

Grant Writing Series

Office for Postdoctoral Scholars

Mahley Auditorium, Gladstone Institutes

Writing a Clear, Compelling Specific Aims Section



SUPPLEMENTAL HANDOUT

EXERCISES & RESOURCES

(to accompany powerpoint slide presentation)

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Exercise 1. Review of 2 Specific Aims Examples

Example 1: NIH RO1, from NIAID

1 Eukaryotic innate immune systems act as effective barriers to infection by microorganisms. Understanding the mechanisms that bacterial pathogens employ to circumvent innate immune systems will improve our ability to control disease. Plants and animals use specific pattern recognition receptors (PRRs) to recognize conserved molecules of microorganisms (known as PAMPs). Plants have numerous PRRs that can recognize specific virulence proteins specifically present in pathogens (known as Avr proteins). Many Gram-negative bacteria use type III protein secretion systems to inject effector proteins into host eukaryotic cells. We have shown that a primary role for many *Pseudomonas syringae* type III effectors is to suppress innate immunity. However, the enzymatic activities and the mechanisms that type III effectors use to suppress innate immunity are not well understood. Identifying the enzymatic activities of type III effectors and their substrates is essential to identify important components of innate immunity and to improve strategies to control bacterial diseases.

2 *Our long-term goal is to elucidate the molecular basis for suppression of innate immunity by type III effectors. The objective of this application is to identify targets of the P. syringae type III effector HopU1, a mono-ADP-ribosyltransferases (ADP-RTs), and to determine its roles in bacterial pathogenesis. The central hypothesis of the proposed experiments is that the targets of the HopU1 ADP-RT type III effector will be components of innate immunity. We formulated this hypothesis based on the literature and on our research on other type III effectors as well as our preliminary data showing that HopU1 suppresses outputs of innate immunity. Recently, we have shown that HopU1 can use several Arabidopsis RNA-binding proteins as high affinity substrates in in vitro ADP-RT assays. Based on our preliminary data, one of these proteins, AtGRP7, plays a role in innate immunity. A major goal of this application is to elucidate the function of this protein as it relates to innate immunity. We are prepared to undertake the proposed research because we have extensive experience in manipulating type III systems, and we were among the first to report that certain type III effectors suppress innate immunity. In addition, our preliminary identification of HopU1's substrates has positioned us well to perform the experiments described in this application. Our research team includes experts in the following areas: type III secretion systems, proteomics and mass spectrometry, Affymetrix microarrays, plant glycine-rich RNA-binding proteins, and animal pathogen ADP-RTs. This qualified group of investigators will insure that our discoveries are linked to basic concepts of pathogenesis and immunity in both plants and animals.*

3 The Specific Aims of this application are as follows:

- 1. Determine the molecular consequence of ADP-ribosylation on the function of AtGRP7 and elucidate the role this protein plays in innate immunity.** *Our working hypothesis* of this aim is that AtGRP7 binds to immunity-related RNAs to enhance the innate immune response and that ADP-ribosylation by HopU1 disrupts its function.
- 2. Identify additional substrates of HopU1 and verify their involvement in innate immunity.** *Our working hypothesis is* that the plant targets for the HopU1 ADP-RTs will be important components of plant innate immunity.
- 3. Analyze the affect that HopU1 has on host-microbe interactions.** *Our working hypothesis of this aim is* that HopU1 type III effector suppresses innate immunity. This is based on our preliminary data and in this aim we will determine to what extent this occurs with HopU1.

4 The proposed research is innovative because, to date, ADP-RTs have not been implicated in the suppression of innate immune surveillance systems. Moreover, RNA-binding proteins have not been described as substrates for ADP-RTs and, therefore, represent novel substrates for this important group of bacterial toxins. Collectively, we expect the outcomes of these experiments will greatly add to our understanding of the activities and roles of type III effectors, particularly in how they suppress innate immunity in eukaryotes.

Example 2: NIH K08, from NIAID

Contact PD/PI: Ambroggio, Lilliam

Metabolomics Evaluation of the Etiology of Pneumonia

Lilliam Ambroggio, PhD, MPH

3. SPECIFIC AIMS AND HYPOTHESES Community-acquired pneumonia causes substantial morbidity and mortality worldwide.¹⁴ In the U.S., CAP is among the most common and costly causes of hospitalization in young children.¹⁵ Clinical examination and chest radiography do not differentiate between viral and bacterial CAP.^{16,17} Conventional diagnostic tests (e.g., sputum cultures) used in adults are not feasible in young children and high yield tests (e.g., lung tap)¹⁸ are too invasive to be routine. Other bacterial diagnostics infrequently identify the causative pathogen (e.g., <7% for blood cultures) limiting their diagnostic utility. Viral tests, while often positive, do not exclude the possibility of bacterial co-infection.^{19,20} Therefore, patients with CAP receive empiric antibiotic therapy, which confers no benefit for children with non-bacterial infections, but places children at risk for adverse drug reactions, treatment-related complications (e.g., *Clostridium difficile* colitis) and antibiotic resistance.²¹ There is a pressing and unmet need for *non-invasive diagnostic tests that permit accurate and timely clinical decision-making in children with CAP.*²²

The interaction between pathogen and host results in a unique metabolic profile that is detectable in the host's urine.⁷ Quantitative metabolomics can simultaneously detect and quantify metabolites (small molecules, < 1 kDa) in a biological sample using ¹H-NMR.^{23,24} Quantitative metabolomics testing of the urine is *simple, noninvasive and rapid* (< 1 hour, as opposed to >24 hours for conventional blood culture techniques).²⁵ We developed a prospective cohort study in our Emergency Department to examine approaches to investigate the etiology of pediatric CAP. Using this cohort, I began investigating the potential for metabolomics to distinguish pathogens in CAP. Over the past 3 years, we enrolled 169 children between 3 months and 11 years of age with CAP. Our preliminary data suggest that distinct urine metabolite profiles cluster by class of pathogen. Evaluating the change in these metabolite profiles in response to antimicrobial therapy also offers a tremendous opportunity to non-invasively gauge appropriateness of initial antibiotic choice. *Urine metabolite profiles may provide a fast, accurate and non-invasive approach for pathogen identification of CAP in children representing a critical innovation in the severely limited current practice.*

My *long-term goal* is to become an independent investigator in infectious diseases epidemiology with a focus on translating bench science research to bedside application. *The overall objective of this application is to combine robust clinical data (e.g. smoking exposure, antibiotic receipt) with metabolomics data using advanced statistical and metabolomics methodologies to inform pathogen-detection in childhood CAP.* I will use a clinical database and specimen biorepository from an established, funded, and fully-operational prospective cohort study of children with CAP. I will acquire knowledge and skills in systems biology sciences and their application to clinically challenging infections such as CAP. To accomplish this objective, I will pursue the following specific aims:

- 1. To characterize the sources of variability in the urine metabolite profiles of healthy children.** The *primary hypothesis* is that between subject variability will be larger than within subject variability in the metabolites in the urine of healthy children and the highest proportion of variance will be attributable to age and sex.
- 2. To determine and compare the urine metabolite profiles of children with viral or bacterial CAP with those of healthy children.** The *primary hypothesis* is that urine metabolite profiles of children who have viral pneumonia will be uniquely different from those of healthy children. In addition, urine metabolite profiles of children who have *Streptococcus pneumoniae* (the most common bacterial pathogen) or *Mycoplasma pneumoniae* (the most common atypical bacterial pathogen) will be distinct from those of healthy children.
- 3. To evaluate longitudinal changes in the urine metabolite profile of children with CAP compared with healthy children.** The *primary hypothesis* is the magnitude of change in the metabolome of a child with viral or bacterial CAP will be greater than the diurnal fluctuation of the metabolome in a healthy child. A *secondary hypothesis* is that longitudinal changes in metabolite patterns of children with CAP will differentiate response from non-response to antibiotics.

Successful completion of these aims will generate preliminary data on distinct metabolite profiles associated with bacterial and viral pneumonia. These findings will inform an R01 application for a large scale, multi-center, validation study that includes more diverse microbial causes of CAP to advance the development of point-of-care diagnostic tests. *This study will further the field of CAP by introducing a method to differentiate bacterial and viral pathogens thereby increasing targeted antibiotic therapy for children.*

EXERCISE 2: THE LOGICAL, TESTABLE, AND FEASIBLE HYPOTHESIS

What are the problems with the following hypotheses? (Too broad? Not logical? Not testable?) Can you revise them?

1. We will test the hypothesis that analogs to chemokine receptors can be biologically useful.

2. We will test the hypothesis that a wide range of molecules can inhibit HIV infection.

-or-

3. Write or revise a hypothesis statement for your own research. It can be for the research you will propose, or for your current research.

EXERCISE 3. On your own: Review a Sample Specific Aims Section

Work through questions 1-3 below, analyzing your own specific aims section (if you brought one), or one of the samples that follow, below.

1. Circle and label the components of this specific aims section:
 - the problem,
 - the gap in knowledge,
 - long term goal statement,
 - hypothesis [or overall goal, if applied research],
 - aims,
 - main techniques that will be used

2. Consider whether the section is written clearly...
 - a) In what way does the first paragraph identify the goal of the proposed research and make a case for why achieving that goal is important?
 - b) Is the problem that the research aims to address clearly defined? How did the author do this?
 - c) How did the author make the hypothesis clear?
 - d) How do you know that the hypothesis is “reasonable”?
 - e) Is each aim brief and specific? If so, how did the author achieve this?
 - f) Examine the language used to state the aims, particularly the verbs. Are they vague (e.g., “To study”, “We hope to show”)? Are they direct and specific (e.g., “To quantify”, “To determine”)?

3. How do formatting and word signals help the reader skim quickly to find the key information?

4. Do you need to use more explicit language? In particular...
 - Abbreviations – do they need to be defined?
 - Wording – do you need to use more precise and simple words?
 - Active voice – do you need to use active voice more? (less passive voice)

Specific Aims - Example (1) of K01 Mentored Career Proposal¹

Among people with diabetes, an estimated 60% suffer from chronic pain.³⁸ Peripheral neuropathy is one of the most common complications of diabetes and often results in chronic pain that impairs functional activities, severely impacts quality of life, and impedes effective diabetes self-management, such as physical activity. Pharmacological treatments for painful diabetic neuropathy (PDN), such as anti-convulsants or anti-depressants, vary in clinical effectiveness and have side effects that can impede quality of life. The increasing incidence of co-occurring diabetes and pain in the US population underscores the need for effective pain management. This need is magnified for vulnerable populations, such as low-income, racial/ethnic minorities with limited access to healthcare, who bear a disproportionate burden of disease and are undertreated for their painful symptoms.^{14, 15, 39, 40} ***Innovative pain management strategies are needed to improve quality of life for vulnerable populations living with chronic conditions like diabetes.*** A growing body of evidence indicates the effectiveness of acupuncture for pain relief and functional improvement for various chronic pain conditions.⁴¹⁻⁴⁴ Acupuncture is a promising treatment for PDN, as indicated by its effects of increased blood flow, nerve conduction velocity, and decreased pain,⁴⁵⁻⁴⁷ but evidence is limited by lack of consistent treatment protocols, small samples, and inadequate comparison groups in prior studies. It is also unknown if acupuncture is a feasible treatment for PDN among vulnerable populations who rarely have access to acupuncture.

My overall career goal is to design and evaluate CAM interventions to improve quality of life among medically underserved populations living with chronic conditions. The objective of the current study is to design, implement, and evaluate a group acupuncture intervention for PDN among underserved patients at San Francisco General Hospital (SFGH), an urban safety-net hospital. Group medical visits (GMVs) combine quality healthcare with educational support in a collective setting and have been shown to improve disease self-management and quality of life among patients with a range of chronic conditions.^{25, 48} Acupuncture is commonly administered in groups in China and in some community settings in the US.⁴⁹ This study proposes to augment GMVs with group acupuncture and will provide necessary preliminary data for an R01 proposal of group acupuncture treatment for PDN in vulnerable populations. The study design uses novel approaches to bridge gaps between internal and external validity that can hinder progress in acupuncture research. ***While therapeutic efficacy is a central question that needs to be addressed, clinical trials can have only limited impact on public health without a consideration of real-world factors.*** To maximize research efforts and facilitate the transition between research and practice, Glasgow recommends evaluating factors that impact the success and outcomes of an intervention, such as reach, adoption, implementation, and maintenance, even during efficacy trials.⁵⁰ Thus, principles from Glasgow's RE-AIM framework will be applied to all study phases, from planning to outcomes evaluation. With senior mentorship from expert researchers at UCSF's Osher Center for Integrative Medicine and the Center for Vulnerable Populations, and international leaders in acupuncture research, the current study leverages expertise and resources in public health approaches to disease management and rigorous CAM research with the following specific aims.

Aim 1: To develop a standardized acupuncture protocol for PDN through formal manualization.

Treatment manualization, initially developed to evaluate complex, heterogeneous psychotherapy interventions, has been successfully applied in clinical acupuncture trials as a means of standardizing protocols while maintaining the flexibility of individualized treatments.⁵¹ In Years 1 and 2, I will develop a formal acupuncture protocol for PDN using mixed-methods by: (1) conducting a systematic literature review and meta-analysis of acupuncture for PDN; (2) assessing inter-rater reliability of acupuncturists' traditional Chinese medicine (TCM) diagnoses for PDN; and (3) developing an algorithm for TCM diagnoses and treatments for PDN. This research will produce a usable treatment manual to assess efficacy of acupuncture for PDN in an RCT.

Aim 2: To evaluate acceptability, feasibility, and preliminary efficacy of group acupuncture for PDN among underserved patients with diabetes. In Years 3-5, I will conduct a pilot trial of 48 SFGH patients with PDN randomized to: (1) usual care provided in group medical visits (GMVs), (2) GMVs augmented with group acupuncture, and (3) GMVs augmented with group sham acupuncture (16 patients in each arm). **Hypothesis 1.** A group acupuncture trial of PDN using a treatment manualized approach is feasible in a safety net setting. Feasibility and external validity will be evaluated with RE-AIM metrics (see section 11C.4- b3). **Hypotheses 2a and 2b.** Compared to usual care or sham acupuncture, group acupuncture will (a) decrease pain severity and

¹ Source: UCSF CTSI (Chao)

(b) improve quality of life among patients with PDN. Weekly pain severity scores and quality of life will be assessed using validated measures.

Specific Aims - Example (2) of K08 Mentored Career Proposal²: Malaria afflicts ~200 million people yearly, with ~430,000 malaria deaths due to *Plasmodium falciparum* (Pf) (1), underscoring the need for a highly effective malaria vaccine. The first licensed malaria vaccine, RTS,S, will provide much-needed reductions in morbidity and mortality. However, its 25.9% efficacy in reducing clinical malaria in the target population of African infants (2) leaves ample margin for improvement. Other malaria vaccine candidates that performed well in experimental studies also proved less effective in pediatric field studies in Africa (3), suggesting that immune factors specific to children in endemic areas may influence vaccine-induced responses. Better understanding of immunity to Pf in naturally exposed populations will provide insights for improving malaria vaccine design. Despite the established role for Pf-specific IgG in conferring immunity to malarial symptoms (**clinical immunity**) (4, 5), unambiguous immune correlates of clinical protection remain elusive (6). Even less is known about immunity to Pf infection (**sterile immunity**) given that years of repeated exposure to the parasite does not reliably confer sterilizing protection from parasitemia (7). Systems biology, which relies on computational modeling of large-scale data sets to elucidate complex biological networks (8), has been used to predict vaccination outcomes (9-12) and classify diseases (13-15) but can also reveal novel insights into host immunity to infection in an unbiased manner (16).

The goal of this proposal is to use systems biology approaches to elucidate correlates and mechanisms of both clinical and sterile immunity to Pf infection in a well-characterized, prospective cohort of Malian children living in an area of intense, seasonal malaria transmission (7, 17, 18). We have defined children as clinically immune or susceptible to Pf infection based on 3 years of malaria surveillance data. Our initial data has shown that clinically immune children at their uninfected baseline have increased B cells and Pf-specific IgG levels—both features typical of asymptomatic carriers of Pf. Identification of uninfected and asymptotically infected children who have been prospectively defined as clinically immune or susceptible provides an opportunity to investigate whether asymptomatic Pf blood-stage infection (**BSI**) confers clinical immunity or is a consequence of clinical immunity. We have also identified a subset of young children who, remarkably, remained free of **BSI** as determined by bi-weekly PCR surveillance during 6 months of intense malaria transmission and who demonstrated Pf exposure by boosting of Pf-specific IgG, suggesting that these children controlled BSI to below PCR-detectable levels—*i.e. they developed the closest functional equivalent to sterile immunity seen in a naturally exposed population*. The present study will take advantage of samples collected from this unique cohort to identify immune parameters that characterize and best predict **clinical immunity** (no symptoms with BSI) or apparently **sterile immunity** (no evidence of active BSI by PCR despite evidence of Pf exposure). Our overall hypothesis is that children who have acquired either clinical or sterile immunity to Pf have distinct pre-infection immune profiles than predict Pf infection or malaria outcomes.

Specific Aim 1: Identify immune parameters predictive of clinical immunity to Pf infection (protection from symptomatic BSI) and sterile immunity to Pf infection (protection from BSI).

We will perform transcriptomics, multiplex cytokine analysis, Pf-specific antibody profiling, and flow cytometry on baseline blood samples and apply these parameters along with parasitological, demographic, and genetic data to both machine learning algorithms and statistical models to determine which parameters best predict clinical or sterile immunity. Although the goal is to identify novel predictors and signatures of both clinical and sterile immunity in an unbiased manner, based on previous data, we hypothesize that Pf-specific IgG antibodies, a B-cell signature, and Pf BSI status will be significant predictors of clinical immunity, whereas either a Type I or Type II interferon signature will predict sterile immunity.

Specific Aim 2: Compare cellular, molecular, and Pf-specific IgG reactivity profiles in the blood of children with clinical immunity vs. children with sterile immunity and relate these profiles and immune outcomes to the in vitro parasite-inhibitory activity of their plasma.

To determine biological signatures that might define clinical and sterile immunity, we will directly compare transcriptomic, cytokine, Pf-specific antibody reactivity, and flow cytometry data between the two immune classes. We hypothesize that the clinically immune signature will be biased towards B-cells and have a predominantly blood-stage specific antibody profile, whereas a signature of sterile immunity will be driven by

² <https://www.niaid.nih.gov/sites/default/files/K08-Tuan-Manh-Tran-Application.pdf>

interferon responses and an antibody profile targeting both pre-erythrocytic and blood-stage antigens. We will quantify the ability of plasma from children in our study to inhibit parasite invasion of human hepatocytes and erythrocytes in vitro and determine whether invasion-inhibitory activity in plasma correlates with protection from BSI or symptomatic BSI after accounting for confounding variables. We hypothesize that plasma from sterilely immune children will inhibit parasite invasion of both hepatocytes and erythrocytes in vitro and that this activity will correlate with increased IgG reactivity intensity against both pre-erythrocytic and blood-stage antigens. Taken together, this work will enhance our understanding of natural immunity to both malarial disease and Pf infection and may identify novel predictors and mechanisms of immunity to Pf within the vaccine target population, thus informing the rational design of malaria vaccines for children in Africa.

Template for Writing the Specific Aims Page of an NIH Grant Proposal³

The Specific Aims page of an NIH grant proposal contains several key components. The sequence of those components can differ, but the components are the same and should appear in the narrative. To see a short video that shows two different sequences of these components in two different proposals (and to see the two proposals themselves) go to the following website:

<http://www.northwestern.edu/climb/resources/written-communication/aims-pages-part-1-the-rhetorical-pattern-of-introductions-in-aims-pages.html>

To develop a powerful Specific Aims page that covers all of the key components, ask and answer the following key questions:

General context & significance: What is the big picture for the research? Why is it important?

Narrowing context: What is known and accepted in your research area?

Your research contribution: Has your previous work contributed? How?

Complication: What is the problem, roadblock, unknown?

Long-term goal: What final “big result” will the research help achieve? Not today, but down the road?

Specific narrow research goal: What is the specific goal of the proposed research in this application?

Summary of research—path to the hypothesis: How does previous research (yours, others’) lead to the overall hypothesis that the aims are designed to test?

Hypothesis: How would you explicitly state what you believe to be the answer to the complication/problem/unknown that you’ve raised?

Qualifications: What makes you (or your team) the right person (or team) to undertake this research?

Specific aims statements:

- Is each aim an elaboration of an element or elements of the logic flow of your overall hypothesis?
- Does each aim have its own “sub hypothesis”, either explicitly stated or implicitly clear? (The aims must be logically connected to the rest of the Aims page—that is achieved by having the aims posit “sub hypotheses” of the proposal’s central hypothesis, which was formulated on the basis of your preliminary data and prior work by you and others).
- Is each aim independent of the others so the failure of one does not invalidate the others?
- Does each aim have a specific, ideal, concrete outcome (in other words, what will the success of the aim show us or teach us)? You can test the independence of the aims by stating what each outcome would ideally be.

Conclusion: Does the conclusion point to the novel nature of the research and the broader impact it might have? (e.g., *“The proposed research is innovative because to date.... Collectively, we expect the outcomes of these experiments will greatly add to our understanding of....”*)

³ Notes are based on two presentations: <http://www.northwestern.edu/climb/resources/written-communication/aims-pages-part-1-the-rhetorical-pattern-of-introductions-in-aims-pages.html> and <http://www.northwestern.edu/climb/resources/written-communication/aims-pages-part-2-specific-aims.html>