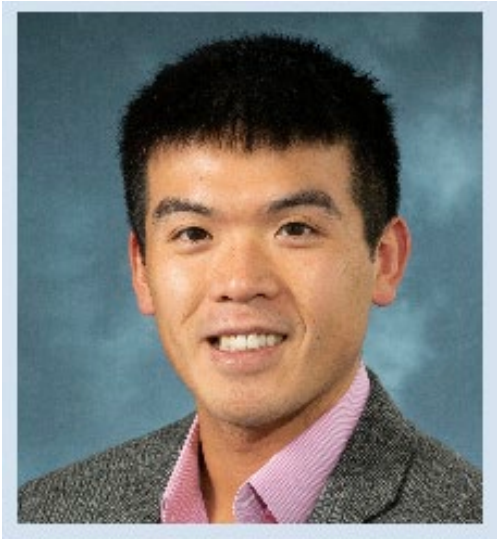
PeDRA
Pediatric Dermatology Research Alliance

The FUNdamentals of Grants



Jeff Yu, MD MS and Beth Drolet, MD

Why You *Should* Apply for a Grant

- Advance science and knowledge
- Advance your own academic career
- Learn a new skillset and gain experience doing research
- Gets you out of clinical obligations
- Sets you up for future grants
- Make some extra $$ on the side**

Why You *Shouldn't* Apply for a Grant

- Not always the best financial decision (hours spent/$ gained)
- Nagging pressure to do the project you said you were going to do
- Administrative and logistically challenging
- Time is probably better spent doing something else


NIH
Languish


Smaller Grant Mechanism
Clinical Trials
Pharma Supported
Philanthropy
…or don’t

If You've Never Failed, You're Not On The Road To Success

Live It Forward
with **Kent Julian**

2023 PeDRA Research Fellowship

KD

Katherine Devenport <katherine.devenport@pedraresearch.org>

Monday, May 1, 2023 at 4:02 PM

To: Yu, JiaDe,MD; sgeorge005@citymail.cuny.edu

George-Yu_PeDRA_...
157.3 KB

Download • Preview

Retention: Partners Retention Default - Delete after 10 Years Expires: 05/01/2033.

External Email - Use Caution

Dear Jeff and Shaina,

Thank you for your application to the 2023 PeDRA Research Fellowship Grant program. I am sorry to inform you that your project was not approved for funding. This cycle produced the largest and strongest group of applications we've ever received, and the Grants Committee needed to make some very difficult decisions on how to allocate limited funds. Details on the review process and comments from reviewers can be found in the attached summary statement.

August 2023 PeDRA EIRG Application

MS

Michael Siegel <mike.siegel@pedraresearch.org>

Friday, September 1, 2023 at 11:46 AM

To: Sandler, Mykayla Lexi

Cc: Yu, JiaDe,MD

Retention: Partners Retention Default - Delete after 10 Years Expires: 09/01/2033.

External Email - Use Caution

Dear Mykayla,

Thank you for your application to the 2023 PeDRA Emerging Investigator Research Grants program. I am sorry to inform you that your project was not approved for funding. This program was extremely popular, and we received many strong applications. Our Grants Committee needed to make some very difficult decisions on how to allocate the funds available.

Going for that hat trick

2023 PeDRA Research Grant Application Submission

P

PeDRA <no-reply@email.zenginehq.com>

To: Yu, JiaDe,MD

Cc: research@pedraresearch.org

Wednesday, September 20, 2023 at 3:10 PM

Retention: Partners Retention Default - Delete after 10 Years Expires: 09/20/2033.

To protect your privacy, some external images in this message were not downloaded. Download external images Go to Settings

External Email - Use Caution

Dear JiaDe,

Your 2023 PeDRA Research Grant Application, Augmenting the Acid Mantle as a Therapeutic Strategy in Atopic Dermatitis: A Randomized, Double-Blind, Placebo-Controlled Trial, has been successfully submitted! Your application will undergo an administrative review followed by an expert review by the PeDRA Grants Committee. Applicants will be notified of award outcomes no later than December 15, 2023.

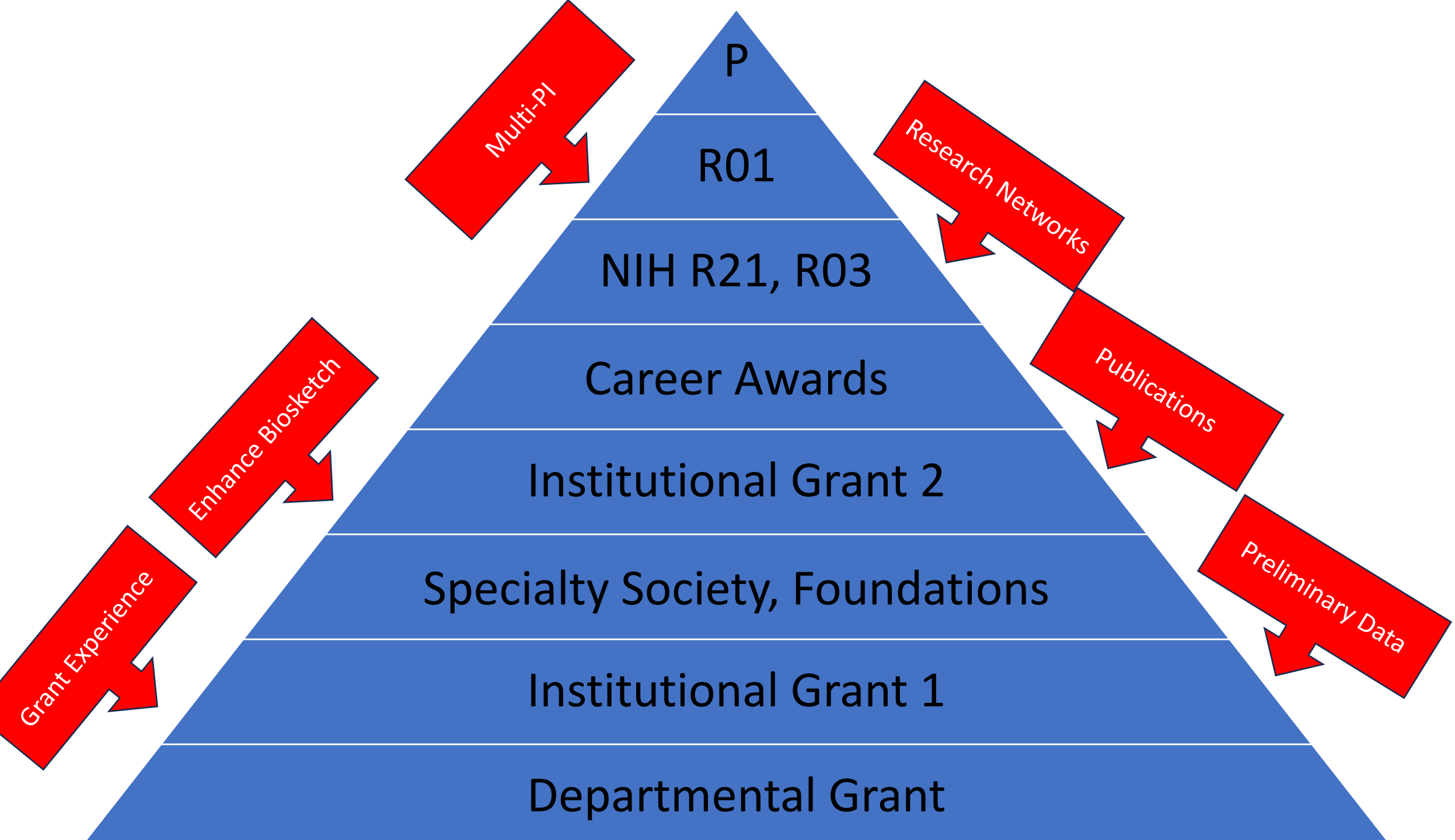
Please note that you are the only recipient of this confirmation email, and should notify your other team members regarding this submission accordingly.

If you have any questions about your application or the review process, please email research@pedraresearch.org.

Sincerely,

The PeDRA Team

Scaffolding Grants


P
R01
NIH R21, R03
Career Awards
Institutional Grant 2
Specialty Society, Foundations
Institutional Grant 1
Departmental Grant
Multi-PI
Research Networks
Publications
Preliminary Data
Enhance Biosketch
Grant Experience

NIH Research Project Grant (Parent R01 – PA-20-185)
~ Checklist, Timeline & Guideline ~

MPI: B. Drolet & H. Chang	Due: 6/05/2023	Submit: 06/02/2023	Project: 4/01/2024-3/31/2029

Title: Genetic and Molecular Basis of G-protein Mutant Vascular Anomalies.

Submit 6/02	Proposal Components	Notes & Pages Limits	Key Person	First Draft Due	Final / Due
☒	Set up Cayuse		LUH	NA	
☒	Set up WISPER		LUH	NA	
☒	Set up Box Folder		LUH	NA	
	1. OTHER PROJECT INFORMATION				
☐	Cover Letter	Optional	BD/HC	--	6/02
☐	Project Summary	30 lines	BD/HC	5/17	6/02
☐	Project Narrative	3 sentences	BD/HC	5/17	6/02
☐	Bibliography	No page limit	BD/HC	5/17	6/02
☐	Facilities & Other Resources	No page limit	BD/HC	5/17	6/02
☐	Equipment	No page limit	BD/HC	5/17	6/02
	2. PERSONNEL				
☐	Biographical Sketches	Drolet, Chang, Ayuso, Arkin, Kendziorski Newton	LUH	5/17	---
	3. BUDGET & BUDGET JUSTIFICATION				
☐	Budget & Budget Justification		LUH/HC	5/12	5/19
	4. RESEARCH PLAN SECTIONS				
☐	Specific Aims	1 page	BD/HC	5/17	6/02
☐	Research Strategy	12 pages	BD/HC	5/17	6/02
☐	Vertebrate Animals	No page limit	BD/HC	5/17	6/02
☐	Multi-PI/PD Leadership Plan	No page limit	BD/HC	5/17	6/02
☐	Letters of Support	No page limit	BD/HC	5/17	--
☐	Resource Sharing Plan	(1) Sharing Model Organisms: If applicable (2) Research Tools: if applicable	BD/HC	5/17	6/02
☐	Other Plans	2 pages Data Sharing & Management (DMS) Plan: Required (includes Genomic Data Sharing Plan, if applicable)	BD/HC	5/17	6/02
☐	Authentication of Key Resources	1 page suggested	BD/HC	5/17	6/02

☐	Human Subjects (for non-exempt human subjects)	<u>Required documents (in addition to what needs to be filled out in study record):</u> ☐ Inclusion of Individuals Across Lifespan ☐ Inclusion of Women and Minorities ☐ Recruitment and Retention Plan ☐ Inclusion Enrollment Report ☐ Study Timeline (Optional) ☐ Protection of Human Subjects ☐ Data Safety and Monitoring Plan (Optional; only if high risk) ☐ Overall Structure of Study Team	BD/HC	5/17	6/02

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☐	Biographical Sketches	Drolet, Chang, Ayuso, Arkin, Kendziorski Newton	LUH	5/17	---
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Specific Aims- Jeff

- Not every grant requires an “Aims Page” but every grant wants to know your aims
- This is by far the most important part of your grant
- Spend most of your time working on this to make it short, tight, clear, poignant
- Tells reviewers what your grant is all about and if they read nothing else, the aims page should tell them what you’re trying to do
- Figures are great to include to clarify your methods

Clear and concise aims.
Keep it to 1 page or less.

1. Aim #1 is the most important.
2. Brief overview of how you will accomplish aim 1 (more explained later)
3. Aims 2 and 3 are bonus aims. Don't make them too ambitious.

PeDRA Grant Application- Project Proposal
JiaDe Yu, MD

SPECIFIC AIMS:

Aim #1: **Compare three over the counter moisturizing ointments (Aquaphor, Vanicream Moisturizing Ointment, Cerave Ointment) versus Vaseline ointment on their respective effects on trans-epidermal water loss in children with atopic dermatitis** Trans-epidermal water loss (TEWL) is a measure of barrier function with greater values signifying greater barrier dysfunction, a hallmark of atopic dermatitis (AD). Emollients such as ointments, the gold standard of emollients given their occlusive and hydrophobic properties, are the foundation of AD treatment. While Vaseline petroleum jelly is the most plain and cheapest moisturizing ointment, other ointments on the market contain additives such as ceramides, humectants, fatty acids, etc that purport to have additional moisturizing efficacy. Whether these ointments with additives are superior to Vaseline in terms of their respective ability to lower TEWL is unknown.

Children with mild to moderate atopic dermatitis will be recruited from the Massachusetts General Hospital Pediatric Dermatology Clinic and randomized to receive one of three moisturizing ointments. Patients will apply the assigned moisturizing ointment on the right volar forearm and apply Vaseline ointment on the left volar forearm. TEWL from the bilateral volar forearms will be measured at time of recruitment (day 0), day 14, and day 28 using a Tewameter TM Hex (C+K Electronic, Köln, Germany).

Aim #2: Measure the cosmetic acceptability of the four ointments (Aquaphor, Vanicream Moisturizing Ointment, Cerave Ointment, and Vaseline) Study participants will complete the previously published 21 question Cosmetic Acceptability Questionnaire[1] at the final visit (day 28) to evaluate the cosmetic acceptability of the ointments.

Aim #3: Evaluate the cost-effectiveness of studied moisturizing ointments The cost of moisturizers differs greatly from $0.31/ounce for Vaseline ointment to $1.42/ounce for Cerave ointment as reported by Amazon (Seattle, WA). In this study, we seek to determine the cost effectiveness of different moisturizing ointments based on their ability to decrease TEWL. We will calculate the dollars per ounce to percent decrease in TEWL ratio for each studied moisturizer ($/oz:%TEWL decrease).

SPECIFIC AIMS

Vascular anomalies represent a diverse group of developmental disorders and affect patient-reported quality of life globally, but particularly mental health and emotional well-being due to severe tissue overgrowth; chronic pain; functional impairment; and life-altering, stigmatizing disfigurement.[1-4] Unfortunately, the care available to these patients suffers from large gaps in clinical practice. Misdiagnosis is common because vascular anomalies result in diverse clinical presentations. Even patients who are correctly diagnosed are likely to undergo invasive and often unsuccessful surgical procedures because no standardized treatment protocols or FDA-approved drugs have been developed to treat vascular anomalies.

The PeDRA-funded Vascular Anomalies registry emerged to address this critical gap in practice. This registry has generated a comprehensive genomic dataset, which we have leveraged for gene discovery. With others, we have demonstrated that many vascular anomalies are caused by mutations in highly conserved oncogenes. This has transformed our fundamental understanding of the pathobiology of vascular anomalies and, for the first time, revealed potential pharmacologic targets. The challenge now is to elucidate the specific downstream effects of these mutations at the mRNA and protein levels, including the effects on signaling and cell-specific expression patterns, of which little is known. Precise information establishing how these mosaic variants drive disease progression is critical to implement therapies that target the activated pathway, penetrate to the appropriate skin depth, and spare normal tissue.

This fellowship project will leverage the PeDRA registry infrastructure to further investigate genotype–phenotype correlations and to elucidate downstream gene expression profiling. This project is the ideal opportunity to mentor Ashley Ng, a medical student with a sustained interest in pediatric dermatology. It will grow her skills in cross-institutional and translational research in gene discovery and expression. Most importantly, it will groom Ms. Ng to join the next generation of physician scientists who advance high-impact research to understand and treat childhood skin disease.

We hypothesize that the spatially resolved transcriptome will elucidate shared expression patterns across three key signaling pathways and will accurately locate the depth of altered signaling within the tissue. This will guide molecularly targeted drug development and will help determine the best mechanism for drug delivery (e.g., topical, injectable, or oral). We will investigate our hypothesis through the following aims:

Aim 1: Clarify genotype–phenotype correlations by expanding the existing PeDRA national registry and leveraging cutting-edge 3D imaging technology

We will genotype the affected skin using targeted, high-depth, next generation sequencing (NGS). High-depth exome sequencing will be used for those negative on the targeted panel. We will then correlate genotype with deep phenotyping, which will include medical information, colorimetric analysis, and 3D photography using the Canfield VECTRA H1 imaging system.

Aim 2: Determine the landscape of mRNA expression with spatial resolution in vascular anomalies

We will perform spatial transcriptomics on patients' diseased skin and anatomically matched, normal skin. The spatial transcriptomics method (Figure 4) uses NGS to provide an unbiased quantitation of mRNA expression differences *in vivo*. This method also boasts the unique advantage of localization of mRNA expression within the tissue architecture to the resolution of individual skin layers (e.g., epidermis, dermis).

This project will advance knowledge regarding vascular anomalies and expand potential therapies and their efficacy. Funding of Ashley Ng's fellowship will accelerate this work as she has experience in PeDRA research. Thus, she is primed to be rapidly immersed in the culture and to advance PeDRA's mission to sustain pediatric dermatology research through this established and thriving registry.

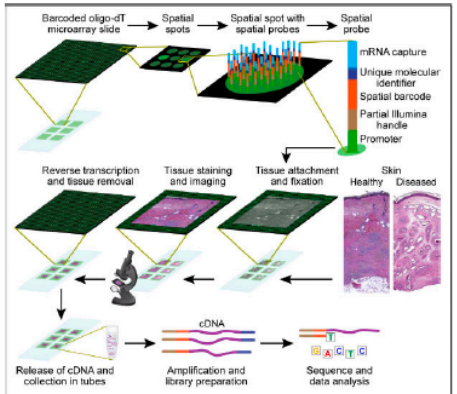

Figure 4. The spatial transcriptomics workflow. Slides are divided into six subarrays (upper left), each with thousands of circular spatial spots. Within the spots are millions of spatial probes that include a location-specific barcode and a poly-A mRNA capture sequence. Thin skin tissue sections are attached to each microarray, fixed in methanol, and imaged with H&E. The tissue is permeabilized to facilitate release of the RNA and then reverse transcription is carried out, generating cDNA on the slide. Tissue is digested away, and the cDNA is collected, amplified, and converted into paired-end libraries. The libraries are sequenced and computational reconstruction is carried out to map expressed genes to their spatial locations, using the H&E image as reference.

Research Proposal

Dermatology care is nearly nonexistent for children who identify as Native, which includes American Indians, Alaska Natives, Native Hawaiians and Pacific Islanders. This lack of access to pediatric dermatology is even more severe for rural communities, such as Native American reservations. We know that common, treatable skin diseases such as acne and eczema are prevalent and inadequately treated amongst Native Americans. Untreated skin diseases are associated with poorer quality of life, sleep disruptions, learning difficulties, mood disorders, and school absenteeism. Nevertheless, there is insufficient data on the prevalence of skin disease, the burden that skin disease has on quality of life, and access to dermatology care in Native American adolescents and young adults.

Our study proposes to answer these questions by surveying Native American adolescents and young adults in large-scale community gatherings. We have already surveyed 278 Native adolescents and young adults in the unique setting of large community powwows. Our survey contains question probes about demographics, including age, education level, and race/ethnicity; healthcare access, including insurance type, access and barriers to dermatologic care, and resources to engage via telehealth; incidence of common skin diseases; and, includes the 30-question Skindex-29 quality of life assessment for adults, and both the Skindex-29 and 22-question Skin-Teen quality of life assessment for adolescents. Utilizing this survey data, we will build a database and conduct data analysis.

Our investigator team is particularly suited to successfully conduct this study with an ideal environment for understanding Native contexts of care through the Centers for American Indian and Alaska Native Health; and with a mentor-mentee team who is a compassionate ally of Native peoples. The objective of this study is to assess the potential scope of unmet need for dermatology care among Native adolescents and young adults, so that there is a nationwide recognition of this unmet need, and program development to increase access to dermatologic care in this population.

We will attain our objective through the following *specific aims:*

Specific Aim 1: To describe the prevalence of skin disease, and its effect on quality of life in Native adolescents and young adults with and without skin disease. Each survey includes a brief demographic questionnaire and items requesting participants to answer scaled items about symptoms, emotions, and functionality as it relates to their health taken from the Skindex-29. We will aggregate this data by demographic characteristic (race/ethnicity, self-perceived skin health issue) and characterize how their skin condition impacts their quality of life.
Working hypothesis: There is a significantly higher prevalence of skin disease among Natives than non-Natives. Skin disease negatively affects quality of life in Native American adolescents and young adults.

Specific Aim 2: To identify barriers to access of dermatologic care for skin disease among Native adolescents and young adults with skin disease. We will link insurance type and population density of current geographic home along with reported barriers to accessing dermatologic care, in order to understand the level of access Native Americans have to dermatologic care. This data will serve as a guide to formation of strategies and programs that will increase access to dermatologic care in this population.
Working hypothesis: Native peoples are less likely to see a dermatologist when they have a skin problem. Native American adolescents and teens experience substantial barriers to accessing dermatology care.

The *expected impact* of this study is recognition of the dermatologic needs of Native adolescents and young adults and their current access to dermatologic care. The results of this survey will inform areas of greatest need and the impact of skin disease in Native adolescents and young adults.

CRITIQUE 1

Significance: 1
Investigator(s): 1
Innovation: 2
Approach: 2
Environment: 1

Overall Impact: This study aims to characterize the effects of postzygotic somatic GNA mutations in vascular anomalies. Three aims will be studied including multi-omics characterization of mutant GNA vascular anomalies and their association with severity: characterization of the effect of mutant GNAs in vascular function in micro physiological systems and in a mouse model. The approach is logical, and the three aims are complementary. The details included in each of the aims are appropriate to evaluate feasibility and scientific rigor. The team is excellent, and the environment has all the required elements to achieve success in this project. There is enthusiastic about the potential of this application.

1. Significance:

Strengths

- Important problem associated with specific organ dysfunction.
- Focus on a group of mutations frequently associated with these disorders (35% of their disease-specific biobank).
- Mutations in these genes are associated with different clinical phenotypes.

Weaknesses

- None noted.

2. Investigator(s):

Strengths

- The investigators have complementary expertise and are qualified to accomplish the proposed aims.

Weaknesses

- None noted.

CRITIQUE 3

Significance: 2
Investigator(s): 1
Innovation: 3
Approach: 3
Environment: 1

Overall Impact: Vascular anomalies represent a group of rare developmental disorders of the vasculature affecting as many as 120,000 infants each year. The study will focus on vascular anomalies that harbor mosaic mutations in *GNAQ* and *GNA11* (collectively, GNA) because they are

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found in a variety of life-altering diseases of the skin, eye, and central nervous system and because patients with these mutations have no targeted treatment options. Applicant has generated three novel Cre-activated mouse alleles (*GNAQ R183Q*, *GNAQ R183G*, and *GNA11 R183C*) that allow us to spatially and temporally control the expression of mutant GNA in mice. The goals are (1) identify molecular signature in mutant GNA vascular anomalies using a multi-omics approach and correlate these to phenotypic severity, (2) determine the effect of mutant GNAs in vascular function using *in vitro* microphysiological systems, and (3) determine the mechanism of mutant GNAs in vascular anomalies using mouse genetic models. Dr. Drolet is an international leader in this field and expert for these diseases.

1. Significance:
Strengths

- Congenital vasculopathies are an important medical issue.

Weaknesses

RESEARCH STRATEGY

A. Background Information

Vascular anomalies and their management. Vascular anomalies represent a group of rare developmental disorders of the vasculature.[1-3] Though most vascular anomalies present during infancy, the management often requires a multidisciplinary team of specialists into adulthood. These disorders are remarkably variable, ranging from simple skin discoloration to debilitating tissue overgrowth and severe structural birth defects (**Fig. 1**). Patients with vascular malformations that harbor *GNAQ* and *GNA11* (GNA) mutations suffer from progressive tissue overgrowth, pain, and life-altering disfigurement. Those with forehead and/or eyelid involvement often have vascular anomalies involving the brain and eye, leading to glaucoma, vision loss, hydrocephalus, seizures, and development delay (e.g., Sturge-Weber syndrome and phakomatosis pigmentovascularis). Despite the severity of these complications, there are no FDA-approved drugs to treat them, and it is difficult to predict severity and associated structural anomalies. The mosaic etiology and resultant clinical heterogeneity has led to over 20 acronyms and specialty-specific nomenclature, further impeding research. Misdiagnosis is common, and physician uncertainty heightens parental anxiety and delays therapy.

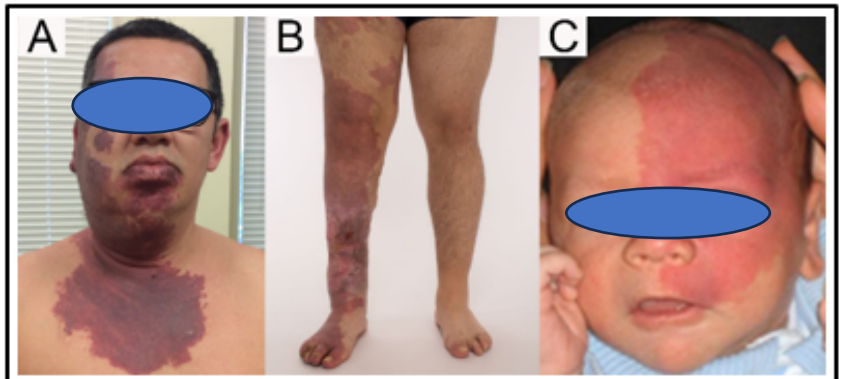

Fig. 1. Patients with vascular anomalies harboring a *GNAQ* R183G mutation. (A) 29-year-old with capillary malformation and overgrowth. (B) 33-year-old with capillary malformation and overgrowth (Klippel-Trenaunay syndrome). (C) 3-month-old with capillary malformation, glaucoma and leptomeningeal enhancement causing seizures (Sturge-Weber syndrome).

Vascular anomalies harbor mutations in oncogenes. Most vascular anomalies are sporadic; however, linkage studies in rare familial cases were instrumental in identifying candidate pathways for non-inherited vascular anomalies. In 2009, Limaye et al. demonstrated that venous malformations harbor mosaic mutations in the angiopoietin receptor gene *TEK* that were absent in germline DNA.[19,20] Mutations that occur after the zygote is formed (postzygotic) but during embryogenesis will affect progenitor cells and give rise to individuals with genetically distinct populations of cells. Embryonic mosaicism explains many of the clinical features observed in vascular anomalies and the co-existence of vascular anomalies and ipsilateral structural birth defects. Targeted high-depth sequencing of affected tissue has revealed that vascular anomalies harbor mutations in highly conserved oncoproteins that tightly regulate the cell cycle and collectively control cell size, proliferation, migration, and apoptosis. These discoveries transformed our understanding of vascular anomalies and, for the first time, revealed potential pharmacologic targets for treatment of these progressive birth defects.

Genetic analysis of vascular anomalies has been accelerated by leveraging techniques used in tumor

B. Significance and Innovation

Vascular anomalies present with wide-ranging and devastating phenotypic manifestations that persist through adulthood. Affected patients often have disfiguring vascular stains; intractable pain; severe tissue overgrowth; and/or brain and ocular manifestations resulting in vision loss, seizures, and developmental delay. Due to the substantial phenotypic heterogeneity in patients and a lack of understanding of the disease mechanisms, there are no uniform guidelines for the treatment of GNA-associated vascular anomalies and related syndromes. The goal of this study is to dissect the mechanisms that lead to the development of GNA-associated vascular anomalies using patient tissue, microphysiological systems (**MPS**), and novel mouse models. Our innovation lies in the multi-disciplinary, yet highly integrated approach to investigation of GNA-associated vascular anomalies. Defining the mutational signature in human tissue, MPS, and mouse models will provide evidence of causality, explain phenotypic variability, and create robust preclinical platforms to test patient-centric therapeutic approaches for this genetic disease.

We focus on *GNAQ* and *GNA11* because mutations in these two genes represent over 1/3 of our biorepository and are associated with life-altering disease for which no treatment options are available. Although mutations in *GNAQ* (R183Q, Q209P) and *GNA11* (R183C, R183S) have been shown to cause an overactive Ras-MAPK pathway and render cells independent of growth factors *in vitro*,[10,11] there is limited direct evidence showing that they cause disease *in vivo*.[13] In-depth knowledge of the biological mechanisms leading to the development of GNA-driven vascular anomalies and the factors that drive disease progression is required before targeted signal transduction inhibitors can be studied in humans. Our aims are designed to fill these knowledge gaps and lay the foundation for the development of targeted therapy (**Fig. 2**).

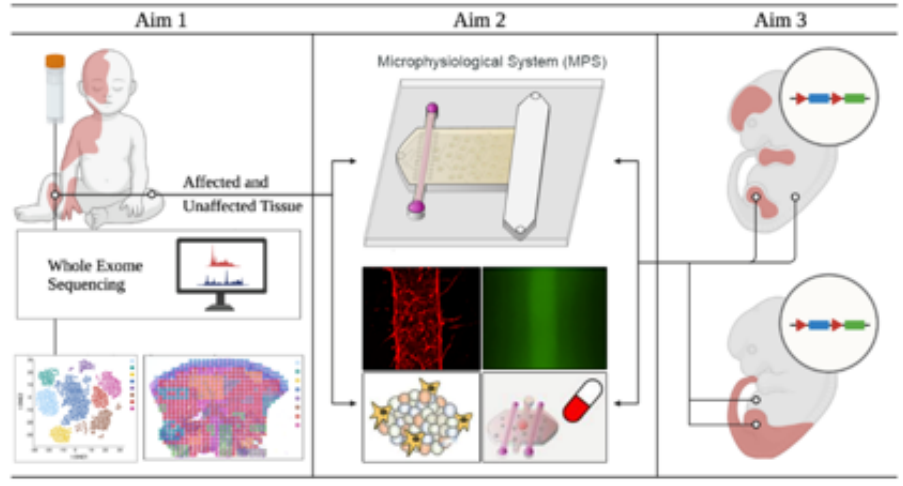

Fig. 2. Overview of the proposed three aims.

To determine what drives phenotypic variation, we will continue to enroll subjects, use high-depth, NGS sequencing to identify alterations in DNA from affected tissue (including but not limited to capillary malformation, phakomatosis pigmentovascularis, Klippel-Trenaunay Syndrome, diffuse capillary malformation with overgrowth, and Sturge-Weber syndrome). We will create an atlas consisting of spatial transcriptomics and single-cell RNA sequencing (scRNA-seq) data from affected and normal

C. Approach

Investigative Team. The multiple-principal investigator (MPI) research group has expertise in genomics, MPS, bioinformatics, and mouse models. In 2012, MPI Dr. Drolet spearheaded the multi-site biorepository and with Dr. Arkin [Co-Investigator (Co-I)] has published extensively on vascular anomalies. MPI Dr. Chang has over 20 years of experience leading investigations in mouse models. Analysis of the genomics, spatial transcriptomics, and scRNA-seq data will be done by Dr. Kendziorski (Co-I), who is an expert in the field of spatial and scRNA-seq analysis and statistical methods and software for scRNA-seq quality control,[28,29] normalization,[30] and downstream analysis.[28,31-34] Dr. Kendziorski's collaboration with Dr. Drolet has led to the identification of an artifact in the 10x Genomics Visium platform, as well as a computational method to adjust for it.[35] Dr. Ayuso is an expert in microfluidics and MPS. He has published and patented multiple *in vitro* devices to study tissue microenvironment, vascular response, and vasculature-tissue interactions.[36-40] In short, the investigative team is well integrated, has a track record of productivity and impact, and has the complimentary expertise to achieve the aims of this proposal.

Aim 1. Identify molecular signatures in mutant GNA vascular anomalies using a multi-omics approach and correlate these to phenotypic severity.

Preliminary Data and Rationale. Given the rarity of vascular anomalies, a multi-site biorepository was created that is a vanguard study of the Pediatric Dermatology Research Alliance (PeDRA). This has expanded to be one of the largest vascular anomalies biorepositories in the US, containing affected tissue, unaffected skin samples, blood, and saliva with robust clinical phenotyping stored in a secure RedCAP database. We have previously published on high-depth targeted NGS of affected tissue from subjects enrolled in our biorepository. We found 83% of our cohort harbored mutations in oncogenes, with *GNAQ/GNA11* being the most common.[25,41,42] Most were identified at low variant allele frequency (VAF), consistent with postzygotic somatic etiology. Since these publications, our cohort has grown to 489 patients. Affected tissue from 178 patients has undergone high-depth NGS, and 63 of these samples harbor mosaic GNA mutations. These patients diverge phenotypically and carry various clinical diagnoses (**Table 1**).

Table 1: Existing GNA Patient Cohort and Expected Enrollment

Clinical Diagnosis	Current	Expected	Total
Klippel-Trenaunay Syndrome	19	14	30
Sturge-Weber Syndrome	12	9	21
Capillary malformation with overgrowth DCMO)	18	11	25
Isolated Capillary malformation	8	7	9
Phacomatosis Pigmentovascularis	5	3	8
	63	**44**	**107**

The mosaic mutations of *GNAQ* and *GNA11* detected in GNA vascular lesions predicted to be inactivating mutations of GTPase activity, rendering the *GNAQ* and/or *GNA11* "switches" in the constitutive "on" position,

expression of mutant GNA proteins can cause vascular anomalies but also tools to dissect the mechanisms for disease pathogenesis.

Experimental Plan.

3.1. Monitor the transgene expression in the conditional overexpression GNA alleles. Our preliminary RT-PCR data show the expression of mutant *GNAQ R183G* can be induced by doxycycline when combined with *Rosa26-LSL-rtTA* and *Sox2-Cre*. We will further examine the transgene expression by western and immunostaining. Just as preliminary data were prepared, we will cross the *tet-GNAQ R183G; Rosa26-LSL-rtTA* transgenic males with *Sox2-Cre* females and inject pregnant females with a single dose of doxycycline (100 mg/kg body weight) at E13.5 to induce the transgene expression. We will collect protein lysates from E15.5 embryos and detect the *GNAQ R183G* expression by western using GNAq antibody (Santa Cruz, #SC-136181). We will also examine the expression of mutant *GNAQ* protein by immunostaining the sagittal sections of E15.5 embryos. In the *Rosa26-LSL-rtTA* allele, Cre expression will turn on both the reverse tetracycline-activated transactivator and EGFP. We will co-stain the sagittal sections with *GNAQ* and EGFP (CST, #2555) antibodies to determine whether EGFP can mark *GNAQ R183G* mutant cells. Similar experiments will be performed on the *tet-GNAQ R183Q* and *tet-GNA11 R183C* mice to see if transgene expression can be induced.

3.2. Determine the effects of mutant GNA overexpression in endothelial cells. For simplicity, we will refer the three tet-ON inducible mouse lines (*tet-GNAQ R183G, tet-GNAQ R183Q,* and *tet-GNA11 R183C*) as *tet-GNA^{mut}* mice. To activate mutant GNA expression in embryonic endothelial cells, we will cross *tet-GNA^{mut}; Rosa26-LSL-rtTA* transgenic males with *Tie2-Cre* females

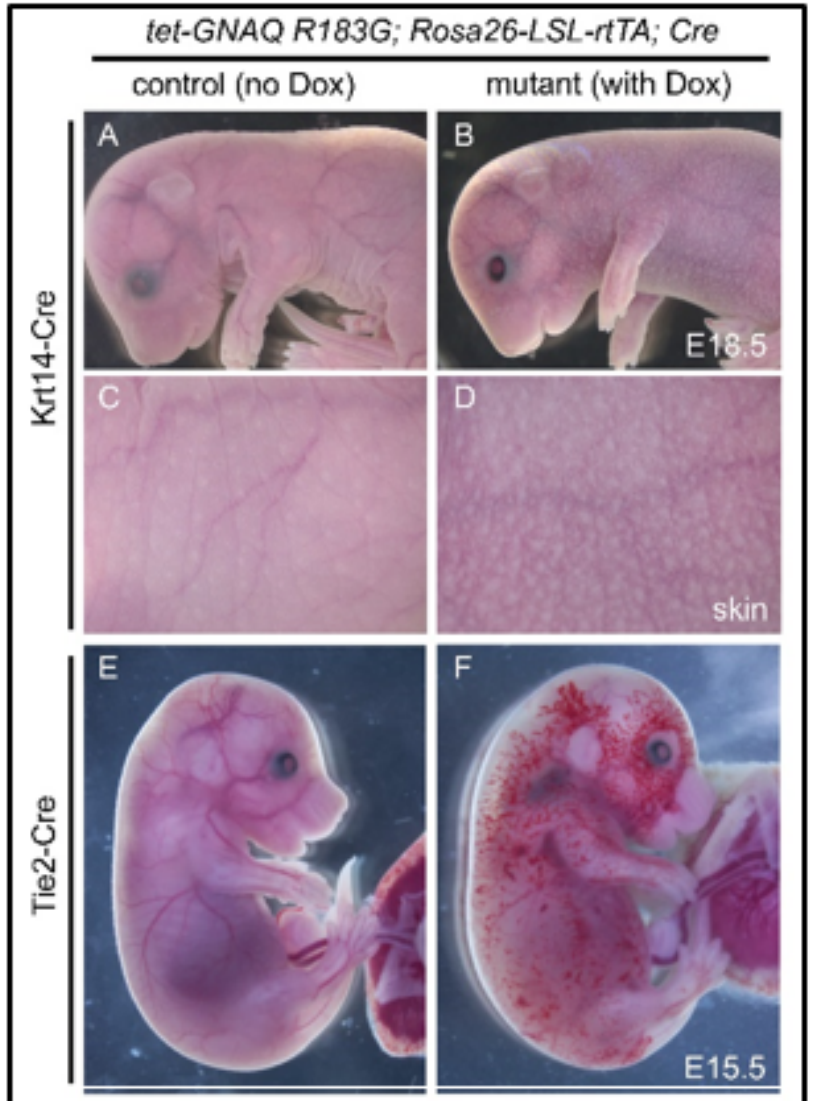

Fig. 8. Overexpression of mutant GNA in developing mouse skin and blood vessels. GNAq R183G mutant mouse embryos show a dramatic skin (A-D) and endothelial phenotype (E-F).

Critical Analysis of Experimental Design and Alternative Strategies. The proposed *in vivo* experiments in Aim 3 should provide definite information about the role of mutant GNA proteins in vascular anomalies, which will deepen our understanding of the disease mechanism and ultimately facilitate the development of novel treatments for this disease. Given our preliminary data demonstrating that the expression of the mutant *GNAQ R183G* can be turned on by a Sox2-Cre and doxycycline injection in the *tet-GNAQ R183G* mice we generated, we expect to see that the *tet-GNAQ R183Q* and *tet-GNA11 R183C* alleles will also function as designed. Like any transgenic lines, tetracycline-inducible system is subjected to genomic integration site-dependent variation. If the *tet-GNAQ R183Q* and *tet-GNA11 R183C* integrated in a heterochromatic region, and we see no induction of the transgene expression in the lines we have generated (currently we have one line each for *tet-GNAQ R183Q* and *tet-GNA11 R183C*), we will generate and screen more transgenic lines to get the one(s) with a robust expression. Alternatively, we can generate conditional overexpression mutant GNA alleles using Rosa26 the knock-in approach.[78] We expect that overexpression of mutant GNA proteins in endothelial cells will cause various vascular anomalies in mice. We expect that overexpression of mutant GNA proteins in a mixed cell population will cause not only the vascular phenotype but also soft-tissue and bone overgrowth, as seen in human patients. Since the efficiency of Cre-induced recombination varies among different alleles, we will adjust the dose of 4-HT injection if we observe a too high or too low recombination efficiency (e.g., all cells or no cells expressing the transgene). Through the unbiased transcriptome analysis on the skin of the *tet-GNA^{mut};Rosa26-LSL-rtTA;Tie2-Cre* and *tet-GNA^{mut};Rosa26-LSL-rtTA;Cdx2-Cre* embryos at E13.5, we expect to identify potential downstream effectors of mutant GNA proteins in the development of vascular anomalies. It is possible the evaluation of gene expression on E13.5 skin (two days after transgene induction) will not capture the initial responses crucial for the phenotype. We will perform additional experiments at earlier time points as a backup.

Scientific Rigor /Statistical Analysis/Reproducibility. We have provided a rigorous experimental strategy with comprehensive and well-controlled experiments, appropriate use of statistical methods, and unbiased analysis and interpretation of data. We will use the necessary replicates of the proper sample size and corresponding controls for all the experiments we propose. To ensure reproducibility over time, we will cryopreserve batches of pre-authenticated cells (by STR profiling analysis) and will not use cells after 20 passages. For the *in vivo* experiments using mouse models in Aim 3, a sample size of 12 (6 males and 6 females) mice per experimental condition is proposed. This sample size is sufficient to detect the anticipated differences in the primary comparisons between experimental groups with adequate power. For an estimate of the required numbers of animals needed to complete the specific aim, please see the Vertebrate Animals section. Quantitative results between and among groups will be analyzed using Student's t-test and ANOVA, respectively. Tukey's Honestly

PROJECT AND COMPANY TIMELINE	Pre-award	Year 1				Post-award Year 2				
Milestone 1		Q1	Q2	Q3	Q4	Q1	Q2	Q3	Q4	Q1
Obtain Institutional Review Board Approval										
Patient enrollment and image collection										
Reliability testing										
Milestone 2										
Obtain Orphan Disease Designation										
Protocol development for first-in-human										
Pre-IND meeting packet										
Concurrent Company work not funded by SEED grant										
Conversion from LLC to C-corp										
Active Ingredient sourcing of GMP grade supply										
Non-GLP toxicology screening										
GLP IND enabling toxicology studies										
Preclinical and Clinical formulation development and analytical testing										
Clinical protocol for FIH and agreement with FDA in pre-IND meeting										
Extended Development Timeline Clinical Proof of Concept: 15 months from FDA "May Proceed Letter."										
File IND										
Engage clinical CRO										
Engage activate clinical sites										
Obtain IRB approvals										
Patient enrollment and dosing										
Clinical phase I report										

Budget- Jeff

- **Pay attention to what you can use the money for**
  - Eg Salary? Travel? Study expenses only?
  - Emerging Investigator Research Grant

    5. Budget – budget may be used to support an awardee stipend or salary, travel to conduct a project at the mentor's institution, and limited research supplies. Direct costs only. No salary support for the mentor will be allowed.

  - Weston Career Development

    Award
    This award provides **$40,000 USD per year for up to two years (direct costs only)** to support research activities and up to 0.2 FTE of salary support for the applicant. For two-year projects, receipt of funding for the second year will be dependent upon satisfactory progress through the first year as demonstrated in an interim report.

  - Dermatology Foundation Career Development Award

    All CDAs offer an annual salary stipend of $55,000 that can be supplemented from institutional sources so that the salary received is commensurate with peers within the institution. A CDA recipient may seek simultaneous grant support from other agencies to provide for the non-salary components of the research being performed under the auspices of an award.

Request for Department 20% Indirect Cost Support

Section A:
Investigator: JiaDe Yu
Sponsor: Pediatric Dermatology Research Alliance
Award Name: PeDRA Research Grant
Award Deadline Date: 9/21/23
Sponsor website:
https://pedraresearch.org/wpcontent/uploads/2023/05/PeDRA_ResearchGrants_2023RFA.pdf

Direct Cost Budget: $39,502

- Salary & Benefits: $0
- Other Direct Costs: $39,502

Indirect Cost Budget (if any): $ no IDC allowed

Section B:
Total support requested: $7,900

Please describe any sundry fund under your supervision which could be utilized to cover the requested indirect costs (including current balance $): none

Be as specific as possible

Line item budgeting

Provide justification
- why you want to buy this and this much?

PeDRA Grant Budget Sheet

Modular Budget

	Cost ($)/Unit	Units Needed	Total Cost ($)
Aquaphor (14oz)	13.73	10	$137.30
Cerave (12oz)	19.88	10	$198.80
Vaseline (13oz)	4.29	20	$85.80
Vanicream Ointment (13oz)	15.99	10	$159.90
Visa Gift Cards	50	75	$3750.00
CURTIS Facility Fee	50/hour	30 Hours	$1500.00
BallHull Plastic 2oz Jars	1	180 jars	$180.00
Tewameter TM Hex	8960.00	1	$8960.00
Total Budget			**$14,971.00**

Budget Justification

Our study seeks to evaluate the efficacy of 4 different over the counter ointments in reducing TEWL on the volar forearms of children with mild to moderate atopic dermatitis. We will purchase the 4 over the counter ointments from Amazon: Aquaphor, Cerave, Vanicream, and Vaseline. We anticipate each patient will use 1-2 fingertip units (~1g) of their assigned ointment twice a day to the right volar forearm or 56g (2 ounces) for the duration of the study which will be packaged in blinded BallHull Plastic Jars. All patients will also receive 2 ounces of Vaseline ointment to use on the left volar forearm. All ointments will be color coded by research staff not directly involved with the study.

The study will be conducted at the MGH Clinical Unit for Research Trials and Outcomes in Skin (CURTIS) with support of research staff and humidity and temperature-controlled facilities. We anticipate each patient will require 45 minutes of time in CURTIS and 2 patients can be evaluated at the same time given the room availability. However, we will budget for 30 hours in case patients take longer or we are unable to evaluate 2 patients at the same time due to scheduling conflicts.

For each patient's time, we will give every enrolled patient a $50 Visa gift card at the completion of the study. Parking for the patients will be validated by the department and incur no extra expense for the study investigator.

Overlapping Grant Funding

There are no overlapping grant funding for this proposed project.

Budget Justification:

Trainee support costs $3500

Round Trip Flight from Newark, NJ to Boston, MA- $300
Housing Boston, MA for ~18 days of clinical observation at MGH - $2700 (Airbnb $143/night plus fees)
Food and incidentals- $500

Travel to present data at national conference $800

Presentation at upcoming meeting (American Contact Dermatitis Society 2023)- registration, flight, hotel

Core costs $700
Biostatistics Consult for Meta-analysis Support- $700 ($100/hour x 7 hours)

What does a pediatric *BAP1* inactivated melanocytic tumor (BIMT) look like?

Project Budget

Total Budget: $13,000.00

Supplies/Immunohistochemical staining: $10,852.20
Travel costs: $2,000.00
Meeting costs: $147.80

Budget Justification:

Cost of immunohistochemical staining:

BAP1 Immunohistochemistry: $56.99/stain
BRAFV600E Immunohistochemistry: $56.99/stain
Blanks: $3.30/blank

Cost/specimen: $56.99/BAP1 stain + $56.99/ BRAFV600E stain + (2 x $3.30/blank)
Cost/specimen = $120.58/specimen

90 specimens total to stain
(30 combined Spitz and dermal nevi; 30 Spitz nevi; 30 dermal nevi)

$120.58/specimen x 90 specimens = $10,852.20

Travel costs:

Conference travel to present findings at PeDRA Annual Meeting: $500
Conference travel to present findings at AAD Annual Meeting: $1,500

Meeting costs:

Meeting costs: $147.80

Dr. Elena Hawryluk will devote 1% effort. Dr. Hawryluk is not requesting salary from the grant if awarded.

Dr. Lyn Duncan, a dermatopathologist at MGH will not devote measurable effort but will serve as a collaborator on this project.

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: Drolet, Beth Ann

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

POSITION TITLE: Professor of Dermatology and Pediatrics

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
Michigan State University, East Lansing, MI	BS	1983–1987	Physiology and Nutrition
Loyola University, Chicago, IL	MD	1987–1991	Medicine
University of Colorado, Denver, CO	Internship	1991–1992	Medicine
Medical College of Wisconsin, Milwaukee, WI	Residency	1992–1995	Dermatology
Medical College of Wisconsin, Milwaukee, WI	Fellowship	1995–1996	Pediatric Dermatology

A. Personal Statement

I am Professor and Chair in the Department of Dermatology at the University of Wisconsin (UW) and hold the Johnson Sture Distinguished Chair. I have served in successive leadership roles, including past-President of Society for Pediatric Dermatology, past-President of Hemangioma of Infancy Group (HIG), and Clinical Vice President of Children's Wisconsin. As a pediatric dermatologist with expertise in vascular anomalies, I provide medical care to children and adults with rare vascular anomalies. My research interrogates molecular profiles of vascular anomalies to identify target pharmacologic intervention to improve medical management for these conditions. I have cultivated and led several NIH-sponsored, inter-institutional registries, biorepositories, and outcomes studies that have advanced the field of vascular anomalies. I have published >150 peer-reviewed journal articles on these subjects. My extensive experience leading multidisciplinary care teams, multi-site research networks, and national research organizations well-qualifies me to serve as Multi-PI for the proposed project entitled *Genetic and Molecular Basis of G-protein Mutant Vascular Anomalies*. I have assembled a multidisciplinary team of experts in microfluidics, mouse genetic models, and biostatistics. Together, we will work collaboratively to establish a multiomics atlas for the broader research community that characterizes human GNA-associated molecular profiles. We will then interrogate these profiles in state-of-the-art microphysiological systems and mouse models.

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

State Economic Engagement and Development (SEED) Research Program Grant Funding
Drolet (PI)
07/01/2022-06/31/2023
"Developing a topical beta blocker formula as a targeted therapeutic for infantile hemangioma"

Dermatology Foundation Career Development Award
Arkin (PI), Role: mentor to PI

Letters of Support

- *Really* important if you are not the expert or if someone else can lend GRAVITAS to your grant

- Most critical for mentorship grants like the Dermatology Foundation where your mentor is *critical* to your success

- Often you can write the letter first to save the recommender time and you can shamelessly plug yourself

Penn Medicine

Department of Dermatology

Hospital *of the* University *of* Pennsylvania

Bruce A. Brod, MD
Director, Occupational and Contact Dermatitis

Oct 1, 2018

To the Medical and Scientific (

I am writing to give my
Foundation Pediatric Dermato
Contact Dermatitis Clinic at th
and in that role I am responsib
mentored many young physici
focus during his fellowship at (
allergic contact dermatitis. I an
pediatric allergic contact derm

MGH 1811 MASSACHUSETTS GENERAL HOSPITAL

HARVARD MEDICAL SCHOOL

VA LOMA LINDA HEALTHCARE SYSTEM

A Division of VA Desert Pacific Healthcare Network

Maryam Asgari, MD MPH
Associate Professor of Dermatology
11201 Benton Street
Loma Linda, CA 92357
(800) 741-8387 - (909) 825-7084
www.lomalinda.va.gov

In Reply Refer To:

September 18, 2018

Dear Dermatology Foundatio

I am delighted to write a stro
Pediatric Dermatology Caree
as faculty at Massachusetts G
clinical and research area of u
a field that is relatively unexp
understanding of the immune

Jerry L. Pettis Memorial VA Medical Center
11201 Benton Street
Loma Linda, CA 92357
(909) 825-7084

Blythe VA Rural Health Clinic
1273 W. Hobson Way

September 26, 2018

To the Dermatology Foundation Medical and Scientific Committee:

I am writing this letter to provide Dr. JiaDe Yu my strongest recommendation as a candidate for the Dermatology Foundation Pediatric Dermatology Career Development Award. I am honored and delighted to be one of Dr. Yu's collaborators and mentors for his prospective Pediatric Allergic Contact Dermatitis Registry.

Currently I serve as the president of the American Contact Dermatitis Society (ACDS)and have specialized in pediatric allergic contact dermatitis (ACD) since completion of my dermatology residency. I have published extensively in ACD in children and strongly endorse Dr. Yu's commitment to advancing this important and yet still under-explored field! The proposed Registry offers a significant opportunity to contribute critical clinical and prevalence data to the field with the creation of the largest multi-facility North American registry of children with ACD. Notably, most published studies in pediatric ACD have focused on a limited number of patients and in a retrospective manner limiting conclusion.

Take Home Messages

- Grants may not be for everyone but it is an important pathway to academic success
- Various grants are available from small institutional grants to large NIH grants
- If you choose to take the plunge, read the grant RFP carefully so you understand exactly what it’s asking for
- Reach out to colleagues for examples of grants that have been successful and ask for as many eyes as possible

Questions?

- Jeff Yu- jiade.yu@mgh.Harvard.edu

- Beth Drolet- bdrolet@dermatology.wisc.edu