & RELATED BUDGET - SECTION C, D, & E, Budget Period 4

UEI*: [redacted]

Budget Type*: ❍ Project ● Subaward/Consortium

Organization: Vanderbilt University Medical Center

Start Date*: 09-01-2026 **End Date*:** 08-31-2027 **Budget Period: 4**

C. Equipment Description	
List items and dollar amount for each item exceeding $[redacted]	
Equipment Item	**Funds Requested ($)***
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)	[redacted]
2. Foreign Travel Costs	
Total Travel Cost	[redacted]

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 4

UEI*: [redacted]

Budget Type*: ❍ Project ● Subaward/Consortium

Organization: Vanderbilt University Medical Center

Start Date*: 09-01-2026 **End Date*:** 08-31-2027 **Budget Period: 4**

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

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

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)*
1 . MTDC	75.0	[redacted]	[redacted]
		Total Indirect Costs	[redacted]

Cognizant Federal Agency (Agency Name, POC Name, and POC Phone Number): DHHS, Region 4, 404-562-7888

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

J. Fee	Funds Requested ($)*

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

L. Budget Justification* File Name: VUMC_Budget_Just.pdf

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

OMB Number: 4040-0001
Expiration Date: 11/30/2025

RESEARCH & RELATED BUDGET - SECTION A & B, Budget Period 5

UEI*: [redacted]

Budget Type*: ❍ Project ● Subaward/Consortium

Enter name of Organization: Vanderbilt University Medical Center

Start Date*: 09-01-2027 **End Date*:** 08-31-2028 **Budget Period: 5**

A. Senior/Key Person

	Prefix	First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base Salary ($)	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits ($)*	Funds Requested ($)*
1.	Dr.	C.	Buddy	Creech	M.D.	Consortium PD/ PI	[redacted]	2.4			[redacted]	[redacted]	[redacted]
2.	Dr.	James	Christopher	Slaughter	Ph.D	Consortium Co-Investigator	[redacted]	0.6			[redacted]	[redacted]	[redacted]

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

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

B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits*	Funds Requested ($)*
	Post Doctoral Associates						
	Graduate Students						
	Undergraduate Students						
	Secretarial/Clerical						
1	Senior Research Assistant	2.4			[redacted]	[redacted]	[redacted]
1	Research Assistant	2.4			[redacted]	[redacted]	[redacted]
2	**Total Number Other Personnel**					**Total Other Personnel**	[redacted]
						Total Salary, Wages and Fringe Benefits (A+B)	[redacted]

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

Funding Opportunity Number: PA-20-185 . Received Date: 2023-04-10T16:10:08.000-04:00

RESEARCH & RELATED BUDGET - SECTION C, D, & E, Budget Period 5

UEI*: [redacted]

Budget Type*: ❍ Project ● Subaward/Consortium

Organization: Vanderbilt University Medical Center

Start Date*: 09-01-2027 **End Date*:** 08-31-2028 **Budget Period: 5**

C. Equipment Description

List items and dollar amount for each item exceeding $[redacted]

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)	[redacted]
2. Foreign Travel Costs	
Total Travel Cost	[redacted]

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 5

UEI*: [redacted]

Budget Type*: ❍ Project ● Subaward/Consortium

Organization: Vanderbilt University Medical Center

Start Date*: 09-01-2027 **End Date*:** 08-31-2028 **Budget Period: 5**

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

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

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)*
1 . MTDC	75.0	[redacted]	[redacted]
		Total Indirect Costs	[redacted]

Cognizant Federal Agency (Agency Name, POC Name, and POC Phone Number): DHHS, Region 4, 404-562-7888

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

J. Fee	Funds Requested ($)*

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

L. Budget Justification* File Name: VUMC_Budget_Just.pdf

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

BUDGET JUSTIFICATION

1. PERSONNEL Buddy Creech, MD, MPH, Co-Principal Investigator (Years 1-5: 2.4 calendar months) is Edie Carell Johnson Chair and Professor of Pediatric Infectious Diseases, Director of the Vanderbilt Vaccine Research Program (VVRP), and Principal Investigator of the NIH-funded Vanderbilt Vaccine and Treatment Evaluation Unit. In 2006, Dr. Creech established the Staphylococcal Research Laboratory, which initially focused on staphylococcal colonization and molecular epidemiology of clinical isolates of *S. aureus.* Working with Dr. Isaac Thomsen (former trainee of Dr. Creech who recently transitioned away from Vanderbilt University Medical Center), the laboratory expanded approximately 15 years ago to focus on the adaptive immune response to staphylococcal infections. Dr. Creech has worked closely with Dr. Liu to design and refine the proposed research studies. Dr. Creech will oversee patient enrollment, sample acquisition, and laboratory experiments of Aim 1 and 2. He and his team will analyze and interpret data generated from this proposal and take co-lead on preparation of scientific abstracts and manuscripts. He will work closely with Dr. Liu to ensure that the proposed scientific aims are met. The majority of Dr. Creech's time is allocated for research (>85%).

Christopher Slaughter, PhD, Biostatistician (Years 1-5: 0.6 calendar months) is Associate Professor in the Department of Biostatistics at Vanderbilt. Dr. Creech has worked with Dr. Slaughter for previous studies and they will continue their collaboration to analyze results from the current proposal. Dr. Slaughter will assist Drs. Creech and Liu with the development of analysis plans for all experiments proposed in Aims 1-3 of the proposal, ensuring that all analyses are technically sound and completely reproducible. He will also assist with manuscript preparation. In the first two years of the proposed award, Dr. Slaughter will assist primarily with analysis design and interpretation of initial data. In later years of the award, this effort will transition to data analysis and manuscript preparation.

Sandra Yoder, BS, MT(ASCP), Senior Research Assistant (Years 1-5: 2.4 calendar months) is an experienced medical technologist with advanced training and experience in flow cytometry whose 25-year career has focused on characterizing the adaptive immune response to infection and vaccination. She played a key role in establishing the Human Immunology Core at VUMC and we were fortunate to recruit her to the VVRP Laboratory approximately 10 years ago. She has extensive experience conducting humoral assays (ELISA, kinetic ELISA, and functional assays), cell-mediated immunity assays (including immunophenotyping, multiparameter flow cytometry, and cytokine analyses), and preparation of samples for multi-omics analysis. Ms. Yoder has nearly 20 years of experience with high parameter flow cytometry specializing in immune monitoring and bead-based detection of soluble proteins. She has extensive experience in development and optimization of flow cytometry stains of more than 10 parameters, and she has broad knowledge of instrumentation and reagents needed to best address complex immune system analyses. She will assist with the conduct of the liquid bead-based serology experiments outlined in Aim 1 and the flow-based experiments outlined in Aim 2. She will also oversee personnel working with patient samples and will oversee biosafety training, compliance, and quality assurance for the laboratory.

Eric Brady, BS, Research Assistant (Years 1-5: 2.4) has worked with the VVRP Laboratory for over five years. He will have primary responsibility for specimen acquisition, processing, labeling, and storage. He will also assist with experiments outlined in Aims 1-3, specifically bead-based serology assays in Aim 1 and functional assays described in Aim 2.

2. NON-LABOR BUDGET

VVRP Laboratory Supplies (Years 1-2: $[redacted] Year 3: $[redacted] Years 4-5: $[redacted]

Funds are requested to support the portion of the experiments that will be conducted in the VVRP Laboratory, as outlined in Aims 1-2. These include multiplex IgG detection on the Luminex platform (Aim 1); complement binding assays (Aim 2); antibody-mediated neutrophil phagocytosis assays (Aim 2); and cytotoxicity assays (Aim 2). The largest initial expenses will be costs related to bead conjugation of staphylococcal antigens for

serologic detection of anti-staphylococcal antibodies as outlined in Aim 1. These experiments will be performed on a Luminex platform, and all necessary equipment exists in the VVRP Laboratory. Additional reagents, such as MagPlex microspheres and Sulfo-NHS are necessary and will be used primarily in the first 3 years of the project. Additional funds are requested for phlebotomy supplies (collection of samples, as well as labels for the samples); reagents and supplies for cytotoxicity assays and neutrophil phagocytosis assays, including CellTiter
Cell Proliferation Assay**,** RPMI-1640 Media, and 96-well plates; ELISA reagents, including blocking buffer, human serum without IgG, M, or A, mouse anti-human IgG, goat anti-rabbit IgG and tetramethyl-benzadine; biological reagents including cell culture materials, FBS, and antibiotics; and chemical reagents for gel electrophoresis and characterization of immunoglobulins.

3. OTHER EXPENSES

Human Subject Reimbursement (Years 1-5: $[redacted]/year): Subjects are compensated $[redacted] for participation in the study, as per our standing, IRB-approved protocol for the enrollment of children and adults hospitalized with *S. aureus* disease. We estimate 20 subjects per year, for a total of $[redacted] in reimbursement per year.

Biostatistics Collaboration Center (Year 1-5: $[redacted]
The Biostatistics Collaboration Center (BCC) is a VUMC sponsored core resource whose goal is to provide for, enhance, and facilitate statistical collaborations involving the design, conduct, analysis or publication of biomedical research at the university. The BCC is a revenue-neutral resource. VUMC annually reviews the BCC to ensure that it is in compliance with all applicable federal and state regulations, including Uniform Administrative Requirements, Cost Principles, and Audit Requirements for Federal Awards. Rates are adjusted annually to ensure that the BCC is operating on a strict non-profit cost recovery basis.

All biostatistics staff/faculty effort is billed directly to each project as a percentage of salary and fringe benefit expenses. In addition, a scientific resource fee of $[redacted] per 100% annual FTE (faculty and staff combined) is billed to each project for allowable costs related to providing biostatistics support beyond what will be directly provided by Dr. Slaughter. The department uses robust computing technologies and innovative methodologies to manage complex analyses. This scientific resource fee covers costs necessary to perform the work of biostatisticians and systems analysts. These resources are directly related to the many technologies used and types of data generated across multiple disciplines that biostatisticians must competently handle.

Travel (Years 1-5: $[redacted]/year): Funds are requested to support transportation, lodging, and meeting fees for the principal investigators to present research findings from this project at two national scientific meetings each year. Costs are estimated as airfare ($[redacted] per trip), hotel ($[redacted]/day for 3 days per trim), and daily expenses ($[redacted]/day for 3 days per trip).

RESEARCH & RELATED BUDGET - Cumulative Budget

	Totals ($)	
Section A, Senior/Key Person		[redacted]
Section B, Other Personnel		[redacted]
Total Number Other Personnel	10	
Total Salary, Wages and Fringe Benefits (A+B)		[redacted]
Section C, Equipment		0.00
Section D, Travel		[redacted]
1. Domestic	[redacted]	
2. Foreign	0.00	
Section E, Participant/Trainee Support Costs		0.00
1. Tuition/Fees/Health Insurance	0.00	
2. Stipends	0.00	
3. Travel	0.00	
4. Subsistence	0.00	
5. Other	0.00	
6. Number of Participants/Trainees	0	
Section F, Other Direct Costs		[redacted]
1. Materials and Supplies	[redacted]	
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	[redacted]	
9. Other 2	[redacted]	
10. Other 3	0.00	
11. Other 4	0.00	
12. Other 5	0.00	
13. Other 6	0.00	
14. Other 7	0.00	
15. Other 8	0.00	
16. Other 9	0.00	
17. Other 10	0.00	
Section G, Direct Costs (A thru F)		[redacted]
Section H, Indirect Costs		[redacted]

Funding Opportunity Number: PA-20-185 . Received Date: 2023-04-10T16:10:08.000-04:00

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

Total Direct Costs less Consortium F&A

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

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

PHS 398 Cover Page Supplement

OMB Number: 0925-0001

Expiration Date: 09/30/2024

1. Vertebrate Animals Section

Are vertebrate animals euthanized? ● Yes ○ No

If "Yes" to euthanasia

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

● Yes ○ No

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

2. *Program Income Section

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

○ Yes ● No

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

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

PHS 398 Cover Page Supplement

3. Human Embryonic Stem Cells Section

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

If the proposed project involves human embryonic stem cells, list below the registration number of the specific cell line(s) from the following list: http://grants.nih.gov/stem_cells/registry/current.htm. 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

OMB Number: 0925-0001
Expiration Date: 10/31/2025

Introduction	
1. Introduction to Application (for Resubmission and Revision applications)	
Research Plan Section	
2. Specific Aims	Specific_Aims_-_final.pdf
3. Research Strategy*	Research_Strategies_-_final.pdf
4. Progress Report Publication List	
Other Research Plan Section	
5. Vertebrate Animals	Liu_Creech_R01_Vertebrate_animals.pdf
6. Select Agent Research	R01_Select_agent_research.pdf
7. Multiple PD/PI Leadership Plan	Multiple_PI_Project_Leadership_Plan.pdf
8. Consortium/Contractual Arrangements	Creech_Facepage_--_flattened.pdf
9. Letters of Support	Liu_R01_Consultancy_Letter_.pdf
10. Resource Sharing Plan(s)	Liu_Creech_R01_Resource_sharing_plan_Final.pdf
11. Other Plan(s)	Data_Management_Sharing_Plan.pdf
12. Authentication of Key Biological and/or Chemical Resources	Authentication_of_key_Bio_resources.pdf
Appendix	
13. Appendix	

Tracking Number: GRANT13855431
Funding Opportunity Number: PA-20-185. Received Date: 2023-04-10T16:10:08.000-04:00

SPECIFIC AIMS

Staphylococcus aureus (SA) is the leading cause of invasive bacterial infections in the US. Despite its status as a priority target for vaccine development, all SA vaccines tested to date have been unsuccessful. One consistent barrier has been the lack of reliable translational animal models that predict vaccine protection in humans. Given the impossibility of controlled human infection models of invasive staphylococcal infections, we have sought to define the adaptive host response following natural invasive SA disease in children and older adults through models that best recapitulate the natural human host environment. We recently demonstrated that the transfer of convalescent sera from children with invasive staphylococcal disease protected mice from SA sepsis, and alpha toxin-specific antibodies purified from protective sera conferred partial anti-staphylococcal immunity. Using the same approach, we discovered non-protective human anti-SA antibodies that abrogated otherwise protective SA vaccines. Vaccine interference occurred because of recall of non-protective memory responses and antibody competition. Based on these findings, we hypothesize that SA infection in humans generates a functional, but limited, antibody response that is beset by non-protective or interfering antibodies. **Development of a successful antibody-based approach to SA must take these factors into account, but they remain poorly defined.** The central objective of this proposal is to define the protective components of the human antibody response to SA and to delineate these from non-protective or suppressive responses, in order to elucidate critical mechanisms of anti-staphylococcal antibody protection. These studies will elucidate critical mechanisms of adaptive staphylococcal immunity and inform novel antibody-based approaches to prevent or treat SA infections. We propose three Aims to address these goals:

Specific Aim 1: To define functionally protective antibody responses to SA antigens in children and older adults with invasive SA infections. SA is a highly evolved human pathogen, and it remains unclear what antigens are produced in the setting of invasive human infections. While certain genes may be present in clinical isolates, this does not indicate expression during human infection and functional recognition by the host. We will leverage our existing infrastructure to develop a prospective cohort of clinical samples from children and older adults with invasive SA infections and age-matched controls. We will define the targets and kinetics of the natural humoral response to SA and follow these patients for serial serologic and clinical assessments, including the possibility of re-infection. In parallel, we will engage our passive transfer model to define protective characteristics of the human adaptive response. Completion of Aim 1 will generate a list of specific candidate antibodies induced during human staphylococcal disease that are associated with protection against *SA*.

Specific Aim 2: To identify and characterize protective and non-protective human antibodies targeting clinically relevant SA antigens. Serologic assessment provides candidate antigen targets for intervention, but functional assays are critical for rational design of targets with a high likelihood of success. Using the cohort established in Aim 1, we will interrogate clinical samples and specific antibodies for a) their ability to confer protection, and b) the mechanisms of protection as defined by a series of *in vivo* and *ex vivo* models. Targeted antibody depletion of serum will be performed against selected antigens identified in Aim 1, and structural and functional profiling will elucidate protective and non-protective antibodies. *In vitro* bacterial killing and cytotoxicity assays coupled with structural analyses will be part of the battery of assays used to characterize protective and non-protective antibodies, and confirmatory assessments will occur by transfer to both wild-type and humanized mouse models. In total, this Aim will reveal the key determinants of the functional, adaptive human response to SA and define candidates for reverse vaccine development.

Specific Aim 3: To determine how prior humoral interaction with SA shapes vaccine efficacy. The concept of original antigenic sin postulates that the immune response to first infection imprints itself on subsequent host responses to infections and vaccination. Using a prototype vaccine that targets a SA surface protein, IsdB, we have shown that immune imprinting and antibody interference impair an otherwise functional antibody response. This aim will determine the impact of pre-existing SA antibodies on both passive and active immunization strategies by defining antibody interference in specific vaccination models. We hypothesize that a truly effective antibody-based intervention must account for antibody interference and suppression, and that subdominant antigen targets or antigens not prone to interference may prove to be good vaccine targets. This Aim seeks to rationally assess putative vaccine candidates by accounting for immune imprinting and interference related to prior host exposure to *SA*.

A. SIGNIFICANCE

A.1. Focus on an unrivalled pathogen that has defied all attempts at control with vaccination. SA is now the most common invasive bacterial pathogen in children in the US[1-3]**.** Highly effective vaccines have profoundly reduced the rates of previously common invasive bacterial pathogens, such as *S. pneumoniae* and *H. influenzae*, but a successful vaccine against *SA* remains elusive[4]. *SA* is unrivalled among bacterial (and perhaps all) pathogens with regard to unsuccessful vaccine attempts. Equally remarkable is that the reason behind the vaccine failures remains unclear. Proposed causes include strain-dependent expression of certain *SA* antigens, poor clinical trial designs, and a mouse model that does not mimic the human condition (**Fig. 1**).

A.2. An entirely new framework that explains failure of human *SA* vaccine trials. To date, animal models have lacked predictive value for development of a successful human vaccine. A key difference between humans and laboratory animals is the frequency of host exposure to SA. Laboratory mice are rarely colonized or infected with human *SA* whereas half of humans are colonized by 1.5 months of age and routinely have elevated levels of SA-specific antibodies (Ab) in their sera[5,6]. In our recently published study of the mechanisms underlying failed IsdB vaccine (Tsai 2022)[7], we showed that, while IsdB vaccine administered to naïve mice is effective, the same vaccine, administered to SA-experienced mice, is ineffective because of immune recall of non-protective IsdB memory response. In preliminary experiments (below), we have extended our finding to several other failed active and passive *SA* vaccines. Predictably, naïve mice were protected by specific vaccination, but the same vaccines were not protective in mice with pre-existing specific anti-*SA* immunity. We thus hypothesize that *pre-existing pathogen-specific immunity critically shapes vaccine efficacy in the SA-experienced host*. Our expanded human study aims to broadly test our hypothesis across failed vaccines.

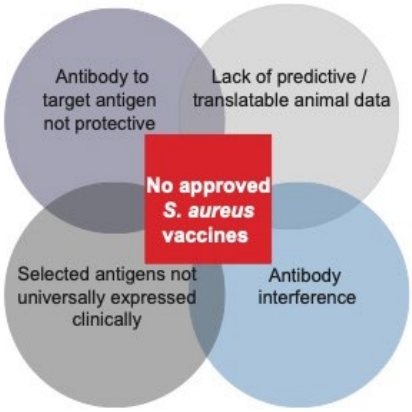


Figure 1. Potential reasons for prior *S. aureus* vaccine failures

A.3. Study of human SA-specific Ab to identify human-relevant candidate vaccine antigens and Ab determinants of protection. Few prior studies have attempted to identify functional, protective *SA* antigens *in humans*. One longstanding challenge to the search for mediators of protection has been the inability to determine which subjects generate a protective adaptive host response. For some pathogens, human challenge models have addressed this barrier, but human challenge is not ethical for invasive *SA* disease. To begin addressing functional immunity to *SA* in humans, we developed an adoptive transfer model wherein transfer of sera from *S. pneumoniae* vaccinated (vs. unvaccinated) individuals led to predictable protection against a pneumococcal challenge[8]. We then used the model to show that children with invasive *SA* infection develop protective sera over the course of convalescence against SA. We showed that purified human serum Ab against the antigen IsdB (shown to be non-protective in our published study) is not protective, whereas natural human Ab to the protective antigen alpha toxin (Hla)[9] conferred partial immunity against *SA* challenge in the model (**Fig. 2A and 12C**). We further identified Fab and Fc features of the IsdB Ab that determined non-protection (Tsai 2022). Hence, our proof-of-principle study of human Ab to *SA* demonstrates the potential to leverage our adoptive transfer platform and structure-function assays to reveal *SA* antigens that induce protective and non-protective responses following invasive disease in humans.

A.4. Studying pediatric and elderly populations to define host Ab responses to invasive *SA* infections. Children represent the ideal host in which to study invasive *SA* infections in humans, for several reasons. First, *SA* disease in children involves uniquely defined phenotypes of invasive infection that are typically hematogenous in origin (e.g., acute osteomyelitis, septic arthritis, or visceral abscesses). This allows for straightforward stratification of disease types and a true assessment of the host-pathogen interactions in the setting of human bloodstream infection. Second, *SA* carriage exerts a variable effect on specific anti-staphylococcal antibodies[10,11]. The pediatric serologic profile, lacking a lifetime of intermittent or persistent nasal colonization, reflects the response to acute infection more directly. Finally, the relative lack of immune-modifying characteristics (e.g., medical comorbidities seen in older individuals[12,13]) is an additional advantage to studying responses in children. In the present study, pediatric sera will be contrasted against sera from older adults with SA infection, to determine whether mechanisms of vaccine failure are affected by aging and a lifetime of SA exposure. Older adults may have increased concentrations of interfering antibodies and potential changes in Ab glycosylation that may impact phagocytosis negatively. Conversely, older patients have a lifetime to accumulate protective antibodies against selective SA antigens. Our proposed experiments will determine if and how antibodies from older adults could lead to better or worse vaccine responses.

A.5. Develop a predictive mechanism-based model of *SA* vaccine efficacy to support human trials. Our recently published study points to two mechanisms that explain inefficacy of IsdB vaccine in *SA* pre-infected mice (Tsai 2022). First, IsdB vaccine recalls ineffective IsdB memory response from prior *SA* infection, akin to the original antigenic sin described with HIV, influenza, dengue, and RSV viruses[14-16]. Furthermore, when protective Ab generated by IsdB vaccine is co-transferred with non-protective IsdB Ab from human sera, protection from the vaccine Ab is significantly dampened by competition (**Fig. 2A** and Tsai 2022). We showed similar evidence of interference with a monoclonal Ab (mAb) to *SA* Clumping factor A (ClfA) Tefibazumab that failed clinical trial[17], when human serum samples with high anti-ClfA titers are added in vivo (**Fig. 2B**). Hence, we suggest that **immune imprinting and direct Ab interference** are potential contributory mechanisms to human trial failures. The proposed research will query both mechanisms to explain the failure of a list of active and passive *SA* vaccines as we aim to build a more predictive model for human *SA* vaccines.

B. INNOVATION

B.1. Technical innovation – Adoptive transfer model of human sera into mice and prior infection model enable human-relevant studies of vaccines against SA. Vaccine studies are traditionally carried out in naïve animals as they have provided a good correlate of human protection. The failure of every *SA* vaccine brought to clinical trial so far has prompted us to reevaluate the existing platform and led us to evaluate pre-infected mice or mice pre-transfused with human anti-*SA* serum Ab as models of the human host. Development of the adoptive transfer model was motivated by the need to directly evaluate human protective immunity in mice. When applied prospectively to the study of invasive infection in children as proposed, it enables the generation of a powerful platform for elucidating critical aspects of the adaptive host response[8]. The complementary tools allow us to address why past vaccines have not worked and what are potential protective vaccine antigens.

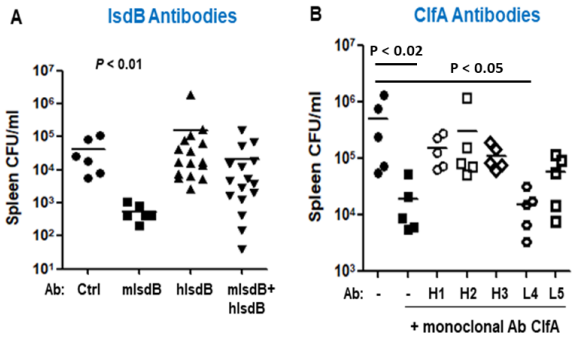


Figure 2. Human serum Ab abrogate efficacy of protective anti-*SA* Ab. (A) Protective murine IsdB-specific Ab (mIsdB) generated by IsdB vaccination were mixed with purified IsdB-specific antibodies from 17 human volunteers individually (hIsdB) in equal molar ratio. Protection against intraperitoneal *SA* (LAC strain, 10^7 CFU) challenge was assessed 4h later. **(B)** Tefibazumab, a ClfA-specific mAb that failed clinical trial was added at 8mg/kg to 5 human serum samples containing low (L4-5) or high levels of ClfA-specific Ab (H1-3). Four hours after Ab transfer, protection was assessed by *SA* challenge as before. Statistics used in all preliminary data are described in the Statistics section at the end of Aim 3.

B.2. Conceptual innovation – Immune imprinting and Ab interference. Original antigenic sin posits recall of an ineffective immune response on encounter with a closely related pathogen, usually a pathogen that has undergone mutations, e.g., influenza and RSV[14,18]. In the case of SA, we have shown that the same antigen presented in the context of *SA* infection versus adjuvant alum can lead to very different outcomes (different epitope specificity and Ab glycosylation) (Tsai 2022). Vaccination leads to protection in naïve mice but recalls the non-protective Ab response in previously SA-infected mice. Furthermore, we showed that Ab competition between protective and non-protective Ab at physiologically relevant concentrations led to interference. *These concepts can potentially explain vaccine failures in SA vaccine trials and may have implication for failures of vaccines to other pathogens.*

B.3. Innovation in vaccine development – targeting of subdominant antigens to achieve protection. Based on our model, we have shown that a vaccine antigen that recalls little prior humoral memory induced robust protection, hence the concept of targeting subdominant antigens to circumvent interference. This concept stands against a current principle that vaccine antigens should elicit a robust Ab response to assure that the antigen is expressed in vivo. In our model, *SA* antigens that induce robust human Ab response may be more likely to induce interference since *SA* has evolved many strategies to suppress specific host adaptive immunity to promote human coexistence.

B.4. Broad scale human study to identify potential vaccine antigens to SA: Our study approach combining human and animal experiments has the potential to identify a refined set of *SA* vaccine candidates encompassing protective antigens (Aim 2.1) and subdominant *SA* antigens that escape interference (Aim 3.3). Our goal is to identify from a list of candidate antigens, the most promising SA antigens that could be advanced to a vaccine trial. Developing the most effective vaccine targets is critical since success or failure of such a vaccine in a human trial could reflect on the validity of the immune imprinting hypothesis.

C. PRELIMINARY DATA AND APPROACH

C.1.A. Adoptive Transfer Model to study existing human anti-*SA* Ab function . We developed the adoptive transfer model specifically to address the aims of this proposal, prompted by the discrepant outcomes from laboratory and human vaccine trials. The model permits sera from invasively infected individuals to be adoptively transferred to a murine model and to measure protective efficacy. We first established proof of concept by using defined pre-and post-vaccine sera from subjects who received the conjugate pneumococcal vaccine, and demonstrated that post-vaccine sera, when transferred to a murine model, potently protected against invasive pneumococcal infection[8]. Given this proof of concept, we developed this model for the measurement of protective efficacy against *SA* disease following invasive human infection. We enrolled a patient cohort of 14 children infected with invasive *SA* disease (bacteremia, osteomyelitis, and endocarditis) at Vanderbilt. Sera were collected at three time points (**Fig. 3**) and screened against a panel of *SA* antigens to ensure an Ab response was generated from infection. The sera were then transferred IP into mice which were infected with *SA* strain LAC 24 hours later for assessment of protection by CFU enumeration. Sera from 6 of the 14 patients induced significant reduction in disease burden compared to control samples, with 2 patient samples conferring complete protection from disease. Importantly, protective efficacy peaked at the convalescent timepoint (V2, 4-6 weeks after enrollment) (**Fig. 3**), the time when post-infection IgG levels peak. These data indicate that human serum from infected individuals contain factors capable of reducing bacterial load in vivo.

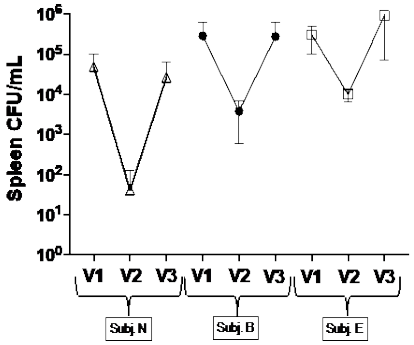


Figure 3: Human sera is protective when antibody levels peak. *SA* burden (CFU) post IP infection is shown acutely (V1), 4-6 weeks post-infection (V2), and at 6 months (V3). Peak reduction in *SA* CFU occurred at 4-6 weeks post infection.

C.1.B. Original antigenic sin: Impact of pre-existing antigen-specific immunity on vaccine efficacy

Mice and humans are fundamentally different in that laboratory mice are largely naïve to human *SA* and humans are colonized early after infancy. As noted previously, all promising vaccines developed in naïve mice have failed in human trials[4]. One of the most notable *SA* vaccine "failures" to date was a vaccine that targeted the iron-regulated surface determinant protein B (IsdB), a crucial antigen for nutrition via acquisition of host iron[19]. Disappointingly, the vaccine lacked efficacy in a Phase 3 clinical trial[19]. Since humans routinely harbor high levels of Ab to IsdB, we sought to determine if prior memory of IsdB has a role in the lack of vaccine efficacy.

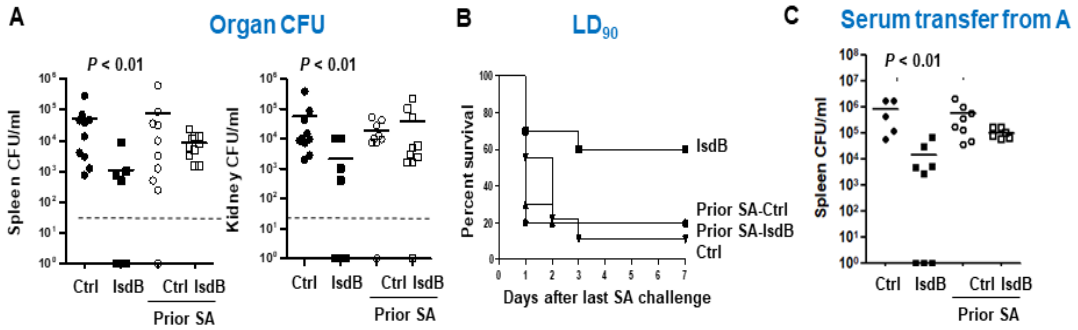


Figure 4. IsdB vaccination confers Ab protection to naïve but not mice previously infected with SA. C57BL/6 mice were injected i.p. with PBS or 10^7 *SA* (LAC strain) 3 times at 7-day intervals. Ten days after the last infection, mice were immunized with either alum (Ctrl) or alum plus IsdB three times at weekly intervals. One week after immunization, the mice were challenged i.p. with **(A)** 10^7 *SA* (LAC) for CFU enumeration or **(B)** $2x10^8$ *SA* for assessment of LD_{90} (n=10 mice per group). **(C)** Adoptive transfer of 100 ml sera from naïve then vaccinated donor mice to naïve recipient mice conferred protection to 10^7 *SA* (LAC) i.p. challenge in the recipient mice.

Prior *SA* exposure undermines IsdB vaccine efficacy. To simulate human exposure, we infected mice by a variety of approaches: through skin infection, single IV infection followed by antibiotic treatment, or through 2-3 IP infections (given humoral response after 1 IP infection yields modest titer of SA-specific Ab). We showed that each of these prior treatments dramatically reduced or abrogated efficacy of IsdB vaccination, and loss of humoral protection is confirmed to be the mechanism and is antigen-specific (**Fig. 4** and Tsai 2022).

IsdB vaccine recalls ineffective anti-IsdB humoral responses – We further showed that protective vaccine-generated Ab are different from non-protective infection induced Ab in two aspects that involve both Fab and Fc fragments: Non-protective Ab have altered Fc α2,3 sialylation and impaired opsonophagocytic (OPK) killing of

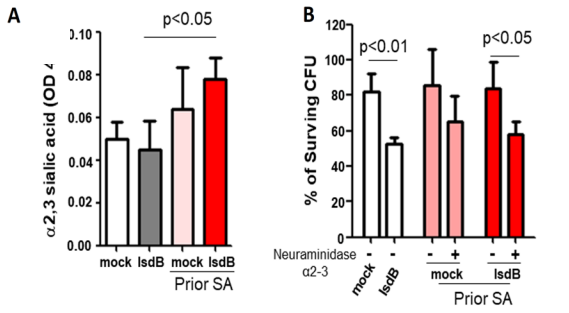


Figure 5. Increased sialylation of Ab contributes to impaired Opsonophagocytic clearance of SA. Mice were infected/immunized as in Fig. 4. **(A)** Reduced α2,3 sialylation of IsdB-specific Ab induced by IsdB vaccine compared to *SA* infection followed by vaccine. **(B)** Sialidase treatment abrogates difference in OPK between protective and non-protective groups.

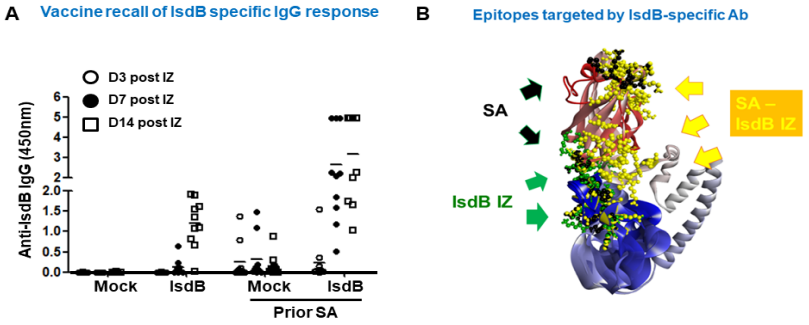


Figure 6. Immune imprinting - Recall of prior IsdB Ab response by IsdB vaccination. **(A)** Naïve and SA-infected mice were immunized with alum+/-IsdB. Shown are IgG titers to IsdB on d3, 7 and 14 post vaccination **(B)** IsdB epitopes targeted by vaccine- and/or infection- generated Ab - IsdB-specific B cells were purified 7 days after the last infection or vaccine, and IsdB epitopes were mapped by B cell receptor sequencing and plotted on the structure of IsdB.

SA compared to protective Ab, that is abrogated with sialidase treatment (**Fig. 5**). Among Fab features, we showed that non-protective and protective IsdB Ab differ in their targeting of protective heme-binding NEAT2 domain (blue domain, **Fig. 6B**). Preliminary data link increased sialylation to IL-10, with anti-IL10 Ab treatment reducing Ab sialylation and improving vaccine protection in vivo (**Fig. 7**). Thus, IL10 induced changes in glycosylation appears to be a mechanism that explains why SA vaccines have failed. Importantly, we showed, in *SA*-exposed hosts, that Ab generated by IsdB vaccine recalls both Fab and Fc features of the ineffective IsdB Ab from prior infection, reminiscent of the concept of Original Antigenic Sin (**Fig. 5, 6**).

Direct Ab interference by non-protective specific Ab – In addition to immune imprinting, we showed that non-protective Ab compete against protective Ab for binding to IsdB and abrogate in vitro and in vivo Ab mediated protection (**Fig. 8**). To query the human relevance of our findings, we purified IsdB-specific Ab from human sera, demonstrated first that they were largely non-protective against *SA* challenge upon adoptive transfer into naïve mice (**Fig. 2A**). When injected along with protective vaccine-generated IsdB-specific Ab, the human antibodies dramatically dampened efficacy of the protective specific Ab against *SA* challenge (**Fig. 2A**).

Influence of prior *SA* exposure on other *SA* vaccines – To probe if interference extends beyond IsdB vaccine, we investigated vaccines against two other cell surface proteins, Fhud2 and IsdA[20,21]. Both vaccines were effective when given to naïve mice but showed no efficacy if the mice were previously infected with *SA* (**Fig. 9**). Conversely, we tested if vaccine against a previously identified "protective" *SA* antigen Hla (alpha toxin) is effective (**Fig. 12**). By adoptive transfer, we showed that human Ab to Hla confer partial protection against *SA* challenge. When naïve and SA-infected mice were immunized against Hla, they were protected in both instances, consistent with our proposed hypothesis that prior specific immunity plays a key role in vaccine function. We also queried if human sera interfered with passive (mAb-mediated) immunization. We showed that the mAb tefibazumab targeting clumping factor A (ClfA, that failed in clinical trial) was effective in naïve mice but ineffective if injected along with human sera with high ClfA titers (**Fig. 2B**). Overall, our data strongly suggest that *pre-existing humoral responses to SA have the potential to modify specific SA vaccine responses and thereby may explain failures of past SA vaccine trials*.

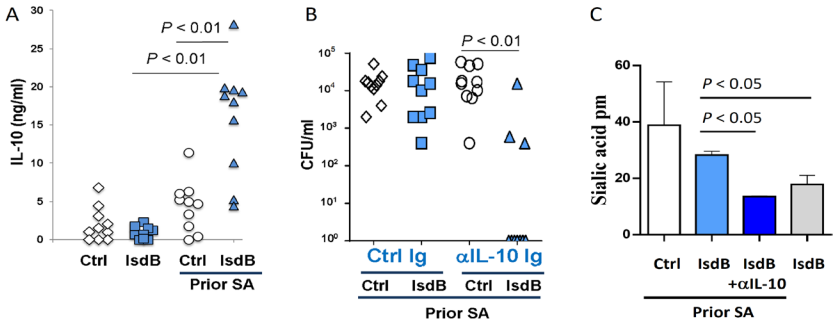


Fig. 7. Role of IL10 in Ab sialylation and vaccine interference. Mice were infected and/or IsdB vaccinated as in Fig. 4. (A) Higher serum IL10 in mice that were SA infected then IsdB vaccinated; (B) Anti-IL10 treatment at the time of IsdB vaccination abrogates vaccine Interference in SA-pre-exposed mice. (C) anti-IL10 Ab reduced Ab sialylation of non-protective IsdB-specific Ab (from SA plus vaccine group) to level of protective Ab (from IsdB vaccine group). Ctrl: control; IsdB: vaccine.

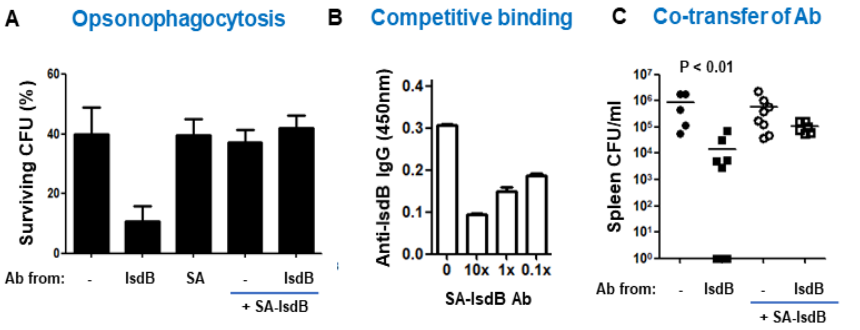


Figure 8. Competition between non-protective and protective IsdB-specific Ab. Protective and non-protective IsdB-specific Ab generated by vaccination in naïve and SA-infected mice respectively (as in Fig. 4) were purified, mixed, and assayed for: **(A)** opsonophagocytic killing of *SA* in co-culture with human neutrophils with equal molar of both Ab; **(B)** competitive binding to recombinant IsdB. Titers reflect binding of protective Ab in the presence of increasing concentrations of non-protective Ab at the indicated molar ratios; **(C)** protection of mice from *SA* challenge following adoptive transfer with protective and non-protective IsdB-specific Ab (1:1 ratio).

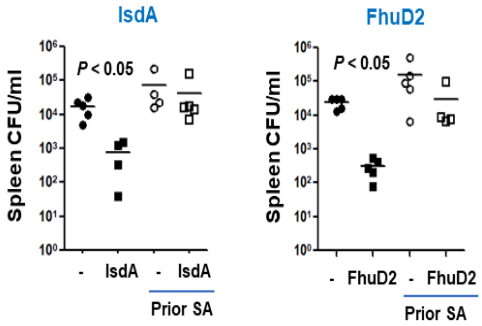


Figure 9. Prior *SA* infection abrogates efficacy of vaccines against *SA* surface antigens IsdA and FhuD2. IsdA and FhuD2 vaccine efficacy in naïve and *SA* infected mice was assessed as in Fig. 4.

RESEARCH DESIGN AND METHODS

Hypothesis: Humans are routinely exposed to *SA* beginning in infancy. We hypothesize that this exposure leads to both protective and non-protective humoral responses depending on the target antigens (Aim 1 and 2A). Determinants of Ab protection include not only which specific antigens are targeted, but also Ab titer and specific Fab and Fc-fragment characteristics (Aim 2B). Further, the humoral immune imprint (protective or non-protective) established by prior *SA* exposure modulates vaccine efficacy (Aim 3A). Finally, we hypothesize that pre-existing, inadequately protective antibodies compete with effective anti-*SA* antibodies to decrease efficacy of passive immunization (Aim 3A and 3B). Targeting of "subdominant" *SA* antigens that show little imprint (low Ab titers after *SA* exposure) is hypothesized as a strategy to overcome vaccine interference (Aim 3C).

We propose three aims to address our hypotheses. Aim 1 will prospectively enroll children and older adults with invasive *SA* disease, perform serial evaluation of Ab titers against a large panel of *SA* antigens, and correlate titers against protection conferred by the serum samples in functional in vitro and in vivo assays, in addition to recurrence of *SA* infection in the patient cohort. Aim 2 will identify and investigate both protective and non-protective specific Ab with the goal of determining what Ab characteristics are drivers of robust or sub-optimal protection. Aim 3 will investigate how non-protective or protective pre-existing humoral responses shape *SA* vaccine responses, in the context of their potential to positively or negatively impact vaccine-mediated protection through immune imprinting or direct Ab interference. Targeting of "subdominant" *SA* antigens will be explored as an approach to circumvent interference from pre-existing humoral immunity. A summary of our hypotheses with the corresponding plan of study is shown in **Fig. 10**.

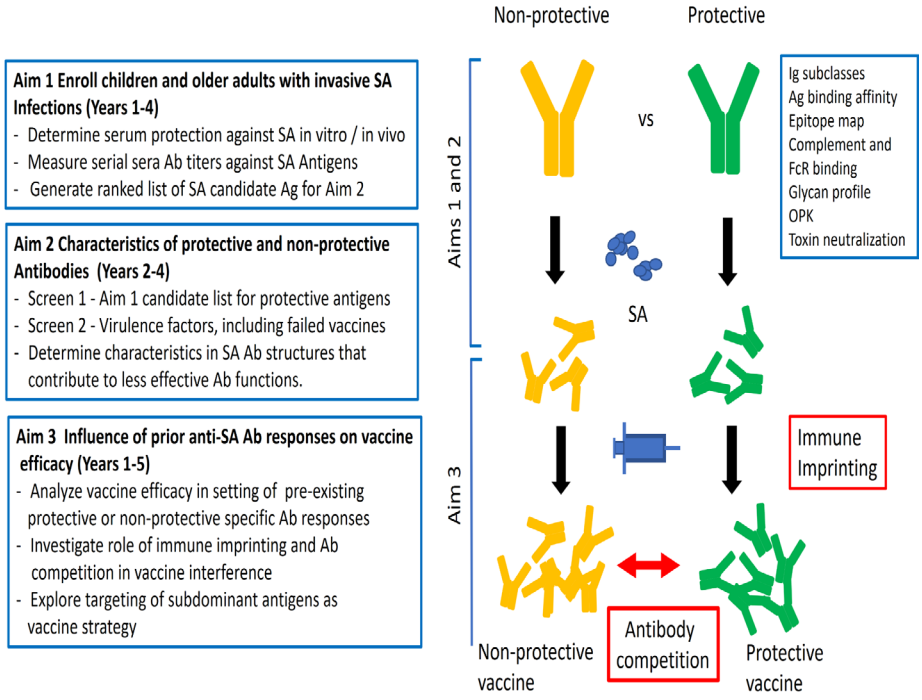


Figure 10: Study schematic and general timeline of events

Specific Aim 1: To define functionally protective antibody responses to *S. aureus* antigens in children and older adults with invasive *S. aureus* infections (Years 1- 4)

Rationale: *SA* is a highly human-specific pathogen, and this is particularly true for certain antigens such as the leukocidins[22]. Prior *SA* vaccine targets have appeared promising in pre-clinical studies but were minimally investigated for importance in human disease and lacked efficacy in human trials. We will determine critical aspects of the host response to *SA* bloodstream infection by defining the antibody response in subjects who survive invasive disease. These subjects are expected to generate the most effective memory responses, of particular importance as recurrent invasive disease in humans is exceedingly rare in the absence of immunodeficiency. To test this, we will leverage our well-established clinical research infrastructure and informatics resources to study the host response to *SA* antigens using samples prospectively obtained from distinctly phenotyped subjects.

Approach: **Subject Enrollment, Invasive *SA* Disease.** Working within a current, IRB-approved protocol for enrollment of subjects with *SA* disease at Vanderbilt, we will target two populations for this work: children (1-17 years of age) and older adults (>65 years of age). We have extensive experience enrolling each of these populations in the setting of invasive SA disease[23-25]. In collaboration with the clinical microbiology laboratory, we are made aware of all cultures growing *SA*, 7 days/week, immediately upon initial culture positivity. Sera and peripheral blood mononuclear cells (PBMCs) will be obtained within 48 h of the growth of *SA* in culture and at multiple timepoints up to 12 months post-infection, and the infecting isolates will be obtained. PBMCs will be stored in anticipation that they could prove useful later, e.g. in relation to IL10 production by SA-specific T and B cells.

Defining the host Ab response to invasive *SA* disease in children and older adults. Given that *SA* is a highly adapted human pathogen, it has remained challenging to identify relevant antigens that are definitively expressed during invasive infection. Detection of a high-titer Ab response (rising in convalescence, and relative to controls) confirms that an antigen is expressed during natural human infection and targeted by the host. We aim, therefore, to define the specific targets of the natural humoral response to *SA,* and the kinetics of this response. We will employ liquid bead array technology, as we have previously used successfully[24,26], to broadly and efficiently measure IgG responses to 65 selected *SA* antigens. Antigens included on the panel will include all relevant aspects of pathogenesis (**Table 1**). All selected antigens are surface-expressed or secreted, allowing immune recognition by the host. Samples from invasive disease will be compared to non-invasive disease and healthy control samples to identify which antigens are most relevant and expressed during invasive disease. Further, we will define the kinetics of this response by measurement at serial timepoints. Patient samples will be measured in biological and technological triplicate to ensure reproducibility of the results. Additional components of characterization studies will include the identification of infecting strain type by multi-locus sequence typing and correlation of strain lineage with virulence factor profiles from the infecting isolates.

Antigen Category	# Antigens on Panel
Nutrient Acquisition	19
Toxins	11
Superantigens	8
IgG-binding	2
Extracellular enzymes	12
Other	13

Table 1. Categories of specific antigens to be assessed by multiplex serology

***In vivo* characterization of post-infection human sera by adoptive transfer.** Following serologic characterization as above, all sera collected at 6 weeks (peak protection based on preliminary data) will be further assessed for their ability to provide protection in the murine adoptive transfer model. If protective, the sera from follow-up time points will also be assessed. In the murine model, patient sera will be adoptively transferred (n=8 mice) by the intraperitoneal (IP) route for 24 hours before systemic challenge with *SA* by IP. Importantly, we will assess rechallenge both with the same clinical isolate obtained from the infected patients, as well as a strain from a distinct lineage, to evaluate breadth of protection. After 48 hours, the kidney and spleen CFUs, cytokines (IL-1β, IL-6 and TNF-α) and kidney histopathology (gross pathology and H&E to assess abscess size and numbers) will be measured. Samples will be selected for advancement to Aim 2 based on evidence of significant protection at any timepoint, defined as $\geq$1 log CFU and/or significant reduction in immunopathology (primary: kidney abscess size and number, and secondary: cytokines) with high protection defined as $\geq$3 log reduction. Sera from serial timepoints will be measured to elucidate the kinetics of the humoral response *in vivo*. Recognizing that some factors may show clear human tropism and efficacy may be missed with wild-type mouse models, we will also use humanized NSG mice as part of the initial screen (UCLA CFAR

core). We have used these mice previously to demonstrate the pathogenic role of human tropic Panton-Valentine Leukocidin[27]. The results from this aim will stand alone in defining the protective humoral response to *SA* in a much more clinically relevant way than *in vitro* assays alone.

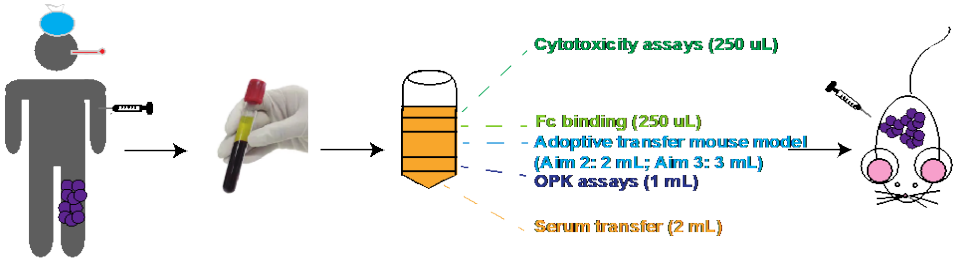


Figure 11. Adoptive transfer model and volumes required for proposed experiments. The studies outlined in the proposal have been carefully designed for feasibility based on realistic patient sample volumes.

Analysis Plan: We anticipate 100 subjects (75 children and 25 older adults) enrolled over the first four years of the study period (with the final year dedicated to completing experimental work). This pediatric sample size is based on 18-22 children admitted to Vanderbilt Children's Hospital with invasive disease per year, consistent with current local epidemiology from 2014-present. Based on our preliminary data, we estimate that 50% of sera will be categorized as non-protective in adoptive transfer, 40% moderately protective, and 10% highly protective. This sample size will provide sufficient power (80%) to detect at least a 1.9-fold increase in the odds of protection per standard-deviation titer increase for each antigen, with a significance level to 0.001 corresponding to a conservative Bonferonni-type correction for 65 antigens. **Generation of a list of candidate antigens for downstream (Aim 2 and 3) investigation:** Samples will be analyzed using logistic regression with specific Ab levels as the continuous variable. This analysis will allow determination of a ranked list of which *SA* antigens are independently predictive of functional protection in human pediatric sera following invasive infection. We have carefully considered the volume of serum to assure feasibility in all 3 Aims (**Fig. 11**). **Age/Sex as biologic variables:** Children and older adults of both sexes will be recruited.

Potential Challenges and Alternative Strategies: The work proposed in this aim depends on the collection of clinical isolates, sera, and cells collected from children and older adults infected with *SA* disease. However, rates of infection are known to vary over time and may wane over the course of the outlined studies. If this occurs, we will draw on our previously collected repository containing >250 invasive samples, consented for future use of leftover samples. The repository lacks sufficient serum volume on enough participants to support the overall proposal but will be able to supplement with a small number of subjects if necessary. The use of a liquid bead array to test the Ab-antigen interaction of patient sera is an attractive option due to its efficiency and reproducibility. However, these arrays can be difficult to use correctly while maintaining the integrity of the proteins in question (e.g., improper conjugation of an antigen to the array would result in a false negative result). To maximize rigor, extensive controls will be used in addition to technical reproducibility and validation by ELISA. Anti-human IgG will be used to confirm the addition of sample and negative controls including buffer and antigen tag controls will also be used to ensure the antigen is properly bound to the array. Our extensive experience in this area allows navigation around these potential pitfalls.

Specific Aim 2: Identify and characterize protective and non-protective human antibodies targeting clinically relevant *S. aureus* antigens (2A: years 1-4; 2B years 2-4)

Rationale: Successful human vaccines against many pathogens have targeted pathogen-expressed antigens that induced protective human Ab immunity, e.g., capsular polysaccharide[28,29]. In preliminary experiments, we have shown that human Ab to a protective antigen (Hla) is protective in both naïve and *SA* experienced hosts (**Fig. 12A**). Conversely, we have shown that several non-protective antigens generate non-protective vaccines in *SA* experienced mice even though protection is achieved in naïve mice (**Fig. 4A and 9**). Hence, we hypothesize that prior host humoral immune interaction with *SA* determines ultimate efficacy of *SA* vaccines. To test our hypothesis, we aim first to define and characterize the vaccine-relevant Ab response to a large panel of *SA* antigens in humans.

Approach: We will study a list of human Ab, preselected to include major virulence factors and vaccine antigens that failed in clinical trials. In parallel, we will characterize protective serum samples advanced from Aim 1 to identify *SA* antigens that are strongly associated with protective Ab responses, as these antigens represent potential targets of intervention for invasive human disease. We will study characteristics of both protective and non-protective Ab to determine what factors and Ab features confer functional protection.

Aim 2.1. Identify protective and non-protective human Ab targeting *SA* antigens

Approach: **1) Preselected *SA* antigens:** we will assess specific Ab against ***10 SA antigens*** *of particular interest*. The list includes well known virulence determinants and major antigens that have been evaluated in murine studies and human vaccine trials: Cell-surface antigens (IsdA, MntC*, ClfA*, SpA, FhuD2, EsxAB), PAMPs (LTA*, Lipoprotein*) and toxins (Hla*, LukED) with * denoting vaccine trials that demonstrated lack of efficacy.[17,20,30-39]. **2) Candidate protective *SA* antigens from Aim 1** - The analysis plan outlined in Aim 1 will result in a ranked list of *SA* antigens that are most likely to induce protective Ab based on titer correlation. Specifically, this rank list will be based on relative titer of specific Ab in protective samples vs. non-protective samples. We will study the **8 highest-ranked protective antigens**. We will purify specific Ab using antigen columns, then evaluate the Ab for protection in the adoptive transfer model. As a complementary approach, specific Ab protection will be evaluated by determination of the protection upon specific depletion of the Ab from that sample, where loss of protection signifies function of the depleted Abs. This approach preserves the interaction between Ab and other serum factors of possible importance**.** In the unlikely case that we find fewer than 8 non-overlapping antigens from the candidate list from Aim 1, we have *a priori* selected 8 alternative antigens of potential importance that we will study (FnBPA, LukPV, PNAG, CSA1a, SdrD, CP5/8, WTA, LukAB)[40]. Therefore, **there will be no critical dependency on Aim 1**.

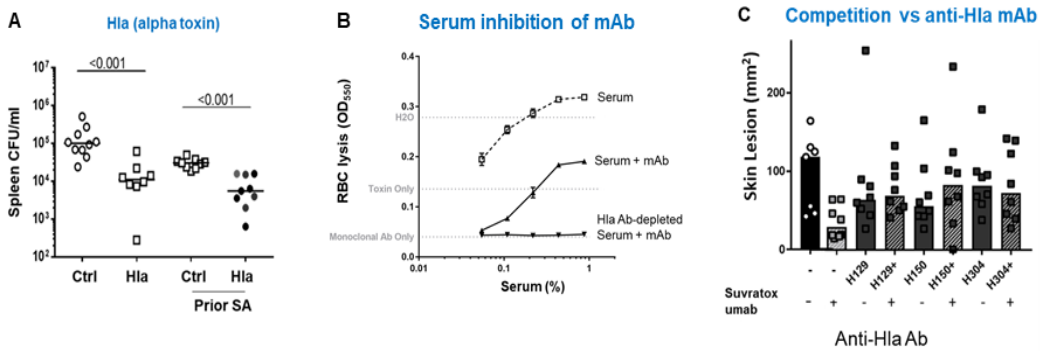


Figure 12. Effect of prior *SA* exposure on Hla (alpha toxin) active and passive vaccines. (A) Hla vaccine efficacy in naïve and SA-infected mice was assessed as in Fig. 4. **(B)** Neutralizing antibody to Hla (Sigma) was mixed with sera from human donors, then added to sheep blood for hemolysis measurement. Significant interference was seen in with 4/11 human Ab. Shown is an example of an interfering Ab profile. Depletion of Hla-specific Ab from the human serum restored full mAb function. **(C)** Suvratoxumab, anti-Hla Ab that was unsuccessful in clinical trial, was injected at 10mg/kg into mice with Hla-Ab purified from human donors (H129, H150, H304, 100 μl serum equivalent). The mice were challenged s.c. with *SA* ($2x10^7$ LAC) and lesions were measured.

- *Contrasting protective and non-protective sera:* Our initial study has shown that ~40-50% of patients who recover from invasive diseases produce transient protective Ab based on in vivo serum protection against *SA* challenge[8]. For each antigen target, we will aim to test protective sera from 5 pediatric and 5 older adult individuals at the peak of protection (usually 6 weeks) and after the protection has subsided (at 6 months). By contrast, some individuals may have moderate to high titer of Ab against the same antigen and yet not be protected. When available, we would like to similarly test the sera from up to 5 such individuals. Analysis of specific Ab that are protective from some individuals but not from others could provide important insight on specific characteristics of the Ab that are particularly important for conferring protection (see Aim 2.2.).

- *SA strains*: the study will utilize primarily LAC, a prototype USA300 epidemic strain of *SA* that circulates within community and healthcare settings. USA300 expresses most common virulence factors, but we will corroborate RNA expression of the specific *SA* antigen by USA300 in vivo prior to proceeding with each study. A collection of other *SA* strains is available if USA300 does not express the antigen. It is possible that some reduced Ab efficacy that we discover between subjects derive from targeting of the same protein with non-conserved *SA* sequences. Thus, we will also evaluate that possibility by comparing function in vitro as described below using LAC and the infecting *SA* isolate obtained from that particular subject.

Experiments: *Purification of specific Ab* – Recombinant *SA* antigens expressed from *E. coli* will be LPS detoxified using a spun-column and purified on an affinity (nickel), then seizing column. Additional QC include appropriate functional assays on each prep to verify and quantify protein function, e.g. cytolysis per μg of toxins. Affinity column will then be generated with the antigens to facilitate purification of specific Ab. Eluate from the column will be used to generate specific-Ab depleted sera (see **Fig. 12B**). *Adoptive transfer of Ab to protect against SA challenge* in mice – We will inject C57BL/6 mice (n=8) IV with the equivalent of 100 μl of purified human specific Ab IV. After 6h, we will challenge the mice with 10^7 late log-phase *SA* (USA300 strain LAC) IV and measure spleen and kidney CFUs and IL-1β, TNF-α, IL-6 and tissue histopathology at 48h. *In vitro functional*

assays – We will evaluate protection using the following assays: For cell surface adhesins, we will perform opsonophagocytic killing (OPK) assays using standard methods. For toxins, cytotoxicity and cytokine secretion in toxin-tropic human and mouse cell lines will be used. For secreted enzymes, we will use assays tailored to test the enzymatic function. OPK: In brief, *SA* preincubated with sera for 15 mins will be added to THP1 or mouse bone-marrow derived neutrophils at MOI of 10:1 for 1 hours and CFU will be enumerated[41]. Toxin neutralization: The selected recombinant toxin will be incubated with control or test sera prior to addition to the appropriate host cells selected based on tropism. Cytolysis and, as appropriate, cytokines from supernatant will be measured[42].
Defining protection – Protection will be primarily evaluated based on the in vivo adoptive transfer assay. In case of marginally significant protection, evidence from the in vitro assays will be used as secondary supportive evidence. Criteria for in vivo protection are the same as in Aim 1, based on CFU and histopathology at 48h.
Anticipated results, pitfalls, and alternative strategies: A hypothesis by Miller, Proctor and Fowler proposed that toxins (TSST, Hla) are protective antigens whereas cell-surface adhesins are not[4]. Our preliminary studies of human IsdB- and Hla- specific Ab are consistent with the hypothesis. More extensive analysis of toxin- and adhesin- specific Ab as proposed above and in Aim 2.2. will provide more clarity on that question. A potential caveat of the study is the use of the adoptive transfer of human sera or Ab into mouse blood. Human serum Ab are largely compatible with mouse Fc and OPK, which has allowed this model to be widely adopted across a broad range of pathogens to gain human relevance[43-45]. We have complemented this in vivo assay with traditional in vitro functional assays, with acknowledgement that each assay is likely to report and predict aspects of human protection. Another pitfall is the use of one single mouse strain for our initial studies. For *SA* antigens that are shown to be highly promising vaccine targets as assessed by experiments proposed in Aim 2.1 and 3.1., future studies will aim to validate the findings across *SA* strains and mice with diverse genetic backgrounds.

Aim 2.2. Perform structural and functional profiling of protective and non-protective human anti-*SA* Ab.

Effective antibody-mediated protection depends on specific interaction of Fab with the pathogen target as well as Fc interaction with host effectors (complement and immune cells). Fab antigen-specificity and affinity determine virulence target neutralization, whereas Fc and Ab glycosylation affect the quality of interaction with complement and Fc receptors and related opsonophagocytic functions[46]. To gain insight on specific determinants of Ab protection (or lack thereof), we will query specific Fab and Fc-mediated functions per our published characterization of IsdB antibodies (Tsai 2022). The use of genetic or pharmacologic inhibitory approaches will corroborate the suggested functions.
Experiments: - *Selecting SA antigens and serum samples* – From the list of antigens evaluated in Aim 2.1., we will **investigate up to 10 anti-*SA* Ab**, ideally 4 against “protective” antigens, and 6 against “non-protective” antigens. We will aim to include toxins, cell-wall anchored adhesins and secreted proteins. Since Ab protection to an antigen could vary among human subjects, we will aim to study serum samples from up to 10 individuals, preferentially picking individual samples that provide highest or lowest levels of protection within the specific antibody group (after controlling for titers), as these would likely provide additional insight towards protective mechanisms. Further, if any of the antigens demonstrated differential efficacy related to age (pediatric vs older adult) in Aim 2.1, this will be further explored in this subaim to identify mechanistic differences related to the effects of aging on antibody characteristics and functions.

- *Profile Ab characteristics and functions:* We will perform 3 types of assays that will inform on antibody protective functions: I. *Titer and Ig class / subclass*. Clearly, low specific titers would lead to non-protection. Therefore, for subsequent functional comparisons, we will use equimolar quantity of the antibodies across subjects. II. *Fab-mediated antigen neutralizatio*n: 1) Ag binding avidity using a urea stringency assay[47], 2) Epitope mapping by BCR sequencing, B cell hybridoma generation and mapping using overlapping peptides as performed for IsdB (Tsai 2022), 3) Neutralization/functional assays, e.g. for toxin neutralization[48], or heme survival assays for assessment of IsdA or IsdB neutralization. III. *Protection related to Fc interaction with complement and phagocytes*: 1) glycan analysis[49], 2) complement binding assay[50], 3) OPK by human neutrophils[41], 4) Ab mediated neutrophil phagocytosis[51].

These initial assays will inform on the potential mechanisms that contribute to suboptimal Ab functions. Thereafter, we will corroborate loss of function by using pharmacologic or genetic approaches, as shown for IsdB-specific Ab (Tsai et al. 2022). Because mapping of epitope is effort and resource intensive, we will limit to 3 Ab targeting *SA* antigens, IsdB (not yet performed on human Ab), IsdA and Hla, which have well-defined protective subdomain(s) based on published structural data[52-54]. For each antigen, we will seek out the 2 most and 2 least neutralizing Ab from the samples and correlate the epitope map for the 4 samples (for each antigen)

to their neutralizing activity in functional assays (heme starvation assays for IsdB and IsdA, and cytotoxicity assay for Hla). **Addressing the short duration of protective memory:** Although protective Ab were noted in 1/3 individual after SA invasive disease, no protection was evident after 6 months (**Fig. 3**). We currently hypothesize that change in Ab titer or glycosylation is most likely the cause. Hence, for up to 3 of the protective antigens that exhibit loss of Ab protection at 6 months, we will compare the structural and functional profile of the same antibodies at 6 weeks and 6 months after SA infection. If Ab titer is suspected to be the reason, we will assess equimolar concentration of the Ab from the different time points. Findings from these studies will inform on a more comprehensive future study that explores the molecular and cellular bases of the short-lived protection as well as potential strategies to overcome the shortened memory (e.g. via adjuvants).

Potential challenges and alternative strategies – Our battery of assays will identify Ab structural and functional characteristics that differentiate protective from non-protective Ab. If questions persist that could not be addressed using the proposed battery of tests, we will perform additional assays, for example, Ab binding to specific FcR's. The finding of IL10 link to non-protective α2,3 sialylation on IsdB Ab begs the question if IL10 universally drives non-protective anti-SA Ab function. If we find similarly modified α2,3 sialylation across non-protective Ab, we will evaluate for increased IL10 secretion by antigen-specific T and B cells from that human donor using the preserved PBMCs from Aim 1. This, along with the use of anti-IL10 (as performed in **Fig. 7** and proposed in Aim 3) will allow us to determine the causal link between IL10 and non-protective glycosylation. Although we propose to compare both protective and non-protective Ab samples against the same antigen, in some studies, it is likely that we will only have fully protective or non-protective Ab samples, with e.g. only one type of glycan profile. The interpretation of protective role of the profile will then depend on comparison to profiles of other anti-*SA* Ab and on specific glycosidase treatment, with further corroboration of phenotype from mouse studies (see Aim 3). All proposed assays have been fine-tuned and published in our study of IsdB Ab (Tsai 2022). Completion of Aim 2 will lead to an understanding of the types of *SA* proteins that induce protective or non-protective Ab and the characteristics of the antibodies that are protective or not protective. Aim 3 will directly assess protection conferred by vaccines targeting these antigens in *SA*-pre-exposed hosts, and will determine the capacity of non-protective Ab to outcompete protective anti-*SA* Ab.

In vitro reproducibility note: Throughout the project, in vitro experiments will be performed using primary cells or cell lines from mice or human of both sexes. To date, we have not observed gender differences for the in vitro cells/assays to be performed here, but we will continue to make note of the age/gender source of the cells used in case such differences become apparent. In vitro experiments throughout the project will be done in triplicate and repeated at least three times to assure reliability.

Specific Aim 3: Determine how prior humoral immune interaction with *S. aureus* shapes vaccine efficacy (3A: Years 1- 4; 3B: 2 – 5; 3C: 3-5)

Hypothesis: Vaccination against non-protective antigens recalls an ineffective memory response in the setting of prior *SA* infection. Furthermore, competition between non-protective and protective vaccine Ab leads to reduced vaccine efficacy. Conversely, vaccination against "protective" antigens recalls a protective Ab response (see **Fig. 12A**). Approach: We will assess how the characteristics of host humoral responses to *SA* antigens, defined in Aim 2.1 and 2.2, predict the efficacy of passive and active *SA* vaccines (Aim 3.1). We will query the roles of immune imprinting and direct Ab competition in vaccine interference (Aim 3.2). We will develop a list of *SA* antigens that induce minimal Ab response in human subjects (subdominant antigens) and query if protective vaccine could be more readily generated to these antigens (Aim 3.3).

Aim 3.1. Determine how the protective function of pre-existing anti-*SA* immunity affects efficacy of specific *SA* vaccines

Experiments: ***Evaluate active vaccines*** – We will select **8 *SA* antigens** from Aim 2.1/2.2 for active immunization study. Given a choice, we will aim for a third to a half of the antigens to be "protective" and to consist of toxins, adhesins and secreted factors. For the protective antigens, we will determine if the vaccines are protective in both naïve and SA-experienced hosts. For non-protective antigens, we will preferentially pick antigens that failed clinical trials, with an aim to assess if these vaccines are ineffective in SA-experienced mice. All antigens should 1) induce protection in naïve mice as a vaccine (this is already confirmed for to the 18 SA antigens listed in Aim 2), and 2) should induce at least moderate level of Ab response to *SA* to permit the effect of prior specific humoral immunity on vaccination to be studied. There are at least 11 antigens from the list in Aim 2 that fit the criteria. Therefore, **critical dependency on Aim 2 will not be an issue.** To simulate exposure to SA (**Fig. 4A**), we will inoculate mice with 10^7 *SA* (LAC) or PBS control IP at weekly intervals x 2-3, to induce a robust specific Ab titer (as a precondition). SA is cleared from organs 3-4 days after infection in this model. Ten days after, we will

immunize the mice with 2.5μg of LPS-depleted, column-purified antigen (see /aim 2.1) in alum at weekly intervals x 3, then challenge the mice 10 days after with the same *SA* strain. We will measure CFU and tissue IL1β, IL6, IL17A, IFNγ, IL23, IL10, TGFβ and histopathology from spleen and kidneys at 48h. To corroborate Ab protection, we will perform adoptive transfer of 100 μl of the serum to naïve mice, then challenge the mice 4h later and evaluate protection based on CFU, cytokines and histopathology as before. For the study of vaccine antigens that demonstrate *human tropism*, we will use humanized mice. To corroborate *specificity* in cases where vaccine interference is observed, we will challenge the mice with an isogenic mutant strain, which would be expected to remove the specific source of interference (as per IsdB findings in Tsai 2022). Knockouts are available for many antigens we are testing, but as needed we will generate the deletion mutant which is a basic skill of the lab.

Evaluate passive vaccines – We will assess the efficacy of **4 anti-*SA* human monoclonal Ab** in mice that have been pre-injected with human sera. The mAbs selected represent mAbs that have failed clinical trials and that we have verified to be available for purchase (LTA, ClfA, lipoprotein, and Hla) from Creative Biolabs. Preliminary data are already presented for two of the mAb (**Fig. 2B and 12C**). Each mAb will be matched with human samples with either high or low levels of specific Ab against the corresponding antigen (5 high and 5 low). We will inject mice IV with 100μL of human sera, followed 4h later with *SA* challenge, then 6h later with the mAb (dosed to simulate the clinical trial). Controls will consist of mAb alone, isotype control and the human serum alone. Readouts at 24h post infection will consist of tissue CFU, cytokines and histopathology per above. To verify that any interference is antigen specific, we will deplete the antigen-specific Ab by passage through an affinity column loaded with the antigen and determine whether interference is abrogated.

Anticipated results, pitfalls, and alternative strategies: This subaim will provide crucial insight into the underpinning of Ab interference with vaccine responses. Based on our hypothesis, efficacy of vaccine will depend critically on efficacy of prior antibody imprint. To date, all our specific vaccine data have closely matched findings from clinical trials. *Reinfection model* – It could be argued that multiple IP infections as proposed does not truly mimic human exposure to SA. We have previously addressed this issue and demonstrated similar vaccine interference after IV infection followed by antibiotic (tsai 2022). In both scenarios, it appears that prolonged exposure to *SA* is what is required to induce a robust *SA* specific humoral response that interferes. Furthermore, transfer of B cells from *SA* infected mice followed by immunization of the recipient is sufficient to induce IsdB vaccine interference arguing against innate training (Tsai 2022). We will selectively corroborate findings using the model of prior IV infection followed by vancomycin which mimics a real-life clinical scenario.

In vivo reproducibility note: We will perform all our experiments with male and female mice. All in vivo experiments will be done with groups of at least 8 age- and sex- matched animals as we have determined that this group size is sufficient to detect the differences being tested using α=0.05 and power=0.8. Experiments will be performed at least twice (n = 4 x 2 exps) to assure reproducibility.

Aim 3.2. Determine the role of immune imprinting and Ab competition in shaping *SA* vaccine efficacy

Our IsdB vaccination study in naïve and *SA* infected mice revealed two important mechanisms of vaccine interference: vaccine recall of a non-protective IsdB Ab response generated from prior infection, and competition between protective and non-protective Ab. We will evaluate these mechanisms in **up to 6 active and 4 passive immunizations** to explain vaccine interference or lack thereof.

Experiments: ***Active immunization*** - *Evaluate immune imprinting:* We will 1) vaccinate naïve or *SA* infected mice as per **Fig. 4A**, then purify *SA* antigen-specific B cells and analyze B-cell receptors for memory clonal expansion that denotes immune imprinting. We will measure *SA* antigen-specific IgM level at 3 and 7 days after vaccination of naïve and *SA* infected mice to look for prompt recall activation of antigen-specific cells.

- *Profile Ab characteristics and functions:* To determine if protective Ab generated by vaccination in naïve mice is different from specific Ab derived from *SA* infection alone or from vaccination of *SA* infected mice, we will assay the Ab from each setting for: titers of Ig classes and subclasses, affinity, glycan profile, OPK, complement binding, cytolysis assays, and selective epitope map as in Aim 2.2. If the analyses point to specific reasons for loss of Ab function, e.g., glycosylation differences, we will perform inhibition experiments using pharmacologic or genetic tools to corroborate the suggested mechanism, e.g., use of specific FcR mutant or removal of unique glycan group to determine if differences in function are reduced. Because of our preliminary data suggesting IL10 linkage to glycosylation to Fc, we will assess if anti-IL10 treatment at the time of vaccination reverses vaccine efficacy and if that is related to reversion of glycosylation per IsdB finding.

Passive immunization **-** We will mix the human mAb to the corresponding human serum samples containing high or low titers of specific Ab in the following assays: 1) competitive binding assays as per **Fig. 8B** for recombinant antigens or antigens on *SA* surface, with detection of biotin-labeled mAb by streptavidin. 2) We will also perform OPK and cytolysis experiments as described above, where appropriate, using different ratios of mAb to the human sera. In all cases where inhibition is observed, we will corroborate the specificity of the inhibition by repeating the experiment with specific-Ab depleted serum samples as shown in **Fig. 12B**. If Aim 2 identifies structural or quantitative Ab differences in aged and pediatric patients that lead to protective differences in vivo, we will assess if these antibodies directly interfere with protective Ab in the infection model.

Potential challenges and alternative strategies: We anticipate that each *SA* vaccine will recall and amplify non-protective humoral responses in approximate proportion to the magnitude of the pre-existing specific humoral response. We anticipate that vaccines will recall Fab- and Fc-specific features of specific Ab that are associated with weak protection – for example, propagation of non-neutralizing Ab (Fab) or glycan profiles associated with poor phagocytic killing of SA (Fc), which we noted with IsdB (Tsai 2022). We anticipate that direct Ab interference through competition would be complex and depend on the titer, affinity and epitope targeted by the competing Ab and the concentration of antigen targets. Our experiments will use mAb and human Ab concentrations that are most likely to be encountered in clinical trials.

Aim 3.3. Target subdominant *SA* antigens to circumvent vaccine interference

If immune imprinting and direct Ab competition are important roadblocks to effective *SA* vaccination, then targeting “subdominant" *SA* antigens that do not induce robust humoral response to *SA* could be a way to circumvent vaccine interference. In support of this strategy, effective experimental vaccines have been developed that target subdominant or pathogen targets that induce low level of Ab[55]. For preliminary support, we show that SdrE, which induces little Ab during *SA* infection can protect both naïve and *SA* infected mice as a vaccine (**Fig. 13**).

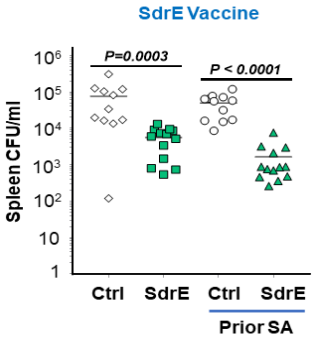


Figure 13. Targeting sub-dominant antigen SdrE. SdrE vaccine efficacy is assessed as in Fig. 4. *SA* infection alone induced 1/100 SdrE-specific Ab compared to IsdB-specific Ab (not shown).

Experiments: - *Identify subdominant SA antigens* – We will collate the list of *SA* antigens that induced low or no Ab in our pediatric study (Aim 1). We will verify that the *SA* antigens induce low specific Ab titer following *SA* infection in mice and perform in vivo transcriptomics study[56] of spleen *SA* at 6h and 24h post *SA* infection to determine expression of the *SA* antigens for our standard infection. We will then investigate **3 antigens** (including SdrE) in passive and active vaccination studies. For some of these antigens, there likely will be already developed vaccines, as is the case for SdrE[20]. Other vaccines will have no reported vaccine studies and require de novo evaluation of these antigens as vaccine candidates (as needed, we will evaluate up to 5 new antigens for vaccine efficacy). *Active vaccines:* We will corroborate efficacy of the *SA* vaccines in naïve mice. If the SA antigen is not protective in naïve mice, we will select an alternative antigen that meets the above criteria. We will then compare protection conferred by the vaccine in naïve and mice infected with *SA* per Aim 2.1 and evaluate the duration of protection in naïve and SA-infected mice for up to 6 months. We will verify that protection is Ab-mediated by adoptive transfer of the serum Ab followed by *SA* challenge as before. *Passive vaccines*: We will assess if the subdominant vaccine is more effective by performing passive transfer of the protective antibodies into mice pre-injected with human sera with low corresponding *SA* antigen-specific Ab. We will then challenge the mice with *SA* and assess as before.

Potential challenges and alternative strategies: This sub-aim addresses the hypothesis that subdominant antigens are more effective vaccine antigens in SA-exposed host. We anticipate that vaccines targeting subdominant *SA* antigens will protect both naïve and *SA* exposed mice because there will be no interfering Ab. Given that a successful *SA* vaccine in humans is likely to consist of multiple targets, subdominant antigens may represent a crucial component of this approach by maximizing efficacy given the absence of interference. Our future goal is to advance the most promising targets from the study to allow testing of the immune imprinting hypothesis in a clinical trial. This justifies our evaluation of more than one subdominant antigen.

Statistics - Two-group analysis will use either unpaired two-tailed *t*-test or a non-parametric Mann–Whitney U-test in the case of missing normality. Comparisons of multiple groups will be performed using one-way ANOVA and subsequent Bonferroni multiple comparisons. If normality or equal variance tests fail, then a Kruskal-Wallis test and subsequent Dunn’s multiple comparisons will be used. Correlation analyses will be performed using linear regression and evaluation of R^2.

PHS Human Subjects and Clinical Trials Information

OMB Number: 0925-0001

Expiration Date: 09/30/2024

Use of Human Specimens and/or Data

Does any of the proposed research in the application involve human specimens and/or data *	● Yes ❍ No
Provide an explanation for any use of human specimens and/or data not considered to be human subjects research.	
Are Human Subjects Involved	● Yes ❍ No
Is the Project Exempt from Federal regulations?	❍ Yes ● No
Exemption Number	❑ 1 ❑ 2 ❑ 3 ❑ 4 ❑ 5 ❑ 6 ❑ 7 ❑ 8
Other Requested Information	

Human Subject Studies

Study#	Study Title	Clinical Trial?
1	Interrogating human anti-staphylococcal antibody responses for staphylococcal vaccine insights	No

Section 1 - Basic Information (Study 1)

1.1. Study Title *

Interrogating human anti-staphylococcal antibody responses for staphylococcal vaccine insights

1.2. Is this study exempt from Federal Regulations * ❍ Yes ● No

1.3. Exemption Number ❑ 1 ❑ 2 ❑ 3 ❑ 4 ❑ 5 ❑ 6 ❑ 7 ❑ 8

1.4. Clinical Trial Questionnaire *

1.4.a. Does the study involve human participants?	● Yes	❍ No
1.4.b. Are the participants prospectively assigned to an intervention?	❍ Yes	● No
1.4.c. Is the study designed to evaluate the effect of the intervention on the participants?	❍ Yes	● No
1.4.d. Is the effect that will be evaluated a health-related biomedical or behavioral outcome?	● Yes	❍ No

1.5. Provide the ClinicalTrials.gov Identifier (e.g. NCT87654321) for this trial, if applicable

Section 2 - Study Population Characteristics (Study 1)

2.1. Conditions or Focus of Study

- Staphylococcus aureus invasive infection (e.g. bloodstream infection)

2.2. Eligibility Criteria

1) Children (age 6 months to 18 years) or adults (>65 years of age) admitted to Vanderbilt University Medical Center with culture-proven Staphylococcus aureus infection.
2) Have provided informed consent and informed assent (when applicable) to participate in the study.

2.3. Age Limits	Min Age: 6 Months	Max Age: 99 Years
2.3.a. Inclusion of Individuals Across the Lifespan	Inclusion__individuals_across_lifespan.pdf	
2.4. Inclusion of Women and Minorities	Inclusion_of_Women_Children_Minorities.pdf	
2.5. Recruitment and Retention Plan	Recruitment_and_Retention_R01.pdf	
2.6. Recruitment Status	Not yet recruiting	
2.7. Study Timeline	Study_Timeline_R01_.pdf	
2.8. Enrollment of First Participant	10/01/2023	Anticipated

INCLUSION OF INDIVIDUALS ACROSS THE LIFESPAN

1. Scientific and Ethical Rationale for Age Range of Participants

As detailed in Aim 1 of the *Research Strategy* Section, we plan to enroll children under the age of 18 (as defined by the NIH Policy on Inclusion of Children) and older than 6 months of age, inclusive. Children under the age of 6 months are excluded due to the significant impact of the maternal IgG profile (transmitted via the placenta to the fetus) on measurements of anti-staphylococcal antibodies in this project.

Children represent the ideal host in which to study the specific questions being investigated in this research proposal, for several reasons. First, *S. aureus* disease in children involves uniquely defined phenotypes
of invasive infection that are typically hematogenous in origin (e.g., acute hematogenous osteomyelitis, septic arthritis, or visceral abscesses). This allows for straightforward stratification of disease types and a true assessment of the host-pathogen interactions in the setting of human bloodstream infection. Second, staphylococcal carriage exerts a variable effect on specific anti-staphylococcal antibodies. The pediatric serologic profile, lacking a lifetime of intermittent or persistent nasal colonization, reflects the response to acute infection more directly. Finally, the relative lack of immune-modifying characteristics (e.g., medical comorbidities and age-related dysregulation of immune responses seen in older individuals) is an additional advantage to studying responses in children.

Children will be enrolled from the Monroe Carell, Jr. Children's Hospital at Vanderbilt (MCJCHV) in Nashville, Tennessee. Children will be approached for the study upon determination of culture-proven *Staphylococcus aureus* disease if they meet specific inclusion and exclusion criteria (*See: Protection of Human Subjects Attachment*).

For children able to provide assent (based on age, maturity, and psychological state), informed assent will be solicited prior to enrollment in the study. Children will be asked to sign an age-appropriate assent form describing the risks, benefits, and purpose of the study. It will be emphasized to children that they may opt out of the study at any time, and that their decision regarding whether to participate in the study will not alter their care or treatment during their hospitalization or emergency department visit in any way. Additional Protections for Children Involved as Subjects in Research (45 CFR Part 46 Subpart D) are addressed under the Protections Against Risk subheading of the Protections of Human Subjects Section.

2. Expertise and Experience of Investigative Team and Facilities Working with Children

The study will exclusively involve subjects admitted to MCHCHV, an accredited 271-bed freestanding children's hospital with ample volume to meet the required sample size for this study over a 5-year period. The principal investigator, Dr. Buddy Creech, is Professor of Pediatric Infectious Diseases at Vanderbilt University Medical Center. Dr. Creech completed 3-year Pediatric Infectious Diseases Fellowship and is an American Board of Pediatrics-certified pediatrician and infectious diseases specialist with extensive experience providing clinical care for hospitalized children. Dr. Creech has extensive experience in pediatric patient-oriented research ranging from multicenter industry-sponsored clinical trials to investigator-initiated, NIH-funded studies.

The Monroe Carell, Jr. Children's Hospital at Vanderbilt is a 277-bed free-standing facility built in 2004, which opened a $[redacted] million 30,000 sq. ft. expansion in May 2012. In. Since 2012, the Children's Hospital has consistently been recognized as one of the top children's hospitals in the nation by U.S. News & World Report.

3. Inclusion of a Sufficient Number of Children to Contribute to the Analysis

The proposed work is focused on the pediatric host response to *S. aureus* disease in children. The estimated sample sizes are based on our knowledge of the current epidemiology of invasive *S. aureus* disease within our hospital catchment area and our previous experience enrolling subjects in this population. We plan to enroll all eligible children during the study period. We anticipate approximately 20 subjects per year, a number that has remained consistent for the past several years in our catchment area. This number will be sufficient to obtain sufficient sera to investigate the questions proposed in Aims 1-3 of the Research Plan.

INCLUSION OF WOMEN AND MINORITIES

This study will be inclusive of all pediatric patients hospitalized at Monroe Carell Jr. Children's Hospital at Vanderbilt (MCJCHV) with culture-proven *S. aureus* infection. All subjects, age 6 months to 18 years, who meet the eligibility criteria (*See: Protection of Human Subjects Attachment*) will be approached for the study, regardless of religion, sex, or ethnic background. We will also enroll 25 older adults (>65 years of age) admitted to Vanderbilt University Hospital (VUH) with culture-proven *S. aureus* bacteremia.

Based on current demographics in the referral area of MCJCHV and VUH, targeted/planned enrollment by race, ethnicity, and sex are provided in the accompanying table (see: **Targeted/Planned Enrollment Table)**.

We anticipate a ratio of 55% females, 45% males based on population demographics and our previous studies.

INCLUSION OF CHILDREN

1. Plans to Include Children and Rationale for Age Range Selected

As detailed in Aim 1 of the *Research Strategy* Section, we plan to enroll children under the age of 18 (as defined by the NIH Policy on Inclusion of Children) and older than 6 months of age, inclusive. Children under the age of 6 months are excluded due to the significant impact of the maternal IgG profile (transmitted via the placenta to the fetus) on measurements of anti-staphylococcal antibodies in this project.

Children will be enrolled from the Monroe Carell, Jr. Children's Hospital at Vanderbilt (MCJCHV) in Nashville, Tennessee. Children will be approached for the study upon determination of culture-proven *Staphylococcus aureus* disease if they meet specific inclusion and exclusion criteria (*See: Protection of Human Subjects Attachment*).

For children able to provide assent (based on age, maturity, and psychological state), informed assent will be solicited prior to enrollment in the study. Children will be asked to sign an age-appropriate assent form describing the risks, benefits, and purpose of the study. It will be emphasized to children that they may opt out of the study at any time, and that their decision regarding whether to participate in the study will not alter their care or treatment during their hospitalization or emergency department visit in any way. Additional Protections for Children Involved as Subjects in Research (45 CFR Part 46 Subpart D) are addressed under the Protections Against Risk subheading of the Protections of Human Subjects Section.

2. Investigative Team and Facilities

The study will exclusively involve subjects admitted to MCHCHV, an accredited 271-bed freestanding children's hospital with ample volume to meet the required sample size for this study over a 5-year period. The principal investigator, Dr. Buddy Creech, is Professor of Pediatric Infectious Diseases at Vanderbilt University Medical Center. Dr. Creech completed 3-year Pediatric Infectious Diseases Fellowship and is an American Board of Pediatrics-certified pediatrician and infectious diseases specialist with extensive experience providing clinical care for hospitalized children. Dr. Creech has extensive experience in pediatric patient-oriented research ranging from multicenter industry-sponsored clinical trials to investigator-initiated, NIH-funded studies.

The Monroe Carell, Jr. Children's Hospital at Vanderbilt is a 277-bed free-standing facility built in 2004, which opened a $[redacted] million 30,000 sq. ft. expansion in May 2012. In. Since 2012, the Children's Hospital has consistently been recognized as one of the top children's hospitals in the nation by U.S. News & World Report.

3. Inclusion of a Sufficient Number of Children to Contribute to the Analysis

The proposed work is focused on the pediatric host response to *S. aureus* disease in children. The estimated sample sizes are based on our knowledge of the current epidemiology of invasive *S. aureus* disease within our hospital catchment area and our previous experience enrolling subjects in this population. We plan to enroll all eligible children during the study period. We anticipate approximately 20 subjects per year, a number that has remained consistent for the past several years in our catchment area. This number will be sufficient to obtain sufficient sera to investigate the questions proposed in Aims 1-3 of the Research Plan.

RECRUITMENT AND RETENTION

Study Recruitment: Dr. Creech and the study team will identify potential study subjects through daily contact with the Infectious Diseases and Hospital Medicine inpatient services, as they have done successfully for prior studies. Potential study subjects will be defined as children 6 months to 18 years of age, and adults >65 years of age, with culture-proven *Staphylococcus aureus* infection from a sterile site (e.g., blood, bone aspirate, or joint fluid). Informed consent will be obtained, as well as informed assent when applicable, and children will be screened for the following exclusion criteria: polymicrobial infection, primary or secondary immune compromise (including long-term oral or parenteral corticosteroids), history of (or current) malignancy, receipt of IVIG or blood products in the past 12 months, and known prior history of invasive staphylococcal disease.

All participating subjects must sign an informed consent form that complies with the requirements of 21 CFR Part 50 and 45 CFR 46. Informed consent is initiated prior to the individual's agreeing to participate in the study and continuing throughout the individual's study participation. Prior to participation in the study, subjects (and their parent/guardian) will receive a comprehensive explanation of the proposed procedures, including the nature and risks of the study and all elements that are part of obtaining proper informed consent. Subjects will also receive a detailed explanation of the proposed use and disclosure of their protected health information, which also includes their serum samples. Subjects (and their parent/guardian) will be allowed sufficient time to consider participation in the trial, after having the nature and risks of the trial explained to them, and having the opportunity to discuss the study with their family, friends or legally authorized representative prior to agreeing to participate. Informed assent will be obtained from all children who are able to provide assent.

Consent (and age-appropriate assent, where applicable) forms describing in detail the study procedures, risks and possible benefits are given to the subject. Consent/assent forms will not include any exculpatory statements. Consent/assent forms are IRB-approved and the subject will be asked to read and review the document. Upon reviewing the document, Dr. Creech or a member of the clinical research team will explain the research study to the subject and answer any questions that may arise. The subjects (or their parent/guardian) must sign the informed consent form and written documentation of informed consent is required prior to starting any procedures/interventions being done specifically for the study including administering study product. For participants 7-17 years, assent to participate in the study will also be obtained. A written script in age-appropriate language will also be used for participants 3-6 years of age. Copies of consent/assent documents will also be provided to study participants for their records (*See: Appendix for sample informed consent and age-appropriate Assent documents*)

Study personnel may employ recruitment efforts prior to the subject consenting; however, before any protocol-specific procedures are performed to determine protocol eligibility an informed consent form will be signed.

Study Retention: By signing the informed consent form, the subject (and their parent/guardian where applicable) agrees to complete all evaluations planned by the study, unless the subject withdraws voluntarily or is terminated from the trial for any reason. The rights and welfare of the subjects will be protected by emphasizing to them that the quality of their medical care will not be adversely affected if they decline to participate in this study.

Retention in the study will be maximized by clear communication of the study timeline and expectations at the time of informed consent and at each study visit. Retention is also maximized by the fact that, in most cases, the majority of study visits / sample collections occur in conjunction with other health care encounters with blood draws, so that study blood samples can be drawn in conjunction with routine care samples.

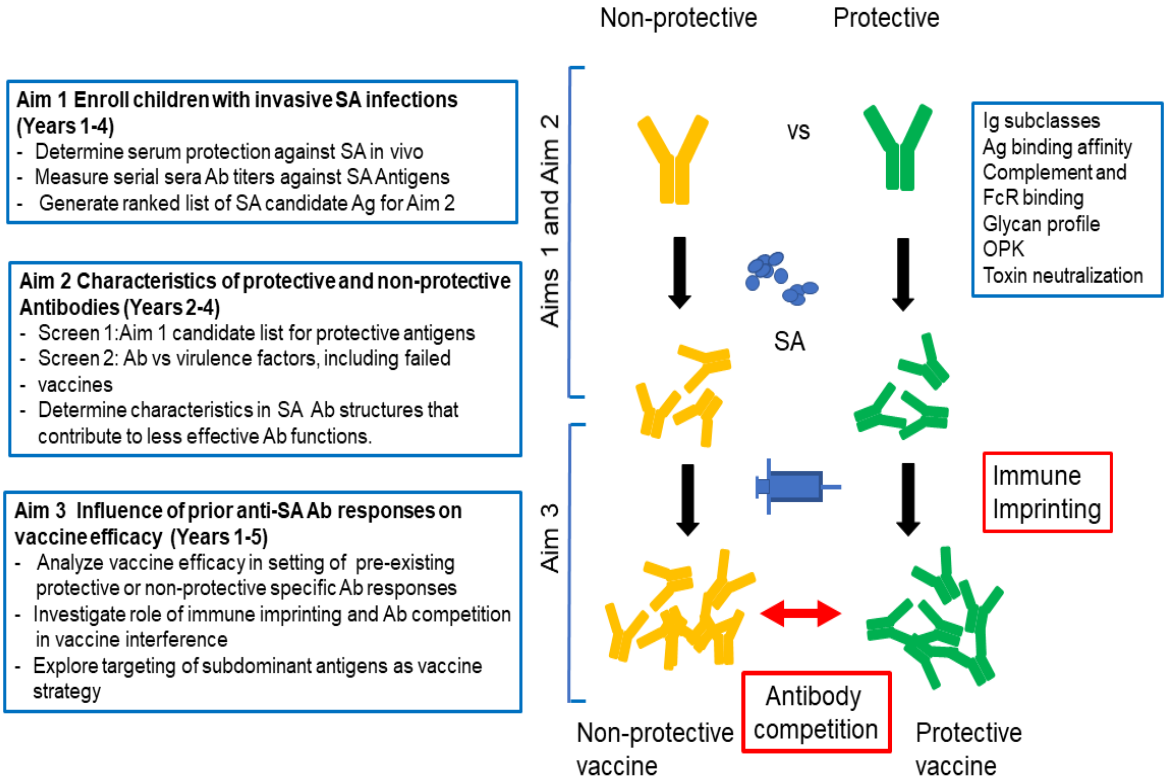
For the late follow-up visits (~6 months after infection), numerous contact methods will be obtain from parents / caregivers (e.g. home, work, and cell phone numbers as well as email addresses). Subjects are reimbursed for time/travel for attending the follow-up visits. We also offer wide flexibility in follow-up visit times to accommodate a variety of caregiver schedules.

STUDY TIMELINE

At the onset of the study period in Year 1 of the award, participant recruitment will begin. We have an active and effective research infrastructure in place from previous awards, and therefore anticipate no delays in the initiation of participant recruitment.

A general schematic of the study timeline is provided below. Briefly, all children admitted to the Monroe Carell Jr. Children's Hospital at Vanderbilt and adults >65 admitted to Vanderbilt University Hospital with culture-proven *Staphylococcus aureus* infection from a sterile site and who meet eligibility criteria (*see: Recruitment and Retention*) will be approached for participation in the study. This will begin at the onset of the award period and continue until 6 months remain in the final year of the award.

In parallel to subject enrollment, Aim 1 (generation of ranked list of candidate antigens based on antibody titers and protection) will proceed during the first four years of the award. The experiments proposed in Aim 2 (characteristics of protective antibodies) will begin in Year 2 and continue through year 4. The third Aim (influence of prior antibody response on protective efficacy) will begin in the 1st year of the award and continue through the final year.



Specific sample collection timeline:

For each subject enrolled in the study, blood will be obtained at 3 pre-specified timepoints, as follows:

- The first sample ("early" sample) will be obtained within one day of enrollment and documentation of informed consent.
- The second sample ("convalescent" sample) will be obtained 4-6 weeks following enrollment.
- The third sample ("late convalescent" sample) will be obtained approximately 6 months following enrollment (window 5 to 7 months following enrollment)

2.9. Inclusion Enrollment Reports

IER ID#	Enrollment Location Type	Enrollment Location
Study 1, IER 1	Domestic	Vanderbilt University Medical Center, Nashville, TN

OMB Number: 0925-0770

Expiration Date: 09/30/2024

Inclusion Enrollment Report 1

1. Inclusion Enrollment Report Title* : Anti-S. aureus humoral responses post invasive diseases

2. Using an Existing Dataset or Resource* : ❍ Yes ● No

3. Enrollment Location Type* : ● Domestic ❍ Foreign

4. Enrollment Country(ies): USA: UNITED STATES

5. Enrollment Location(s): Vanderbilt University Medical Center, Nashville, TN

6. Comments: We plan to enroll all children admitted to the Monroe Carell Jr. Children's Hospital at Vanderbilt with culture-proven Staphylococcus aureus infection who meet eligibility criteria and provide informed consent / assent to participate in the study, regardless of race, ethnicity, or sex. Planned enrollment is estimated based on local demographics. In addition, 25 adults with invasive S. aureus infection will be enrolled.

Planned

Racial Categories	Ethnic Categories				Total
	Not Hispanic or Latino		Hispanic or Latino		
	Female	Male	Female	Male	
American Indian/ Alaska Native	0	0	0	0	0
Asian	4	3	0	0	7
Native Hawaiian or Other Pacific Islander	0	0	0	0	0
Black or African American	11	8	0	0	19
White	23	17	20	14	74
More than One Race	0	0	0	0	0
Total	38	28	20	14	100

Cumulative (Actual)

Racial Categories	Ethnic Categories									Total
	Not Hispanic or Latino			Hispanic or Latino			Unknown/Not Reported Ethnicity			
	Female	Male	Unknown/ Not Reported	Female	Male	Unknown/ Not Reported	Female	Male	Unknown/ Not Reported	
American Indian/ Alaska Native	0	0	0	0	0	0	0	0	0	0
Asian	0	0	0	0	0	0	0	0	0	0
Native Hawaiian or Other Pacific Islander	0	0	0	0	0	0	0	0	0	0
Black or African American	0	0	0	0	0	0	0	0	0	0
White	0	0	0	0	0	0	0	0	0	0
More than One Race	0	0	0	0	0	0	0	0	0	0
Unknown or Not Reported	0	0	0	0	0	0	0	0	0	0
Total	0	0	0	0	0	0	0	0	0	0

Section 3 - Protection and Monitoring Plans (Study 1)

3.1. Protection of Human Subjects	Protection_of_Human_Subjects.pdf
3.2. Is this a multi-site study that will use the same protocol to conduct non-exempt human subjects research at more than one domestic site?	❍ Yes ● No ❍ N/A

Single IRB plan attachment

3.3. Data and Safety Monitoring Plan

3.4. Will a Data and Safety Monitoring Board be appointed for this study?	❍ Yes ● No

3.5. Overall structure of the study team

PROTECTION OF HUMAN SUBJECTS

1. Risks to Human Subjects

a. Human Subjects Involvement, Characteristics, and Design

The primary goal of this study is to define the naturally occurring, functional antibody response to selected toxins of *Staphylococcus aureus* following human infection. To accomplish this, a prospective cohort of pediatric subjects admitted to the Monroe Carell, Jr. Children's Hospital at Vanderbilt (MCJCHV) with documented *S. aureus* infection will be enrolled. The relevant characteristics of the subjects, including inclusion/exclusion criteria and anticipated number in each group, are detailed below.

The target population of this cohort will be children over 6 months of age admitted to MCJCHV with invasive *S. aureus* disease. Invasive disease is defined as the isolation of *S. aureus* from an otherwise sterile site (such as blood, bone, or joint fluid). Approximately 18 children are estimated to be enrolled each year, for a total of 90 subjects over the proposed 5-year award period. This estimate is based on our current local epidemiology of invasive pediatric *S. aureus* disease within our catchment area, as defined by local studies and our own experience enrolling subjects of this nature for the past 7 years.

Eligible subjects will be identified by referral from the infectious diseases, orthopedic, and hospitalist services at MCJCHV, as well as notification from the clinical microbiology lab. We have established a robust electronic algorithm via the Sentri7® software that interfaces with the clinical microbiology laboratory and notifies our research team of new positive cultures for *S. aureus* in real time. All children admitted to MCJCHV **will be considered for the study** if they meet the following **inclusion criteria**: age between 6 months and 18 years, with culture-proven *Staphylococcus aureus* disease from an otherwise sterile site. Subjects will be **excluded** if they meet any of the following **exclusion criteria**: known primary or secondary immune deficiency; history of malignancy, bone marrow transplant or solid organ transplant; receipt of IVIG or blood products in the past 12 months; pregnancy; or any condition that, in the opinion of the investigator, might interfere with study objectives or confer additional risk to the subject.

This study will be inclusive of all subjects who meet the eligibility criteria for each cohort, regardless of religion, sex, or ethnic background.

Children older than 6 months of age will be the only vulnerable population included in this project. Enrolling children in studies to understand the immune response to *S. aureus* is highly important for several reasons:

- The relative lack of characteristics that may alter the immune response to *S. aureus* (e.g., certain medical comorbidities and age-related dysregulation of immune responses seen in older individuals) may allow for more accurate determination of the humoral response to infection.
- The pediatric serologic profile, lacking a lifetime of intermittent or persistent nasal colonization, may reflect the response to acute invasive *S. aureus* disease more directly.
- Children with staphylococcal disease have uniquely defined phenotypes of invasive disease (e.g., acute hematogenous osteomyelitis), allowing for more straightforward stratification of disease types.

No aspect of this observational study will affect treatment decisions or alter the routine care of the children enrolled in the study in any way. No investigational products will be used. The results of the testing in this study will not be not be used to make clinical decisions in the treatment of the subjects enrolled in the study.

b. Study Procedures, Materials, and Potential Risks

Up to two 10mL tubes of blood (20 mL total, and no more than 1.0 ml/kg if subject <20 kg) will be obtained from each subject at a maximum of four separate time points: Immediately upon enrollment in the study; 4-6

weeks later; 6 months later; and (optionally) 1-3 years later for certain subjects with a high yield of functional antibodies. This volume of blood meets the minimal risk description for all subjects in the study. When applicable, the infecting bacterial isolate will be obtained from the Clinical Microbiology Laboratory at Vanderbilt University Medical Center (VUMC).

Clinical data will be collected from each subject via the Vanderbilt Electronic Medical Record System (EpicLeap). Information obtained from EpicLeap will include: age of the patient, admitting and discharge diagnosis, results of microbiologic cultures, duration and height of fever, and laboratory values including white blood cell count, hematocrit, erythrocyte sedimentation rate (ESR), and c-reactive peptide (CRP). Only Dr. Creech and Key Study Personnel will access patient charts for data collection.

Data will be stored in a de-identified manner by unique subject identification number. All data will be managed stored electronically in the Vanderbilt Research Electronic Data Capture (REDCap) database, a secure, HIPAA and FISMA-compliant web-based data collection system. Only Dr. Creech and Key Study Personnel will have access to the REDCap database.

Subject confidentiality is strictly held in trust by Dr. Creech and the study personnel. Access to study documents will be limited to Dr. Creech, key study personnel, and the Vanderbilt IRB. This confidentiality is extended to cover testing of biological samples, in addition to the clinical information relating to participating subjects. The study protocol, documentation, data, and all other information generated will be held in strict confidence. No information concerning the study or the data will be released to any unauthorized third party.

All data and information generated as part of the study (other than a subject's medical records) will be kept confidential by Dr. Creech and other study personnel. This information and data will not be used by Dr. Creech or other study personnel for any purpose other than conducting the study. These restrictions do not apply to: (1) information which becomes publicly available through no fault of the study personnel;
(2) information which it is necessary to disclose in confidence to an IRB solely for the evaluation of the study;
(3) information which it is necessary to disclose in order to provide appropriate medical care to a study subject; or (4) study results which may be published.

No risk is conferred to the subjects in this study other than a small volume of blood obtained (adhering to the minimal risk description). Thus, potential study-related risks are limited to local reactions at the site of blood draw and systemic reactions related to venipuncture and blood sampling. Every effort will be made to obtain the blood sample simultaneous with other laboratory tests required for the routine care of the patient, thus reducing unnecessary blood draws. The subject has the ability to opt out of the blood draw at any time.

2. Adequacy of Protection Against Risks

a. Informed Consent and Assent

Dr. Creech will identify subjects in accordance with the eligibility criteria detailed in the Research Plan. All subjects must sign an informed consent form that complies with the requirements of 21 CFR Part 50 and 45 CFR 46.

Informed consent is a process that is initiated prior to the individual's agreeing to participate in the study and continuing throughout the individual's study participation. Prior to participation in the study, subjects (and their parent/guardian, if age <18) will receive a comprehensive explanation of the proposed procedures, including the nature and risks of the study and all elements that are part of obtaining proper informed consent. Subjects will also receive a detailed explanation of the proposed use and disclosure of their protected health information, which also includes their serum samples. Subjects (and their parent/guardian, if age <18) will be allowed sufficient time to consider participation in the trial, after having the nature and risks of the trial explained to them, and having the opportunity to discuss the study with their family, friends or legally authorized representative prior to agreeing to participate. Informed assent will be obtained from all children who are able to provide assent.

Consent (and age-appropriate assent, where applicable) forms describing in detail the study procedures, risks and possible benefits are given to the subject. Consent/assent forms will not include any exculpatory

statements. Consent/assent forms will be IRB-approved and the subject will be asked to read and review the document. Upon reviewing the document, Dr. Creech or a member of the clinical research team will explain the research study to the subject and answer any questions that may arise. The subjects (or their parent/guardian, if age <18) must sign the informed consent form and written documentation of informed consent is required prior to starting any procedures/interventions being done specifically for the study including administering study product. (See: Appendix for sample informed consent and age-appropriate Assent document)

Study personnel may employ recruitment efforts prior to the subject consenting; however, before any protocolspecific procedures are performed to determine protocol eligibility an informed consent form will be signed. Subjects will be given a copy of all consent forms that they sign.

By signing the informed consent form, the subject (and their parent/guardian, if age <18) agrees to complete all evaluations required by the trial, unless the subject withdraws voluntarily or is terminated from the trial for any reason. The rights and welfare of the subjects will be protected by emphasizing to them that the quality of their medical care will not be adversely affected if they decline to participate in this study. ***b. Protections Against Risk***

This observational study is expected to confer no more than minimal risk to the study subjects. Every effort will be made to obtain the blood sample simultaneous with other laboratory tests required for the routine care of the patient, thus reducing unnecessary blood draws, and the subject (or their parent/guardian, if age <18) has the ability to opt out of the blood draw at any time. Data will be kept strictly confidential using an electronic database (REDCap). Subjects will have code numbers and will not be identified by name. Subject confidentiality is strictly held in trust by Dr. Creech and study personnel.

For participants 7-17 years, assent to participate in the study will also be obtained. A written script in age-appropriate language will also be used for participants 3-6 years of age. All participants will have written informed consent and when necessary verbal informed consent. Copies of consent/assent documents will also be provided to study participants for their records (*See: Appendix for sample age- appropriate Assent document*).

c. Vulnerable Subjects

This observational, no-more-than-minimal-risk study involves the recruitment and enrollment of children between the ages of 6 months and 18 years (Children under the age of 6 months are excluded due to the effect of maternally transferred antibodies on measurements of anti-staphylococcal antibodies in this project).

The study will exclusively involve subjects admitted to MCHCHV, an accredited 271-bed freestanding children's hospital with ample volume to meet the required sample size for this study over a 5-year period. The principal investigator, Dr. Creech, completed a 3-year Pediatric Infectious Diseases Fellowship and is an American Board of Pediatrics-certified pediatrician and infectious diseases specialist with extensive experience providing clinical care for hospitalized children.

Dr. Creech has experience in pediatric clinical research ranging from multicenter industry-sponsored clinical trials to investigator-initiated prospective cohort studies. Shanda Phillips, RN, will be the clinical research coordinator for the study. Ms. Phillips is an experienced clinical research nurse, with more than 10 years of experience conducting pediatric clinical studies.

3. Potential Benefits of the Proposed Research to Human Subjects and Others

Participation in this study provides no direct benefit to the subjects. The potential benefits to humankind, however, are significant. These include an improved understanding of how our body responds to *S. aureus* infection, and the assessment of the immune response against potential targets of intervention against this organism.

4. Importance of the Knowledge to be Gained

Staphylococcus aureus is the most common invasive bacterial pathogen in children in the United States, and is responsible for substantial morbidity, mortality, and health care expenditures in all age groups. Antibiotic-resistant strains of this pathogen are increasingly common, and there is an urgent need for improved therapeutic and preventive agents. This research is designed to advance our understanding of the functional immune response to key staphylococcal antigens which may be of high importance study the feasibility of novel treatment modalities. The data generated from this study may identify critical elements in the host response against this organism, and may identify potential targets for *S. aureus* intervention or prevention.

Section 4 - Protocol Synopsis (Study 1)

4.1. Study Design

4.1.a. Detailed Description

4.1.b. Primary Purpose

4.1.c. Interventions

Type	Name	Description

4.1.d. Study Phase

Is this an NIH-defined Phase III Clinical Trial? ❍ Yes ● No

4.1.e. Intervention Model

4.1.f. Masking ❍ Yes ● No

❑ Participant ❑ Care Provider ❑ Investigator ❑ Outcomes Assessor

4.1.g. Allocation

4.2. Outcome Measures

Type	Name	Time Frame	Brief Description

4.3. Statistical Design and Power

4.4. Subject Participation Duration

4.5. Will the study use an FDA-regulated intervention? ❍ Yes ● No

4.5.a. If yes, describe the availability of Investigational Product (IP) and Investigational New Drug (IND)/ Investigational Device Exemption (IDE) status

4.6. Is this an applicable clinical trial under FDAAA? ❍ Yes ● No

4.7. Dissemination Plan

Delayed Onset Studies

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

Principal Investigator/Program Director (Last, First, Middle): Liu, George Y.

VERTEBRATE ANIMALS

1. Description of the proposed use of animals in this work

Detailed description of each animal experiment is provided in the research design section. Unless otherwise specified, each control and experimental group will consist of ten 8-10 week old male or female C57BL/6 mice or humanized NSG mice purchased from the CFAR UCLA animal core facility. Experiments in Aim 1 and 2 will consist of adoptive transfer of human sera or anti-SA antibodies into naïve mice followed by challenge with S. aureus. Experiments in Aim 3 will, in addition, test active vaccination of mice with recombinant S. aureus antigens in naïve mice or mice previously infected with S. aureus.

Aim 1	
Adoptive transfer study of human sera into mice	2400 mice
Aim 2	
2.1 Adoptive transfer study of human anti-S. aureus antibodies into mice	1800 mice
2.2 characterization of protective and non-protective antibodies	30 mice
Aim 3	
3.1 Study of passive and active anti-SA immunizations in naïve and SA-experienced mice	920 mice
3.2 Study of mechanisms of vaccine suppression	200 mice
3.3 Study of passive and active immunizations targeting subdominant SA antigens	540 mice

In all, approximately **5890 mice** will be budgeted for the project. This is a project that is reliant on screen of vaccine candidates using mice, and thus a high number of mice are required. We will employ highly experienced postdoctoral researchers to reduce waste. All of the proposed experiments have either been approved or are pending approval under UCSD IACUC protocol **#** [redacted]

2. Justification:

Mice have been the standard model for studies of human *S. aureus* infections. The murine models proposed are well established and availability of knockout mice allows mechanism of immunopathology to be studied.

3. Minimization of Pain and Distress:

Appropriate effort will be made always to ensure minimum discomfort and distress to the mice during procedures. Mice will be appropriately anesthetized with isoflurane. If animals appear to be unthrifty or suffering during procedures, then they will be euthanized.

Euthanasia: The only method to be used for euthanasia in this proposal follows the current guidelines established by the American veterinary medical Association panel on euthanasia. Mice will be euthanized by inhalation of isoflurane. This will be followed to cervical dislocation to assure death.

SELECT AGENT RESEARCH

This proposal does not make use of pathogens listed on the select agent list. Performance of all *S. aureus* infection experiments will adhere to BSL2 guidelines and will be performed by laboratory staff who have completed BSL2 training.

Multiple Principal Investigator Project Leadership and Management Plan

PIs Dr. Liu and Dr. Creech are members of the Pediatrics Department at UCSD and Vanderbilt University Medical Center (VUMC), respectively. Dr. Liu is an infectious diseases physician and head of a *S. aureus* basic / translational pathogenesis lab. Dr. Creech, also a pediatric infectious diseases physician, is a patient-oriented investigator who has vast experience with studies of *S. aureus* infections in children and adults. In addition, he has conducted many vaccine trials in children and adults.
PI#1 (George Liu, MD, PhD) and PI#2 (Buddy Creech, MD) will jointly provide oversight of the entire proposed program and the development and implementation of all policies, procedures and processes. In these roles, PI#1 and PI#2 will be responsible for the implementation of the Research Agenda, the Leadership Plan, and the specific aims. They will ensure that systems are in place to guarantee institutional compliance with US laws and NIH policies including biosafety, animal research, and data and facilities.

• *Roles and areas of responsibility of the PIs*

Areas of responsibility are defined by the core expertise and prior experience of each PI. PI#1 will oversee all animal experiments (e.g., adoptive transfer *in vivo* models) proposed in Aims 1 – 3, to be conducted at UCSD. In addition, PI#1 will oversee Fc-fragment experiments such as glycan analysis and immunoglobulin subclass. PI#2 will be responsible for human subject enrollment and sample acquisition as outlined in Aim 1. PI#2 will also oversee the subset of experiments to be conducted at VUMC, including all serologic assays, complement binding assay, antibody-mediated neutrophil phagocytosis, and cytotoxicity assays. PI#1 and PI#2 will work closely for all three proposed studies involving patient samples. PI#1 will serve as contact PI. He will be responsible for communication with NIH and submission of annual reports.

• *Process for making decisions on scientific direction and allocation of resources*

Both PIs will make scientific decisions impacting the overall project, and will assume fiscal and administrative management including maintaining communication among all key personnel through meetings at least every three months.

• *Fiscal and management coordination*

Each PI will be responsible for financial oversight and management issues, and relevant record-keeping. The department (Pediatrics) at UCSD and Vanderbilt University will be the site for immediate fiscal interactions with the internal accounting system at each institution.

• *Publication and intellectual property (if needed) policies*

The Technology Transfer Office at both institutions will be responsible for handling any Intellectual Property issues arising. Publication authorship will be based on the relative scientific contributions of the PIs and key personnel.

• *Procedures for resolving conflicts*

In the highly unlikely event that a potential conflict develops, the PIs shall meet and attempt to resolve the dispute. If they fail to resolve the dispute, the disagreement shall be referred to an arbitration committee consisting of 1-3 impartial senior personnel from a neutral institution agreed upon by both PIs.

• *Data sharing and communication among investigators*

This issue will be greatly facilitated by having all investigators communicate on monthly basis. All key personnel will meet at least once every 3 months and much more frequently during studies involving both labs. Project-related data sharing will be set up via emails or internet-enabled tools.

REFERENCES CITED

1 Kaplan, S. L. Staphylococcus aureus Infections in Children: The Implications of Changing Trends. *Pediatrics* **137**, doi:10.1542/peds.2016-0101 (2016).

2 Klevens, R. M. *et al.* Invasive methicillin-resistant Staphylococcus aureus infections in the United States. *JAMA* **298**, 1763-1771, doi:10.1001/jama.298.15.1763 (2007).

3 Sutter, D. E. *et al.* Changing Susceptibility of Staphylococcus aureus in a US Pediatric Population. *Pediatrics* **137**, doi:10.1542/peds.2015-3099 (2016).

4 Miller, L. S., Fowler, V. G., Shukla, S. K., Rose, W. E. & Proctor, R. A. Development of a vaccine against Staphylococcus aureus invasive infections: Evidence based on human immunity, genetics and bacterial evasion mechanisms. *FEMS Microbiol Rev* **44**, 123-153, doi:10.1093/femsre/fuz030 (2020).

5 Lebon, A. *et al.* Dynamics and determinants of Staphylococcus aureus carriage in infancy: the Generation R Study. *J Clin Microbiol* **46**, 3517-3521, doi:10.1128/JCM.00641-08 (2008).

6 Kluytmans, J., van Belkum, A. & Verbrugh, H. Nasal carriage of Staphylococcus aureus: epidemiology, underlying mechanisms, and associated risks. *Clin Microbiol Rev* **10**, 505-520 (1997).

7 Tsai, C. M. *et al.* Non-protective immune imprint underlies failure of Staphylococcus aureus IsdB vaccine. *Cell Host Microbe*, doi:10.1016/j.chom.2022.06.006 (2022).

8 Tsai, C. M. *et al.* Adoptive Transfer of Serum Samples From Children With Invasive Staphylococcal Infection and Protection Against Staphylococcus aureus Sepsis. *J Infect Dis* **223**, 1222-1231, doi:10.1093/infdis/jiaa482 (2021).

9 Fritz, S. A. *et al.* A serologic correlate of protective immunity against community-onset Staphylococcus aureus infection. *Clin Infect Dis* **56**, 1554-1561, doi:10.1093/cid/cit123 (2013).

10 Verkaik, N. J. *et al.* Anti-staphylococcal humoral immune response in persistent nasal carriers and noncarriers of Staphylococcus aureus. *J Infect Dis* **199**, 625-632, doi:10.1086/596743 (2009).

11 Verkaik, N. J. *et al.* Induction of antibodies by Staphylococcus aureus nasal colonization in young children. *Clin Microbiol Infect* **16**, 1312-1317, doi:10.1111/j.1469-0691.2009.03073.x (2010).

12 Jacobsson, G., Colque-Navarro, P., Gustafsson, E., Andersson, R. & Mollby, R. Antibody responses in patients with invasive Staphylococcus aureus infections. *Eur J Clin Microbiol Infect Dis* **29**, 715-725, doi:10.1007/s10096-010-0919-x (2010).

13 Weksler, M. E. & Szabo, P. The effect of age on the B-cell repertoire. *J Clin Immunol* **20**, 240-249, doi:10.1023/a:1006659401385 (2000).

14 Monto, A. S., Malosh, R. E., Petrie, J. G. & Martin, E. T. The Doctrine of Original Antigenic Sin: Separating Good From Evil. *J Infect Dis* **215**, 1782-1788, doi:10.1093/infdis/jix173 (2017).

15 Tripp, R. A. & Power, U. F. Original Antigenic Sin and Respiratory Syncytial Virus Vaccines. *Vaccines (Basel)* **7**, doi:10.3390/vaccines7030107 (2019).

16 Oxford, J. S., Frezza, P. & Race, E. Challenges and strategies for AIDS vaccine development. *Vaccine* **11**, 612-614, doi:10.1016/0264-410x(93)90303-f (1993).

17 Weems, J. J., Jr. *et al.* Phase II, randomized, double-blind, multicenter study comparing the safety and pharmacokinetics of tefibazumab to placebo for treatment of Staphylococcus aureus bacteremia. *Antimicrob Agents Chemother* **50**, 2751-2755, doi:10.1128/AAC.00096-06 (2006).

18 Vatti, A. *et al.* Original antigenic sin: A comprehensive review. *J Autoimmun* **83**, 12-21, doi:10.1016/j.jaut.2017.04.008 (2017).

19 Fowler, V. G. *et al.* Effect of an investigational vaccine for preventing Staphylococcus aureus infections after cardiothoracic surgery: a randomized trial. *JAMA* **309**, 1368-1378, doi:10.1001/jama.2013.3010 (2013).

20 Stranger-Jones, Y. K., Bae, T. & Schneewind, O. Vaccine assembly from surface proteins of Staphylococcus aureus. *Proc Natl Acad Sci U S A* **103**, 16942-16947, doi:10.1073/pnas.0606863103 (2006).

21 Mishra, R. P. *et al.* Staphylococcus aureus FhuD2 is involved in the early phase of staphylococcal dissemination and generates protective immunity in mice. *J Infect Dis* **206**, 1041-1049, doi:10.1093/infdis/jis463 (2012).

22 Spaan, A. N., van Strijp, J. A. G. & Torres, V. J. Leukocidins: staphylococcal bi-component pore-forming toxins find their receptors. *Nat Rev Microbiol* **15**, 435-447, doi:10.1038/nrmicro.2017.27 (2017).

23 Leuzzi, R. *et al.* Dissecting the Human Response to Staphylococcus aureus Systemic Infections. *Front Immunol* **12**, 749432, doi:10.3389/fimmu.2021.749432 (2021).

24 Radke, E. E. *et al.* Hierarchy of human IgG recognition within the Staphylococcus aureus immunome. *Sci Rep* **8**, 13296, doi:10.1038/s41598-018-31424-3 (2018).

25 Thomsen, I. P. *et al.* Children with invasive Staphylococcus aureus disease exhibit a potently neutralizing antibody response to the cytotoxin LukAB. *Infect Immun* **82**, 1234-1242, doi:10.1128/IAI.01558-13 (2014).

26 Bennett, M. *et al.* A high-throughput liquid bead array assay confirms strong correlation between SARS-CoV-2 antibody level and COVID-19 severity. *iScience* **24**, 102052, doi:10.1016/j.isci.2021.102052 (2021).

27 Tseng, C. W. *et al.* Increased Susceptibility of Humanized NSG Mice to Panton-Valentine Leukocidin and Staphylococcus aureus Skin Infection. *PLoS Pathog* **11**, e1005292, doi:10.1371/journal.ppat.1005292 (2015).

28 Bonten, M. J. *et al.* Polysaccharide conjugate vaccine against pneumococcal pneumonia in adults. *N Engl J Med* **372**, 1114-1125, doi:10.1056/NEJMoa1408544 (2015).

29 Peltola, H., Kayhty, H., Virtanen, M. & Makela, P. H. Prevention of Hemophilus influenzae type b bacteremic infections with the capsular polysaccharide vaccine. *N Engl J Med* **310**, 1561-1566, doi:10.1056/NEJM198406143102404 (1984).

30 Francois, B. *et al.* Efficacy and safety of suvratoxumab for prevention of Staphylococcus aureus ventilator-associated pneumonia (SAATELLITE): a multicentre, randomised, double-blind, placebo-controlled, parallel-group, phase 2 pilot trial. *Lancet Infect Dis* **21**, 1313-1323, doi:10.1016/S1473-3099(20)30995-6 (2021).

31 Gurtman, A. *et al.* The development of a staphylococcus aureus four antigen vaccine for use prior to elective orthopedic surgery. *Hum Vaccin Immunother* **15**, 358-370, doi:10.1080/21645515.2018.1523093 (2019).

32 Magyarics, Z. *et al.* Randomized, Double-Blind, Placebo-Controlled, Single-Ascending-Dose Study of the Penetration of a Monoclonal Antibody Combination (ASN100) Targeting Staphylococcus aureus Cytotoxins in the Lung Epithelial Lining Fluid of Healthy Volunteers. *Antimicrob Agents Chemother* **63**, doi:10.1128/AAC.00350-19 (2019).

33 Yu, X. Q. *et al.* Safety, Tolerability, and Pharmacokinetics of MEDI4893, an Investigational, Extended-Half-Life, Anti-Staphylococcus aureus Alpha-Toxin Human Monoclonal Antibody, in Healthy Adults. *Antimicrob Agents Chemother* **61**, doi:10.1128/AAC.01020-16 (2017).

34 Aman, M. J. Integrated BioTherapeutics. *Hum Vaccin Immunother* **14**, 1308-1310, doi:10.1080/21645515.2018.1440105 (2018).

35 Bagnoli, F. *et al.* Vaccine composition formulated with a novel TLR7-dependent adjuvant induces high and broad protection against Staphylococcus aureus. *Proc Natl Acad Sci U S A* **112**, 3680-3685, doi:10.1073/pnas.1424924112 (2015).

36 DeJonge, M. *et al.* Clinical trial of safety and efficacy of INH-A21 for the prevention of nosocomial staphylococcal bloodstream infection in premature infants. *J Pediatr* **151**, 260-265, 265 e261, doi:10.1016/j.jpeds.2007.04.060 (2007).

37 Landrum, M. L. *et al.* Safety and immunogenicity of a recombinant Staphylococcus aureus alpha-toxoid and a recombinant Panton-Valentine leukocidin subunit, in healthy adults. *Hum Vaccin Immunother* **13**, 791-801, doi:10.1080/21645515.2016.1248326 (2017).

38 Shi, M. *et al.* A protein A based Staphylococcus aureus vaccine with improved safety. *Vaccine* **39**, 3907-3915, doi:10.1016/j.vaccine.2021.05.072 (2021).

39 Weisman, L. E. *et al.* A randomized study of a monoclonal antibody (pagibaximab) to prevent staphylococcal sepsis. *Pediatrics* **128**, 271-279, doi:10.1542/peds.2010-3081 (2011).

40 Armentrout, E. I., Liu, G. Y. & Martins, G. A. T Cell Immunity and the Quest for Protective Vaccines against Staphylococcus aureus Infection. *Microorganisms* **8**, doi:10.3390/microorganisms8121936 (2020).

41 Liu, G. Y. *et al.* Staphylococcus aureus golden pigment impairs neutrophil killing and promotes virulence through its antioxidant activity. *J Exp Med* **202**, 209-215, doi:10.1084/jem.20050846 (2005).

42 Cohen, T. S. *et al.* Staphylococcus aureus alpha toxin potentiates opportunistic bacterial lung infections. *Sci Transl Med* **8**, 329ra331, doi:10.1126/scitranslmed.aad9922 (2016).

43 Dekkers, G. *et al.* Affinity of human IgG subclasses to mouse Fc gamma receptors. *MAbs* **9**, 767-773, doi:10.1080/19420862.2017.1323159 (2017).

44 Jacobsen, H. *et al.* Influenza Virus Hemagglutinin Stalk-Specific Antibodies in Human Serum are a Surrogate Marker for In Vivo Protection in a Serum Transfer Mouse Challenge Model. *mBio* **8**, doi:10.1128/mBio.01463-17 (2017).

45 Duehr, J. *et al.* Tick-Borne Encephalitis Virus Vaccine-Induced Human Antibodies Mediate Negligible Enhancement of Zika Virus Infection InVitro and in a Mouse Model. *mSphere* **3**, doi:10.1128/mSphereDirect.00011-18 (2018).

46 Chen, X. *et al.* Glycosylation-dependent opsonophagocytic activity of staphylococcal protein A antibodies. *Proc Natl Acad Sci U S A* **117**, 22992-23000, doi:10.1073/pnas.2003621117 (2020).

47 Pichler, D. *et al.* Marked Increase in Avidity of SARS-CoV-2 Antibodies 7-8 Months After Infection Is Not Diminished in Old Age. *J Infect Dis* **224**, 764-770, doi:10.1093/infdis/jiab300 (2021).

48 Sharma-Kuinkel, B. K. *et al.* Characterization of alpha-toxin hla gene variants, alpha-toxin expression levels, and levels of antibody to alpha-toxin in hemodialysis and postsurgical patients with Staphylococcus aureus bacteremia. *J Clin Microbiol* **53**, 227-236, doi:10.1128/JCM.02023-14 (2015).

49 Lu, L. L. *et al.* A Functional Role for Antibodies in Tuberculosis. *Cell* **167**, 433-443 e414, doi:10.1016/j.cell.2016.08.072 (2016).

50 Fischinger, S. *et al.* A high-throughput, bead-based, antigen-specific assay to assess the ability of antibodies to induce complement activation. *J Immunol Methods* **473**, 112630, doi:10.1016/j.jim.2019.07.002 (2019).

51 Ackerman, M. E. *et al.* A robust, high-throughput assay to determine the phagocytic activity of clinical antibody samples. *J Immunol Methods* **366**, 8-19, doi:10.1016/j.jim.2010.12.016 (2011).

52 Tanaka, Y. *et al.* 2-Methyl-2,4-pentanediol induces spontaneous assembly of staphylococcal alpha-hemolysin into heptameric pore structure. *Protein Sci* **20**, 448-456, doi:10.1002/pro.579 (2011).

53 Bennett, M. R. *et al.* Human VH1-69 Gene-Encoded Human Monoclonal Antibodies against Staphylococcus aureus IsdB Use at Least Three Distinct Modes of Binding To Inhibit Bacterial Growth and Pathogenesis. *mBio* **10**, doi:10.1128/mBio.02473-19 (2019).

54 Sheldon, J. R. & Heinrichs, D. E. Recent developments in understanding the iron acquisition strategies of gram positive pathogens. *FEMS Microbiol Rev* **39**, 592-630, doi:10.1093/femsre/fuv009 (2015).

55 Lindahl, G. Subdominance in Antibody Responses: Implications for Vaccine Development. *Microbiol Mol Biol Rev* **85**, doi:10.1128/MMBR.00078-20 (2020).

56 Thanert, R., Goldmann, O., Beineke, A. & Medina, E. Host-inherent variability influences the transcriptional response of Staphylococcus aureus during in vivo infection. *Nat Commun* **8**, 14268, doi:10.1038/ncomms14268 (2017)

Form Approved Through 02/28/2023 OMB No. 0925-0001

Department of Health and Human Services
Public Health Services

Grant Application

Do not exceed character length restrictions indicated.

LEAVE BLANK—FOR PHS USE ONLY.

Type	Activity	Number
Review Group		Formerly
Council/Board (Month, Year)		Date Received

1. TITLE OF PROJECT *(Do not exceed 81 characters, including spaces and punctuation.)*

Interrogating human anti-staphylococcal antibody responses for S. aureus vaccine insights

2. RESPONSE TO SPECIFIC REQUEST FOR APPLICATIONS OR PROGRAM ANNOUNCEMENT OR SOLICITATION ☐ NO ■ YES
(If "Yes," state number and title)

Number: PA-20-185 Title: NIH Research Project Grant (Parent R01 Clinical Trial Not Allowed)

3. PROGRAM DIRECTOR/PRINCIPAL INVESTIGATOR

3a. NAME (Last, first, middle)	3b. DEGREE(S)	3h. eRA Commons User Name
C. Buddy Creech	MD, MPH	CREECHCB

3c. POSITION TITLE
Professor of Pediatrics Infectious Diseases

3d. MAILING ADDRESS *(Street, city, state, zip code)*
[redacted]

3e. DEPARTMENT, SERVICE, LABORATORY, OR EQUIVALENT
Pediatrics

3f. MAJOR SUBDIVISION
Infectious Diseases

3g. TELEPHONE AND FAX *(Area code, number and extension)*
TEL: [redacted] FAX: [redacted]

E-MAIL ADDRESS: [redacted]

4. HUMAN SUBJECTS RESEARCH	4a. Research Exempt	If "Yes," Exemption No.
☐ No ■ Yes	■ No ☐ Yes	

4b. Federal-Wide Assurance No.	4c. Clinical Trial	4d. NIH-defined Phase III Clinical Trial
[redacted]	■ No ☐ Yes	■ No ☐ Yes

5. VERTEBRATE ANIMALS ■ No ☐ Yes — 5a. Animal Welfare Assurance No.

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	Through	7a. Direct Costs ($)	7b. Total Costs ($)	8a. Direct Costs ($)	8b. Total Costs ($)
09/01/2023	08/31/2028	[redacted]	[redacted]	[redacted]	[redacted]

9. APPLICANT ORGANIZATION
Name Vanderbilt University Medical Center
Address [redacted]

10. TYPE OF ORGANIZATION
Public: → ☐ Federal ☐ State ☐ Local
Private: → ■ Private Nonprofit
For-profit: → ☐ General ☐ Small Business
☐ Woman-owned ☐ Socially and Economically Disadvantaged

11. ENTITY IDENTIFICATION NUMBER
[redacted]
DUNS NO. [redacted] Cong. District TN-007

12. ADMINISTRATIVE OFFICIAL TO BE NOTIFIED IF AWARD IS MADE
Name D. Clinton Brown, MBA, CRA
Title [redacted]
Address [redacted]
Tel: [redacted] FAX: [redacted]
E-Mail: [redacted]

13. OFFICIAL SIGNING FOR APPLICANT ORGANIZATION
Name D. Clinton Brown, MBA, CRA
Title [redacted]
Address [redacted]
Tel: [redacted] FAX: [redacted]
E-Mail: [redacted]

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.
(In ink. "Per" signature not acceptable.)
[redacted] Digitally signed by [redacted] Date: 2023.04.03 16:26:43 -05'00'

DATE 04/03/23

RESOURCE SHARING PLAN

Plan for Intellectual Property and Sharing of Research Resources

Intellectual property and data generated under this project will be administered in accordance with both University and NIH policies, including the NIH Data Sharing Policy and Implementation Guidance of March 5, 2003.

Ownership of sole or joint inventions developed under the project will be owned by the institution(s) employing the inventor(s). Inventors shall be determined by U.S. Patent law, Title 35 SC. University and Participating investigators/institutions will disclose any inventions developed under the project and such inventions will be reported and managed as provided by NIH policies. Sole inventions will be administered by the institution employing the inventor. Joint inventions shall be administered based on mutual consultation between the parties. Similar procedures will be followed for copyrights.

Materials generated under the project will be disseminated in accordance with University/Participating institutional and NIH policies. Depending on such policies, materials may be transferred to others under the terms of a material transfer agreement.

Access to databases and associated software tools generated under the project will be available for educational, research and non-profit purposes. Such access will be provided using web-based applications, as appropriate.

Publication of data shall occur during the project, if appropriate, or at the end of the project, consistent with normal scientific practices. Research data which documents, supports and validates research findings will be made available after the main findings from the final research data set have been accepted for publication. Such research data will be redacted to prevent the disclosure of personal identifiers.

We will adhere to the NIH Grant Policy on Sharing of Unique Research Resources, including the Sharing of Biomedical Research Resources Principles and Guidelines for Recipients of NIH Grants and Contracts. All data obtained from the studies will be made available through publications in a timely fashion. Protocols developed will be shared with other researchers upon request. All mutants generated will be made available per NIH guideline.

Sharing Model Organisms

As for our plan to share materials and our management of intellectual property, we will adhere to the NIH Grant Policy on Sharing of Unique Research Resources including the Principles and Guidelines for Recipients of NIH Research Grants and Contracts on Obtaining and Disseminating Biomedical Research Resources (PDF) issued December 23, 1999. All 'model organisms' generated by this project will be distributed widely or deposited into a repository/stock center making them available to the broader research community, either before or immediately after publication, in accordance with University policies. If we assume responsibility for distributing the newly generated model organisms, we will fill requests in a timely fashion. In addition, we will provide relevant protocols and published genetic and phenotypic data upon request. Material transfers will be made with no more restrictive terms than in the Simple Letter Agreement (SLA) or the Uniform Biological Materials Transfer Agreement (UBMTA)and without reach through requirements. Should any intellectual property arise which requires a patent, we will ensure that the technology (materials and data) remains widely available to the research

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

1. Cultured cells: Primary murine bone marrow-derived neutrophils and human THP1 cell line will be maintained in culture. THP1 cell line was frozen down when first obtained from vendor (ATCC) and was maintained in liquid nitrogen cell banks. We will routinely screen cell cultures for microbial contamination such as yeast, gram-positive and gram-negative bacteria using a Cell Culture Contamination Detection Kit (Thermo Fisher Scientific, Catalog number: C7028).

2. Mice: Mice, including humanized NSG mice will be purchased from Jackson Laboratory and the UCLA CFAR animal core facility. If additional mice are obtained from collaborators, they will be confirmed by SNP scanning services provided by Jackson Laboratory. Otherwise, genotypes will be tracked via standard mouse genotyping protocols.

3. DNA constructs: All DNA constructs generated during the course of the project will be verified by sequencing.

4. Bacterial strains: *S. aureus* strains used for the study (including LAC) or acquired from Aim 1 were or will be frozen at -80 on first acquisition. For all experiments, streaks of the original frozen stocks will be used.