Reinforcement of Last Seminar's Major Points

Major Strategies for getting grants

- Be aggressive
- Choice of study section
- Knowing PO, SRO
- Get help http://www.jefferson.edu/university/research.html
- Be nice, get to know reviewers
  - Always be friendly
  - Promote your work
  - Schmooz (professionally)

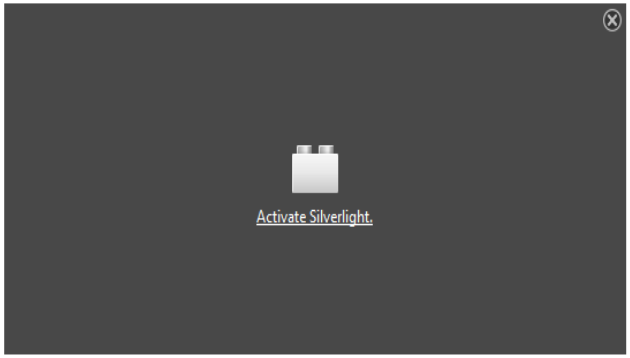
Major Strategies for getting grants

- Be nice, get to know reviewers
  - Get review experience
  - Get on YOUR study section
    - https://public.csr.nih.gov/ReviewerResources/BecomeAReviewer/ECR/Pages/default.aspx
  - Learn specific reviewer's biases

Early Career Reviewer (ECR) Program

Training and other Opportunities

Information about upcoming NIH Early Career Reviewer Opportunities



Thinking about becoming an Early Career Reviewer? Listen to the experiences of ECRs who have served on NIH Peer Review study sections

Early Career Reviewer (ECR) program

» Discover how the ECR program can help you Jumpstart Your Career

Benefits

» Find out how serving as an ECR can benefit your career

What are the qualifications for becoming an ECR?

» Learn about the program qualifications

ECR Resources

» Training Resources
» NIH Early Career Reviewer Training Webinar
» Research Funding
» Diversity Programs

ECR Program

» Apply NOW
» Update your Information
» Make a Referral

FAQ's

» For Applicants
» For Reviewers
» More ...

Major Strategies for getting grants

- Assemble a competitive team
  - Political clout
  - Diversity and depth of approaches
- Learn to interpret reviews (again, get help)
  - ND is not the kiss of death
- Use multiple funding sources
- Grant cycles are important
- Pay attention to ESI deadline
- Establish independence (ideally, real)

Major Strategies for writing grant
(most relate to showing reviewer love)

- Start early
- K.I.S.S. - Clarity rules
- Give reviewer statements to paste into his/her review
- Cartoons, Tables, Flow charts
- Give reviewer the option of speed reading (provide exoskeleton with nested layers of detail)

Specific Aims

- CLARITY, CLARITY, CLARITY (K.I.S.S)
- Convey *s*ignificance sufficiently
- State central hypothesis
- Point to why you can now address the question
  - Recently/published new data
  - New tool or collective expertise
- Cartoons/Design Schema can work here
- Limit detail (big picture more important)

Optimizing β-adrenoceptor signaling bias in asthma

Specific Aims

Agonists of the beta2-adrenoceptor (β2AR), commonly referred to as *β-agonists*, have been a cornerstone of asthma treatment for nearly half a century. Inhaled β-agonists are the drug of choice for rescue from life-threatening bronchoconstriction, and combination treatments including β-agonists are currently first line therapies for preventative (maintenance) asthma treatment.

However, despite their utility, β-agonists used in long-term asthma management have problems. Chronic use of β-agonists can result in loss of their bronchoprotective effect[1-7], loss of disease control (reviewed in[8-10]), and even mortality[11-14]. This has resulted in a black box warning for products containing long-acting β-agonists (LABAs). Although the severity of these problems has been debated, it is clear that β-agonists are far from perfect drugs. This sentiment has been articulated repeatedly in NIH Program announcements declaring the need for safer, more efficacious alternatives to asthma and COPD treatment.

Despite this increasing awareness of the limitations of β-agonists in asthma treatment, almost no progress has been made in the last 15 (arguably 50!) years to address this problem. Drug development has focused almost exclusively on extending the duration of action of inhaled LABAs, ostensibly ignoring the recent explosion of G protein-coupled receptor (GPCR) biology and pharmacology science that reveals new and exciting ways to exploit signaling capabilities of GPCRs.

Recent studies by our group have established that β2AR agonism plays a permissive role in the development of allergic lung inflammation and associated airway hyperresponsiveness (AHR). These studies suggest a paradigm shift in the role for β2AR agonism in asthma, akin to that which occurred 15 years ago in congestive heart failure (CHF) when treatment shifted from βAR agonism to βAR antagonism. However, our current understanding of β2AR biology and receptor pharmacology allows us to entertain a more refined solution to the asthma β2AR paradox. Specifically, the now established concept of *biased* signaling by GPCRs allows us to consider the possibility of selectively activating specific β2AR signaling events that are therapeutic while avoiding or even inhibiting those that are deleterious.

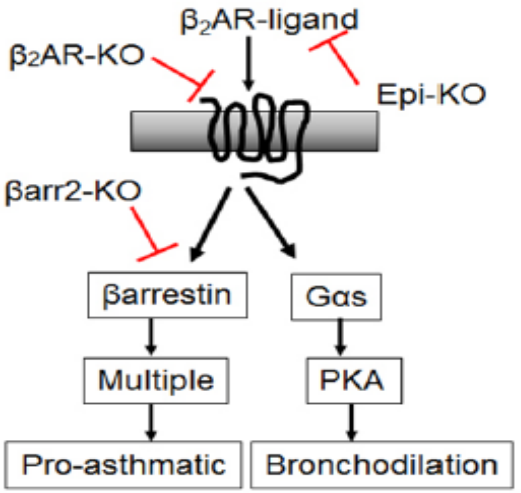


Figure 1. Biased signaling holds the key to the β2AR paradox in asthma. Our published work has shown that the asthma phenotype is significantly diminished in mice with genetic deletion of β2AR (β2AR-KO), βarrestin2 (βarr2-KO) or phenyl-N-methyl transferase, the enzyme required for epinephrine synthesis (Epi-KO). The β2AR has the capacity to activate both a G protein- and a βarrestin-dependent signaling pathway. Studies proposed herein seek to link these pathways with protective and pathogenic roles, respectively, in asthma. Consequently, biased β2AR ligands that avoid/antagonize only the βarrestin-dependent signaling pathway may be better asthma therapeutics.

Herein we propose studies to: 1) detail the mechanism by which β-agonists promote pathogenic allergic lung inflammation that limits their utility; and 2) identify therapeutic strategies, including specific orthosteric ligands and allosteric modulators of the β2AR that enable β2AR signaling that mediates bronchodilation, yet prevents signaling which is pro-inflammatory and pathogenic.

Accordingly, we propose the following Specific Aims.

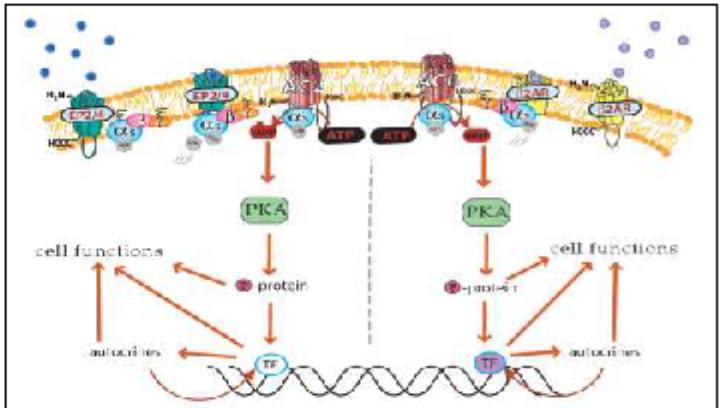
Aim 1. Utilizing genetic approaches, establish the requirement and sufficiency of β2AR agonism in airway epithelial (AE) cells in mediating allergic lung inflammation, mucin production, and AHR.

PKA-dependent regulation of airway epithelial function

G protein-coupled receptors (GPCRs) transduce extracellular signals into discrete intracellular signals that regulate cell, tissue, and organ system function. They are the targets of approximately half of all therapeutic drugs. The beta-2-adrenoceptor (β_2AR) is a GPCR expressed on airway smooth muscle (ASM), and is targeted by β-agonists in the treatment and prophylaxis of asthma. EP2/4 receptors, which are stimulated by prostaglandin E2 (PGE_2), belong to the same subclass of GPCRs and have recently emerged as a potential therapeutic target in asthma [1,2].

The cyclic AMP (cAMP) –dependent protein kinase, a.k.a protein kinase A or *PKA*, is a key effector of GPCRs coupled to the heterotrimeric protein Gs. In the lung, GPCRs (such as the β_2AR) that increase PKA activity have been shown to regulate the function of multiple cells, although conclusive demonstration of PKA's role in such regulation has been confounded by a lack of specific and effective tools for inhibiting PKA. Indeed, Zieba et al. [3] challenged the long held assumption that PKA mediated the relaxant effect of β-agonists, asserting instead that the cAMP effector Exchange Protein Activated by cAMP (Epac) is the key mediator of β-agonist-stimulated airway smooth muscle (ASM) relaxation.

To further confound our understanding of PKA function, we have discovered that not only the quantitative but also the *qualitative* nature of PKA activity appears dependent on the activating GPCR, and on the cellular compartmentalization of signals. In this Ruth L. Kirschstein NRSA Fellowship application, we will characterize the PKA-dependent regulatory effect of physiologically- and clinically- relevant GPCR agonists on airway epithelial functions that play a role in asthma pathobiology. These studies are aided by the recent development of cutting edge tools and approaches, and represent a unique opportunity for the fellow to apply his strong graduate training in cell biology to develop a fruitful program in airway cell biology relevant to obstructive lung diseases. Our central hypothesis is that *ligands of beta-2-adrenoceptors (β_2ARs) and (prostaglandin-activated) EP receptors differentially regulate epithelial cell functions via qualitatively distinct, compartmentalized PKA signaling.*



Concept Model. Compartmentalized signaling dictates quantitative/qualitative effects of β_2AR and EP receptors on airway epithelial cell functions.

Specifically, we propose to:

Aim 1. Characterize differential PKA-dependent *signaling* in airway epithelial cells by beta-agonists and EP receptor subtype agonists. We will employ newly developed molecular and genetic tools in murine and human airway epithelial cultures to demonstrate that differential regulation of PKA signaling occurs as a result of compartmentalization of signaling elements.

Aim 2. Demonstrate the dependence of beta-agonist and EP receptor agonist regulation of epithelial cells *functions* on compartmentalized signaling, and PKA. We will characterize the PKA-dependent regulation of gene regulation and cytokine production by different β-agonists, PGE_2, and EP subtype selective agonists in epithelial cells subject to the multiple inhibitory strategies targeting compartmentalized signaling components, and PKA.

Successful completion of the proposed studies will significantly advance the fields of receptor and asthma biology, and develop those skills critical to a future independent academic investigator.

Research Strategy: Significance, Innovation

- Arguably the most important section
- *Scientific* significance and gap in knowledge
- Basic science and clinical application (have both)

"..will advance both the field of receptor biology and the development of new drugs for asthma."

- Include Premise
- Note innovation in **concept** *AND* **Approach**

Premise

- Include as a titled subsection in Significance:

Premise. The *potential* of PGE_2 as an asthma therapeutic, at least with respect to its bronchorelaxant properties, has been recognized for years. The bronchodilator effects of PGE_2 have been demonstrated in a range of patients (normal, asthmatic, and chronic bronchitis) [1-6]. However, the effects of PGE_2 are complicated by the existance of multiple EP receptor subtypes, and cough remains a significant and insurmountable side effect[7-9]. However, our recent published studies and preliminary data presented herein suggest we are finally able to overcome this limitation by selective activation of specific EP receptor subtypes enable by recently developed EP ligands....

What NIH says re: “Premise”

- From: https://www.nih.gov/research-training/rigor-reproducibility/updated-application-instructions-enhance-rigor-reproducibility

“The scientific premise for an application is the research that is used to form the basis for the proposed research question; NIH has always strived to fund projects that are based on a strong foundation. Moving forward, NIH expects applicants to describe the general strengths and weaknesses of the prior research being cited by the investigator as crucial to support the application. It is expected that this consideration of general strengths and weaknesses could include attention to the rigor of the previous experimental designs, as well as the incorporation of relevant biological variables and authentication of key resources.”

Research Strategy: Approach

(explaining how you are going to test your hypotheses and interpret your results)

3 basic approaches:

1. Provide your preliminary data first in a “Preliminary Data” section prior to an “Approach/Methodology” section;
2. No Preliminary Data Section and integrate your preliminary data into your “Approach/Methodology” section;
3. Have a “Preliminary Data” section but be liberal with inclusion of data in “Approach/Methodology” section.

Preliminary Data section

1. Tell the story of how you got to question at hand; typically some combination of published (by you or others) and unpublished (presented here) data.
2. Can also serve as a “Background” section.
3. ONLY your solid, unequivocal “A” data.
4. Be strategic in how much to present: enough but not too much, statistically significant IF your proposed work is primarily extending these data.
5. Good idea to summarize your findings at end of section as a mean of justifying pursuit of you hypotheses.

Approach/Methodology section

1. Divided by Aims.
2. An initial paragraph discussing overall Design (with cartoon or Flow chart) prior to each Aim's approach can be helpful.
3. Carefully consider the depth of each Aim (subAims?) and the density of approach (relates to ambition, readability).

Approach/Methodology section

1. Divided by Aims.
2. An initial paragraph discussing overall Design (with cartoon or Flow chart) prior to each Aim's approach can be helpful.
3. Carefully consider the depth of each Aim (subAims?) and the density of approach (relates to ambition, readability).

Each Aim

Suggested organization:

State Aim exactly as in Specific Aims Page. Then…

1. Hypothesis (eses)
2. Rationale (optional)
3. Design/Methodology/Approach
4. Expected Outcomes, Data Analysis and Interpretation (If not included in (3))
5. Experimental Considerations and Alternative Approaches

1. Hypothesis (eses):

Just state it. One or multiple. If an alternative hypothesis is possible but still attractive, state it as well.

2. Rationale:

Why you think the hypothesis is true; point to your preliminary data and if valid, some logic.

Keep this section to a few lines. Unless you feel the hypothesis is controversial or the reviewer will need convincing.

3. Design/Methodology/Approach

A. Detail the experiments you will perform to test the hypotheses. Typically this involves many types of experiments. Consider separating the types of experiments into their own section starting by italicizing or bolding the name of the experiment or specific experimental approach.

B. Establish the feasibility of the specific approach (e.g., siRNA-mediated knockdown) by either referencing studies or showing feasibility data.

C. Calculate whether referencing an approach will suffice.

D. Consider if you can group “Experimental Detail” in a section.

E. CONTROLS, CONTROLS, CONTROLS.

4. Expected Outcomes, Data Analysis and Interpretation

Detail: What data are presented, how crunched, what you expect, and what each outcome means. Provide all possible interpretations. If experiments do not support hypothesis, provide alternative interpretations and note how another experiment proposed will further clarify, or note here or on the “Alternative Approaches” section below what other experiments you will pursue.

If complicated, a table listing experiment, type of data, and interpretation can help.

4. Experimental Considerations and Alternative Approaches

Experimental Considerations = Potential Problems.

Note what might go wrong but whenever possible note how your data support your hypotheses and your experience or feasibility data argue feasibility.

.

4. Alternative Approaches

A. Extremely Critical to grant.

B. Here is opportunity to assert ROBUSTNESS

C. Ideally, you've proposed multiple approaches in order to beat the question to death. But if at all possible…

List even more approaches here that might not be the "A" approaches but are reasonable and others have tried. Include, for example:

a. Other sequence within transcript to target with siRNA

b. Alternative inhibitory strategies (e.g., other small molecule inhibitors of different structure of mechanism of action).

c. an additional transcriptome or proteome approach (IHC, ICC, or biochemical/pulldown/fractionation).

4. Alternative Approaches

C. Note Approaches here that would test alternative hypotheses, or if results suggest another direction, how you would pursue them.

D. You might care to preface (C) with: “Although our preliminary data support our proposed hypothesis, the possibility of (alternative outcome) could be examined by (alternative approach

E. For either additional/redundant approaches, or a new approach to test an alternative hypothesis, supplying a feasibility figure often not a bad idea.

Other ~~Technology and~~ Stuff

Timelines- too many reviewers expect one. Consider whether text describing when things will get accomplished is sufficient.

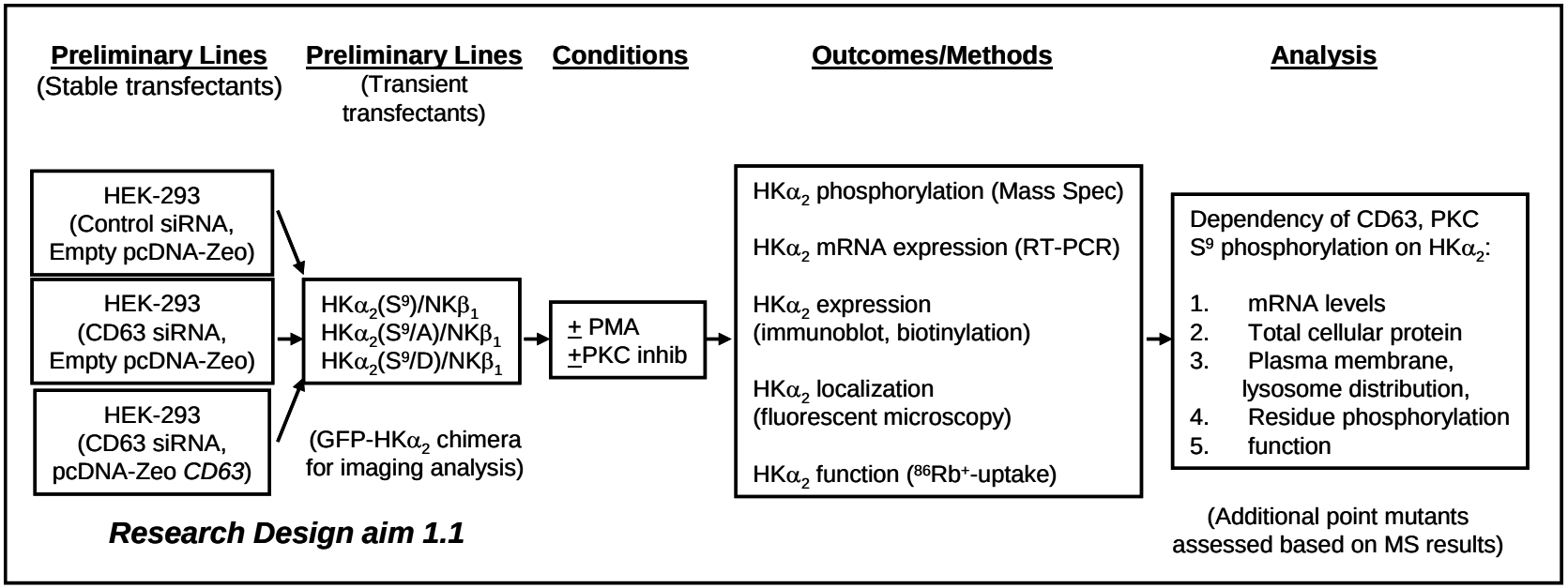
Organization of Approach- making life easy for Reviewer:

a. Within each Aim (or collectively) consider frontloading important information thus allowing the reviewer to either skip or skip detail then move on to next Aim or end of grant.

b. Start with general approach (describing early in Aim) and then get progressively detailed.

c. Consider cartoons/Design Schema to give snapshot of approach, data generated, and expected outcomes.

d. Consider all Stats at end of Methodology; offload Power Analyses to Vertebrate Animals or Human Subjects.


Preliminary Lines
(Stable transfectants)
Preliminary Lines
(Transient transfectants)
Conditions
Outcomes/Methods
Analysis
HEK-293 (Control siRNA, Empty pcDNA-Zeo)
HEK-293 (CD63 siRNA, Empty pcDNA-Zeo)
HEK-293 (CD63 siRNA, pcDNA-Zeo CD63)
HKα2(S9)/NKβ1
HKα2(S9/A)/NKβ1
HKα2(S9/D)/NKβ1
(GFP-HKα2 chimera for imaging analysis)
± PMA
±PKC inhib
HKα2 phosphorylation (Mass Spec)
HKα2 mRNA expression (RT-PCR)
HKα2 expression (immunoblot, biotinylation)
HKα2 localization (fluorescent microscopy)
HKα2 function (86Rb+-uptake)
Dependency of CD63, PKC S9 phosphorylation on HKα2:
1. mRNA levels
2. Total cellular protein
3. Plasma membrane, lysosome distribution,
4. Residue phosphorylation
5. function
(Additional point mutants assessed based on MS results)
Research Design aim 1.1

Other Stuff:

Lastly, if you expect the reviewers are going to raise certain questions that were not addressed in the application, consider a section:

"**Additional Project Experimental Considerations, Issues**"

Additional Project Experimental Considerations, Issues. The challenges posed in the study of a receptor activated by reduced pH/protons are admittedly daunting: limited pharmacological tools, a need to impose numerous controls to exclude nonspecific actions of ↓pHo or indirect activation of the receptors; and the inherent difficulty of working with typically low abundance proteins (most GPCRs) in a primary cell type. Our overall approach considers multiple strategies and techniques, several cutting-edge, in an attempt to overcome these difficulties, and we have considerable experience with all of them. Importantly, we have made considerable progress in characterizing OGR1 and refining our approaches to enable focused and the controlled reductionist and integrative studies proposed. Importantly, we have obtained key reagents, in the form of transgenic/knockout mice, and receptor ligands, that make these studies possible. Additional questions that may remain pertaining to the rationale and logic of the proposed studies are posed below.

How will you control for variability among ASM cells due to donor variability? Getting a handle on the biological variability in the role of OGR1 is one of our goals here, and we design our studies accordingly: we examine numerous ASM cultures derived from multiple donors, and Core B can obtain ASM tissue for cultures at a frequency exceeding the needs of this project. Similarly, Dr. Canning's access to human airways at JHU exceeds our anticipated needs for *ex vivo* contractile studies. In previous studies we observed variability in various receptor-dependent signals that were either dissociated from[23;64;65] or correlated with[64-66] a specific ASM function. This question is further discussed in *Introduction* and in the PPGs' *Overview*.

Why use multiple species? Although the focus is on human ASM, the use of both guinea pig and mouse is required in order to: 1) clarify the true role of OGR1 (therefore OGR1-/-); and 2) provide insight into OGR1 function and the utility of OGR1-targeting drugs *in vivo*. The complexity of pH effects on the airway represents a significant challenge; the guinea pig model is established, it is the most logical and feasible approach available that will enable us to sort out the differential effects of decreased extracellular pH on reflex versus ASM OGR1 on ASM contraction. **No other system affords this level of control and interpretation.** Studies of pH-dependent airway responsiveness and OGR1 function in human subjects cannot occur without first performing these types of studies using these specific models. Although we recognize that potential species-specific difference may complicate interpretation, we would prefer to take advantage of these models, and deal with the challenge of data interpretation.

Where will completion of these studies place us and how will we then proceed? Should our hypotheses be proven correct, we will have established that OGR1 is an important GPCR in ASM capable of regulating ASM contraction via either its sufficiency to do so, or by its ability to influence contraction promoted by other pro-

Introduction (Response to Reviewers)

Main Points:

- Most critical part of a resubmission.
- You have only 1 page so strategy is critical.
- If you are not responsive and respectful to the reviewers YOU WILL GET HAMMERED.
- EVERY reviewer should be addressed, unless they had NO concerns.
- When you get your Summary Statement back, sit down with a highlighter and highlight concerns, jot notes in margin re: what you think response will be.

STRATEGY for Introduction:

- Initial thankful paragraph, noting how great the comments have helped you generate what you feel is a much improved application. Note how/if you tracked changes.
- In this initial paragraph also note how below details how and where you changed the application.
- Group response to concerns by:

  (Reviewer: concerns); or

  (Concerns; (list reviewer(s) who raised it))
- Ok to paraphrase/edit concern if you don't quote it directly
- Only rebut if no option and watch your tone.
- Reference studies if possible.
- Continue to stroke reviewer throughout page; "this is an important issue…thank you for bring this to our attention…We agree…", etc.
- If no room to expound on concern note you address it (where in grant). OK to speak to reviewer and mentions concerns raised in the 12 pages: "To address the concern of xxxx, we have…"