Worksheet, Hypothesis to Specific Aims

Jan 25, 2021

Class Discussion

- Introductions/ Proposal Worksheet
- Questions about Qualifier or Fellowship

Lecture

- Hypothesis to Specific Aims
- Specific Aims and Specific Aims Page
- Active verbs
- Preliminary data
- Significance/ Innovation
- Model/ Concept Figure

1-Discuss Qualifier Proposal Worksheet as a group

- Proposed project title
- Overall goal of project
- What is the gap in knowledge your project will address
- Hypothesis to be tested
- Major scientific approaches/methods you plan to use
- Particular strengths to address now
- Aim 1 statement
- Aim 2 statement

Title

Should be an accurate statement of long-term goals

Include keywords

Limit abbreviations

81 characters, including spaces and punctuation.

Environmental Enrichment-Induced Recovery of Neuronal and Glial Maturation Following Perinatal Hypoxia	5F32NS106723-02
Examining How Graphic Cigarette Warnings Motivate Young Adults to Quit Smoking	1F31CA239567-01
Mechanisms Governing Latency in Clinically Relevant HIV-1 Strains	1F31AI147814-01
Placental Allopregnanolone is Essential for Development of Cortical GABAergic Signaling	1F31HD098886-01
Investigating the effect of alcohol exposure on human cortical development using a 3D in vitro model	1F32AA028163-01

Examples (what do you think?)

Research Question

A tentative question, often from a ”hunch”

Which patients presenting with coma are most likely to return to the ED?

Why are numbers of children with obesity increasing?

question might go in several directions

Think: how might impact which direction to pursue?

FINER Tool:

Characteristics of a good research question

F-feasible; resources, time, stat power, expertise
I-Interesting; to investigator, subjects
N-Novelty; innovation, funder mission?
E-Ethical, safeguard interests of participants (IRB etc)
R-Relevant

Nice questions at scalelive.com

If feasible but not valid, unethical!

You can't decide without a grounding in the literature.

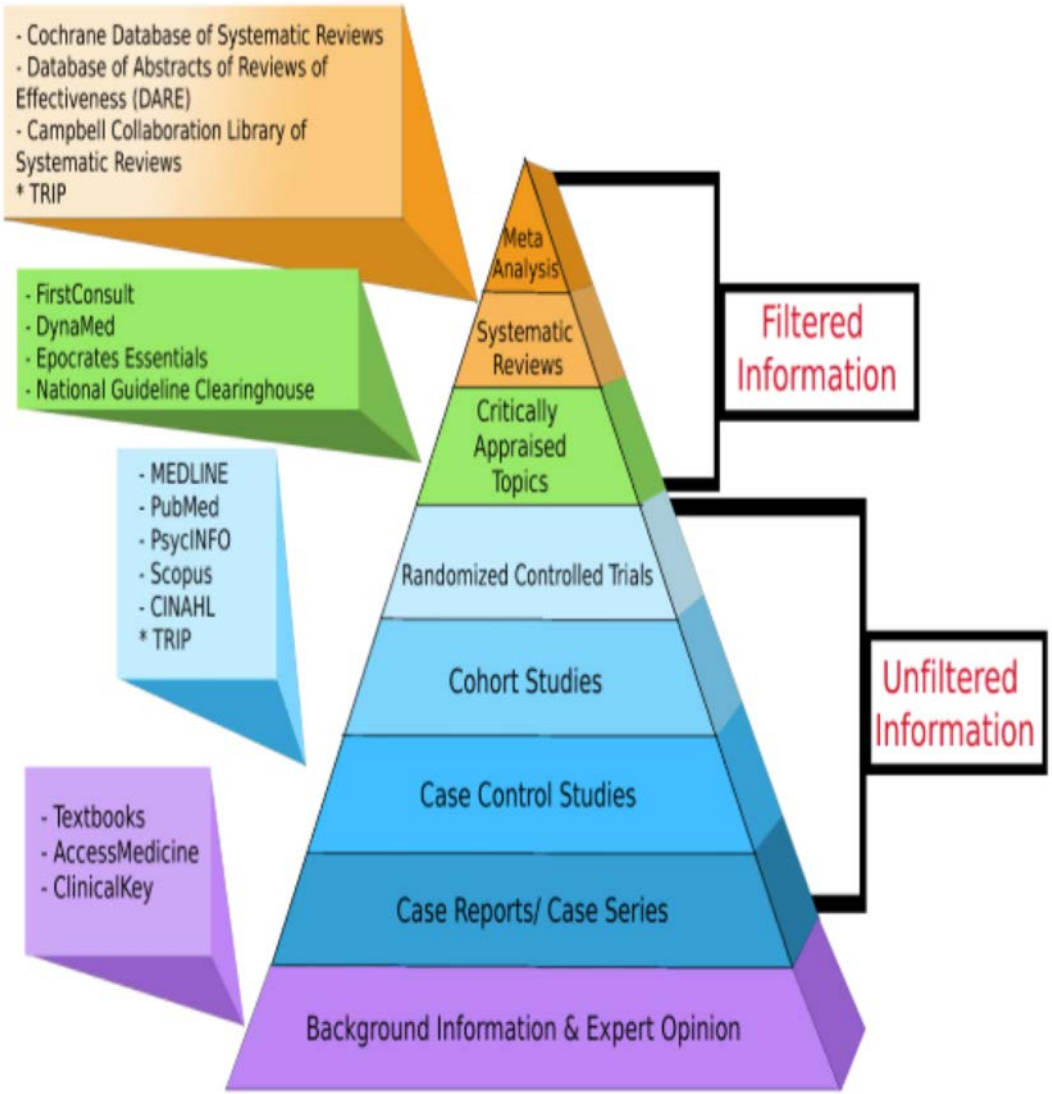
Right about now, you need to know more

- Is question important?
- Has it been studied before?
- Can you answer this now?
- How strong is premise?

What is the knowledge gap?

Insight into new methodology?

Do a good literature review!



http://guides.himmelfarb.gwu.edu/c.php?g=27724&p=4447440

Construct a Hypothesis

Scientific Method:

1. Ask a Question
2. Do Background Research
3. **Construct a Hypothesis**
4. Test Your Hypothesis by Doing an Experiment
5. Analyze Your Data and Draw a Conclusion
6. Communicate Your Results

- **The question comes first.** Clearly identify the question you are interested in studying.

- **A hypothesis is a statement.** The hypothesis is an educated, testable prediction about what will happen. It might be right or wrong, but it can be tested.

Make sure your hypothesis is "testable”

To prove or disprove your hypothesis
you need to be able to do an experiment
and take measurements or make observations
to see how your variables are related.

To create a "testable" hypothesis make sure you:

- Think about what experiments are needed to do the test.
- Identify the variables in the project.
- Include the independent and dependent variables in the hypothesis statement.

State how you will test hypothesis

In general--in Aims

In particular--in Approach

Describe sample sizes, blinding, statistics, controls, replication

Cite papers, but do not expect reviewer to read

Anticipated outcomes and alternative approaches will get spelled out in Approach.

Aims give general examples of your experimental approach…

Specific Aims *Page*-Engage the reader!

Statement of problem or question to investigate
Why this is innovative
Highlight Approach
Why your research needs to be done

Provide 2 or 3 aims, related but not dependent
Don’t forget active verbs, high-order study

Specific Aims Page-Tell a compelling story

- Write this section of your application first
- Get lots of feedback / chalk talk
- Revise it again and again

Include everything important and exciting

- without a lot of detail
- should be simple and easy to read
- Limit jargon, non-experts should understand

Each aim

concise sentence/phrase
should stand alone
not depend on outcome of the other aim

(Different from “Abstract” or “Summary” usually written last)

Use active, measurable verbs

Knowledge Level: List, define, describe
Comprehension Level: compare, identify, explain

Analysis Level: The successful student will examine the learned information critically.

analyze	inspect	test
distinguish	categorize	critique
differentiate	catalogue	diagnose
appraise	quantify	extrapolate
calculate	measure	theorize
experiment	relate	debate

Synthesis Level: The successful student will create new models using the learned infor

develop	revise	compose
plan	formulate	collect
build	propose	construct
create	establish	prepare
design	integrate	devise
organize	modify	manage

Words to avoid in applications

If…whether (or not)….

opportunity for a negative outcome and dead end
sows doubt in the mind of the reader
[*Try: These data provide new opportunity for…]*

"objective in this proposal is to better understand…"

when do you finish "better understanding?"
[*Try: Define the mechanism, elucidate the primary contributing factors…]*

to the best of our knowledge, no earlier studies…

Raises the question that you don't know.
[*Try: Few if any detailed studies into the underlying causes have been published.]*

Where does this fit in Specific Aims ***Page***

Sample structure:

- *First paragraph*: Capture attention-hook, what's known, the gap you will address, why it's important
- *Second Paragraph*: Your solution to fill the gap
- Hypothesis-logical, testable
- *Each Aim*: One sentence or phrase followed by general approach
- *Summary*: What new things we will know, why the application should be supported now
- Consider: Models/Charts/Diagrams

Example of the First paragraph

Capture readers' attention, hook, the gap you will address:

Below is an example of an introductory paragraph:

Viruses are thought to be involved in 15% to 20% of human cancers worldwide, thus providing critical tools to reveal common mechanisms involved in human malignancies. As the etiologic agent of adult T cell leukemia/lymphoma (ATLL), human T cell leukemia virus type I (HTLV-1) is just such a virus. HTLV-1 encodes a potent oncoprotein, Tax, which regulates important cellular pathways including gene expression, proliferation, apoptosis, and polarity. Over the years, Tax has proven to be a valuable model system in which to interrogate cellular processes, revealing pathways and mechanisms that play important roles in cellular transformation. Although the Tax oncoprotein has been shown to transform cells in culture and to induce tumors in a variety of transgenic mouse models, the *mechanism by which Tax transforms cells is not well understood.* A large number of Tax mutants have been generated and their biological activities have been thoroughly characterized, primarily in cell culture systems. *Currently, a major obstacle in the field* is that the transforming activity of Tax mutants cannot be compared using available transgenic models due to random transgene integration sites, variable transgene copy number, and inconsistent transgene expression levels, making it difficult to link the biological activities of Tax mutants with their transforming potential.

Color Key: Hook | Known Information | Gap in Knowledge | Critical Need

Figure 1. The Introductory Paragraph. Sections of the paragraph have been color coded to highlight each critical component.

The Second Paragraph

In this paragraph, your goal should be to introduce the solution that fills the gap in knowledge. It is critical to convince your reviewers

Biosciencewriters.com here

Example of the 2nd paragraph

introduce your solution

- Hypothesis
- Approach
- How your work will advance the field
- Keep your wording simple, relevant, and to the point.

(Seu) AIM 1 To determine the origin and functional significance of lung resident memory B cells (BRMs). Since we observe an early seeding of lung BRMs we hypothesize that the BRMs in the lung should maintain their mutation pattern and proportion early and late during the response, due to early seeding and establishment. Further, the GC derived CD73+swig+ subset should be the most protective, as they have gone through rigorous selection and differentiation through (SHM and AM) in the GC. We will test these hypotheses by analyzing the repertoire of the B cell population in the lung, which should remain constant if our hypothesis is correct. To identify the lung BRM with the greatest inclination to form ASCs, we will image live BRMs post challenge from Blimp-YFP reporter mice (YFP+ will be ASCs) to determine ASC formation. (Model1)

Parkinson disease (PD) is a neurodegenerative disorder characterized by a progressive loss of dopamine producing neurons in the substantia nigra pars compacta resulting in tremor, rigidity, bradykinesia and postural instability in 3-5% of people above age 65. Although dopamine replacement based therapies are quite effective at alleviating symptoms in PD, **they fail to halt neuronal loss** (Obeso, 2010). Alpha-synuclein aggregation is found in Lewy bodies in injured dopaminergic neurons in PD (Baba, 1998), and alpha-synuclein gene duplications and triplications cause PD in a dose-dependent manner (Ross, 2008). These observations demonstrate this protein's importance in PD pathogenesis **although the mechanisms by which it produces toxicity remain unclear**. Recently, research has focused on the possibility that immune activation may be important for PD neurodegeneration: reactive microgliosis has been observed by PET imaging in vivo (Gerhard, 2006) and in PD brains post mortem (McGeer, 1988, Imamura, 2003). A polymorphism in the HLA region has been found to be associated with late-onset PD (Hamza, 2010!.

Our lab has pursued the idea that alpha-synuclein (a-syn) itself may be the trigger for immune activation in PD. We have shown that targeted overexpression of a-syn in the substantia nigra (SN) of mice driven by an adeno-associated virus vector recapitulates the reactive microgliosis observed in human PD (Theodore, 2008) and leads to a 30% reduction in the total number of dopaminergic neurons six months postinjection (St. Martin, 2007). Furthermore, knocking out microglial Fc-gamma-receptors reduces this a-syn induced neuronal degeneration, suggesting that interactions between innate and adaptive immunity are important (Cao, 2010).

The complement system is a critical part of the innate immune system, and is involved in not only the immune response to infection of the CNS, but also the immune response to many native CNS pathologies, including neurodegenerative diseases (Alexander, 2008). In PD, Lewy bodies are positive for C3d, C4d, C7 and C9 (Yamada, 1992), and C1q and C9 mRNA expression are increased in the SN of PD patients (McGeer, 2004). **The overall hypothesis of this study is that activation of complement is required for mediating the dopaminergic neurotoxicity of alpha-synuclein in vivo.**

Completion of the following experiments will determine whether the complement system contributes to neuronal loss in an alpha-synuclein mouse model of Parkinson disease. This study will determine whether the complement system is a potential target for future immune system-based

What do you notice?

Examples of aim statements

- Give your aim an active title phrase that clearly states the objective in relationship to the hypothesis.
- describe briefly each of the aims you will use to test your hypothesis.
- Include a brief summary of the experimental approach and anticipated outcomes for each aim.

Aim 1 will establish an innovative mouse model for HTLV-1 Tax tumorigenesis. *Targeting vectors containing silenced wild-type or mutant Tax genes will be knocked in to the Rosa26 locus of C57BL/6 mice. These mice will then be crossed with homozygous Lck-CRE mice, thereby excising the stop cassette and generating mice that express wild-type or mutant Tax proteins specifically in T cells.*

Goals of specific aims, I

- State the general purpose or objectives of your research
- Give your rationale for choosing the project
- Show how your aims build on your preliminary studies
- State your hypothesis
- Describe briefly each of the aims to test your hypothesis.
- Ideally, the aims should be related, *but not dependent*, upon each other.
- Be sure all objectives relate directly to the hypothesis you are setting out to test.
- Explain why you chose the approach you selected.

Watch Out!

A common Specific Aim might ask “Does A cause B?”

However, your project could come to an end if A doesn’t turn out to cause B.

It’s better to design an aim where the result doesn’t depend on only one outcome, but where one or more different outcomes would also be of interest.

The question becomes “Does A cause B or non-B,” so make sure the “non-B” outcomes make sense

Goals of specific aims II

- State the technologies you plan to use.
- Note expertise to do a specific task (or collaborators).
- Describe past accomplishments related to the project.
- Describe preliminary studies and new, relevant findings
- Explain the area's biology.
- Show how the aims relate to one another.
- Describe expected outcomes for each aim.
- Explain how you plan to interpret data from the aim.
- Describe how to address any pitfalls with contingency plans.

Aim 1: Define the role of TLRs and IL-1R in *S. aureus*-mediated perturbation of osteoclastogenesis.

Based on preliminary studies that suggest a MyD88-mediated mechanism of OC perturbation by bacterial components *in vitro,* I hypothesize that *S. aureus* modulates pre-OC cell biology through TLR recognition or IL-1R signaling upstream of MyD88. To test this hypothesis, we will perform osteoclastogenesis assays on bone marrow (BM) cultures from wild-type and immune-deficient mouse strains, including TLR2, TLR9, and IL-1R-deficient mice, with and without RANKL stimulation, components of *S. aureus*, TLR agonists, or recombinant IL-1 to (i) identify changes in expression of TLRs and factors known to modulate osteoclastogenesis, (ii) define the activation status of intracellular signaling cascades and transcription factors, and (iii) investigate the functionality of OCs induced by bacterial components with bone resorption assays. Taken together, these data will detail how bacterial stimulation modulates OC differentiation and function through TLR and IL-1 signaling.

~~Aim 2: Elucidate the role of skeletal cell-specific~~ MyD88 signaling on pathogen clearance and bone remodeling during *S. aureus* osteomyelitis.

Aim 1 will identify *in vitro* changes caused by *S. aureus* during osteoclast differentiation, including alterations in OC signaling and function. Our *in vitro* assays demonstrate that MyD88 in skeletal cell precursors could be responsible for downstream changes following *S. aureus* stimulation. Interestingly, preliminary data obtained in our *S. aureus* osteomyelitis model shows that MyD88 is also necessary to limit bacterial replication and dissemination to other organs. Based on these data, I hypothesize that innate sensing of *S. aureus* by skeletal cells *in vivo* impacts bacterial clearance and alters bone remodeling during osteomyelitis. To test this hypothesis we will induce osteomyelitis in wild-type mice and mice with skeletal cell-specific MyD88 deletion to

What do you think?

Elevated mTOR signaling causes abnormal social behavior in several genetic mouse models of autism, and mTOR signaling may also be involved in conditioned fear memory. We investigated the mTOR pathway as a potential mechanism for the phenotype of progranulin-deficient mice based on reports of altered S6 kinase expression and phosphorylation in progranulin-deficient model systems, and of interaction of progranulin with insulin/IGF-1 signaling. We found increased phosphorylation of Akt and ribosomal protein S6 in the amygdala of Grn+/– mice relative to wild-type, which suggests increased signaling through the mTOR pathway. We therefore **hypothesize that progranulin deficiency causes abnormal social behavior, conditioned fear, and amygdala dysfunction through elevated mTOR signaling.** We propose to investigate this hypothesis with the following specific aims.

Specific Aim 1 **will test the hypothesis that subjects with schizophrenia have increased dopaminergic inputs in the nucleus accumbens core and shell compared to normal** control cases by measuring the density of immunolabeling of tyrosine hydroxylase, a synthesizing enzyme of dopamine, using (SA1.1) optical densitometry, and (SA1.2) measuring protein levels using western blot assays.

Specific Aim 2 **will test the hypothesis that subjects with schizophrenia have increased synapses, both symmetric (inhibitory) and asymmetric (excitatory), in the nucleus accumbens core and shell** using stereology at the electron microscopic level to determine density of synapse types.

What do you think?

Final paragraph on Specific Aims Page

Expected Outcomes: Specifically state your expected outcomes for this project. Use plain language. What do you expect to see at the completion of each aim (if not in aims).

Innovation: What would completion of this proposal bring to the field that is not present currently?

Impact: State how your project would help those who need it, (i.e. the development of a new treatment, vaccine, disease model or diagnostic tool) Include a broad impact statement about how your proposal will benefit the people or other subjects that you mentioned in the opening paragraph.

Self-Check—Specific Aims (NIAID)

1. I keep to the one-page limit.
2. Each of 2-3 aims is a focused, concrete objective I can achieve
3. My aims highlight the significance of the research
    1. how generate knowledge that may improve human health
    2. how it will move my field forward.
4. My text states how my work is innovative.
5. I describe the biology to the extent needed for readers.
6. I give a rationale for choosing the topic and approach.
7. I tie the project to preliminary data.
8. I explicitly state my hypothesis.
9. My aims can test my hypothesis and are logical.
10. I can design and lead 2-3 experiments to accomplish each aim.
11. I use language that an educated person can understand.
12. has bullets, bolding, or headers so reviewers can spot my aims
13. The closing paragraph emphasizes the significance of the work

Next Time: SPECIFIC AIMS page draft rubric

-Peer reader: What might improve SA page?
-Discuss with research advisor?

Category	Excellent/ Acceptable 1 pt each	Adequate (Needs minor revisions) 0.75	Not Adequate (Needs major revisions) 0.5	Poor (Not acceptable) 0	Score
Intro and Background: **Should critically evaluate existing knowledge (knowns) and specifically identify the gaps that the project is intended to fill (unknowns). Importance of the research and the impact of the study on advancing scientific knowledge should be concisely stated.**	very clearly explains background and gaps in knowledge	clearly explains background and gaps in knowledge but does not relate the two	somewhat explains background and gaps in knowledge, though unclearly or without relating the two	intro section is unclear and does not relate background and gaps in knowledge	
Objectives and Rationale: **Should clearly state the study goals and clarify the thought process used to generate the central hypothesis**	objectives and rationale are clearly stated and easy to follow	objectives and rationale are stated but may not be clear given background	objectives and rationale are stated but it is not clear how hypothesis was generated	objectives or rationale are not stated and it is not clear how hypothesis was generated	
Primary Goals	clearly stated goals that reflect intro and objectives given	stated, but goals are not clear given intro and objectives	some stated, but goals do not reflect intro and objectives given or are not clear	goals are not stated	
Specific Aims: **Should be relevant to study the question, address the proposed hypothesis, and fill in the stated knowledge gaps in the field.**	all aims are clearly stated and relevant to background and objectives given	aims stated and relevant but are not clear given background and objectives	some aims stated but not clear or relevant to background and objectives given	specific aims are not stated or are not relevant to background and objectives given	
Hypotheses: **Should correspond with the stated specific aims and must be testable using proposed approach**	**all clearly stated, match study aims, and testable using proposed approach**	**stated, but all may not match study aims. all testable using proposed approach**	**some stated but may not be clear, do not match study aims, and are not testable using proposed approach**	**hypotheses not stated or not testable using using proposed approach**	