Writing a Grant: Focus on Mentored Awards

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Personal Disclosures

- **Funded by two institutes at NIH, AHRQ, PCORI and six foundations**
  - **Less knowledgeable about other institutes and funders**
- **Most of this talk is my opinion based on my experience**

Outline

- Types of grants
  - Mentored awards
  - Independent funding (“R” awards)
- Timelines and deadlines
- Key parts of mentored awards
  - Mentor and environment
  - Career development plan
  - Specific aims and science

Mentored Awards

- Fellowship grants
  - Individual NRSA (F32)
  - Specialty society fellowship awards
  - Foundation awards
- Most common K-level awards
  - K08 (“basic” science)
  - K23 (patient-oriented)
  - K12 or KL2 awards (institutional)
  - Other career development awards

Independent Awards: “R” Awards

- Don’t worry about these yet – years away
- “Independence” is an archaic term
  - Still important for promotion
- Independent awards
  - R01, VA Merit Award, PCORI, others
- Multiple project grants
  - Program Project Grants, “U” grants
- Increasing recognition of “team science”
  - Multiple PI mechanisms

Which awards should you apply for and when?

- **Individualized: work with your mentor and your mentoring committee**
- **Apply for multiple awards**
  - **Overlap in science is fine if you can't accept more than one**
  - **Overlap in science is NOT fine if you can accept more than one**

NIH: Which Institute and which study section?

- Picking the "right" institute is key
  - NIH has 27 institutes and centers
  - AHRQ is a separate institution
  - PCORI is a new funding entity
- Picking the "best" study section is also important (often more important)
  - Expertise in your science and clinical area
- Use your mentors and advisors to make these decisions

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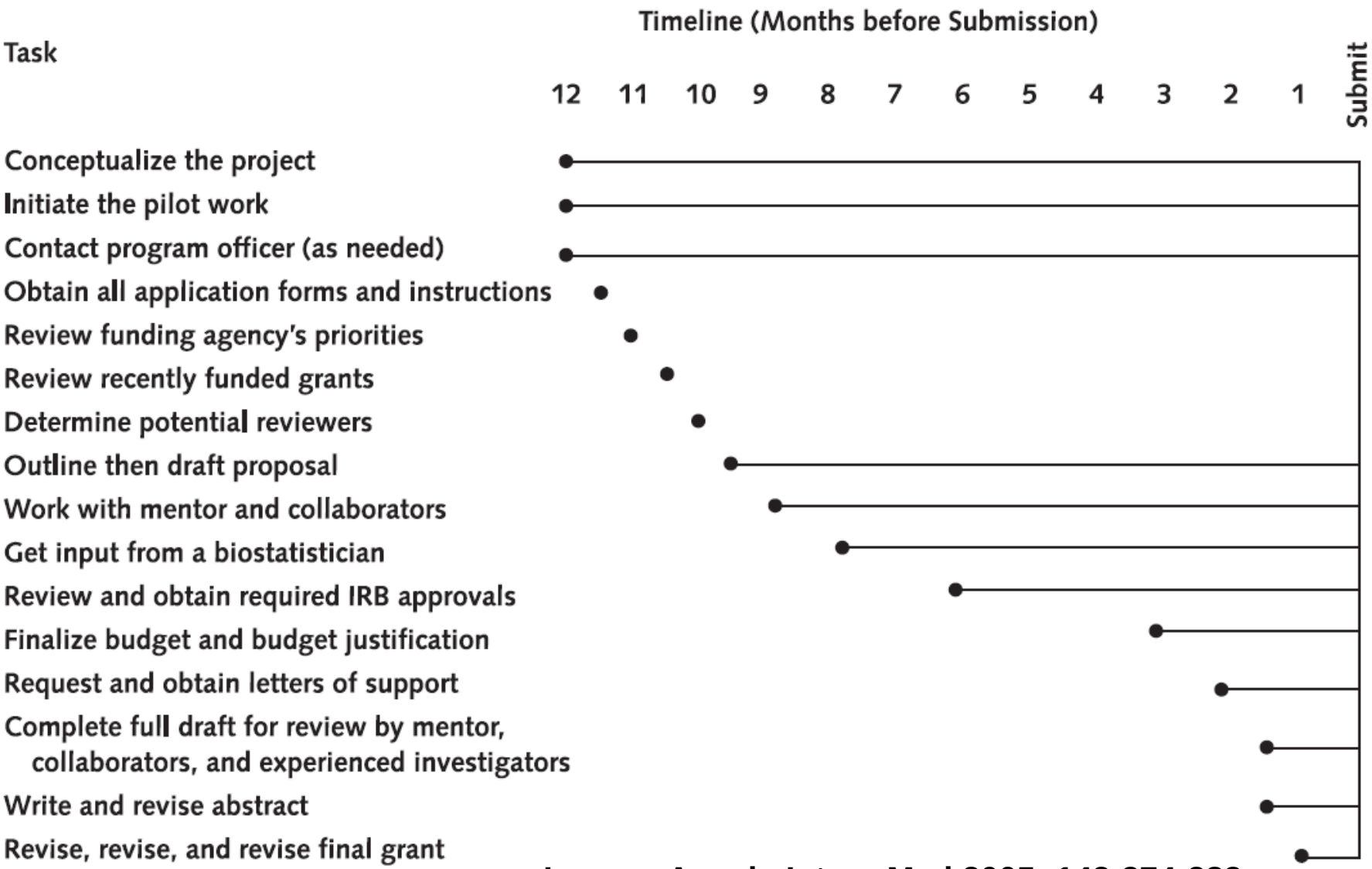
Getting Started: Three Most Important Steps

1. Read the instructions
2. Read the instructions
3. Read the instructions

Timeline for Writing a Grant



Inouye, Annals Intern Med 2005; 142:274-282

Grant Deadlines

- Grant deadline to NIH is always at least a week later than the REAL deadline for you!
  - UW internal review process
  - Mentor/advisor review process
    - The more it is reviewed the better it will be!!
- To get thorough advisor reviews, you need to give reviewers 2 weeks
- Deadline for GOOD draft is a least 1 month before the NIH deadline

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Mentors

- Picking a mentor may be the most important decision you make
- Mentor/co-mentor can work well
- Mentors for NIH mentored grants
  - Should be currently funded by NIH
  - Should have a track record for mentoring to K- and R-level awards
- Accessible and fun to work with

Environment

- Mentor's lab/program important, but only one part
- Use other resources at UW
  - Transcend Department/School boundaries
  - Get creative – diversity is a strength
- Tap into the ITHS
- Distant mentor can be ok, but be careful
  - Clear role and mechanism of involvement
  - Clear connection with distant mentor

Career Development Plan

- Identify your learning objectives
- Make them specific and make them fit your specific aims
- Provide concrete tasks you will do to achieve these objectives
  - Courses, workshops, practicums
- Use tables and figures

Specific Aims: The Most Important Part

- The one thing everyone will read
- The essence of your grant proposal
- Write them first and revise them often
  - Like a good poem
- There is no “one right way”
  - Depends on your project
  - Depends on the investigator

Writing a Good Specific Aims

1. Interesting
2. Cohesive: aims must fit together
3. Novel
4. Feasible
5. Specific: All terms operationally defined
6. Identify a testable hypothesis
7. Publishable regardless of result
8. Builds on investigator's experience

Interesting, Novel, and Feasible

- Ultimate challenge is finding aims that satisfy these three criteria
- Easy to write aims that are either
  - Interesting and novel but not feasible or
  - Feasible but not interesting or novel
- Create new knowledge
- Improve health or health care

Identify a Testable Hypothesis

- Aims should include hypotheses
  - Qualitative research is the exception
- Hypothesis should be important whether it is supported or refuted
- Even qualitative research needs to articulate the importance of the aim
- Critically ask yourself “Who cares?”
  - Where is this going?

Operational Definitions

- Every term clear and defined
- “Critical illness”
  - Defined by what?
- “Improve outcomes”
  - What outcomes
  - How will you measure the outcomes

Specific and Concise

- Your aims should “tell it all”
  - What are you going to do
  - How your are going to do it
  - Methods and analysis
- Concise
  - Not too long and no run-on sentences
  - Beautifully written - poetry
  - Most important thing you will write

Fatal Flaws

- Too long or too hard to follow
  - Assume reviewers are tired, cranky, and not experts in your science
- Too much overlap between aims
  - Not really separate aims
- No connection between aims
  - Not really one project
- “Interdependence of aims”

“Interdependence of Aims”

- Important fatal flaw in the eyes of some reviewers
- No aim should depend on the success of a prior aim
  - If aim 2 depends on the success of aim 1 in order to be done, these aims need to be two separate grants
  - If unavoidable, have an explicit “plan B”

One Approach to Putting Together the Aims Page

- Start with "Statement of the Problem"
  - Why is this problem REALLY important?
- List the specific aims
- Finish with long-term objectives
  - For mentored award include your training goals
- When finished first page, reader knows
  - What the problem is that you are addressing
  - What you are going to do to address it
  - What the long-term goals of this research are
  - Why she or he should care

Good Preliminary Data Strengthens Any Grant

- Important for getting funding
  - Aims should build on prior research and preliminary data
- Can build on your mentor's experience and preliminary data
- Choose your early fellowship projects in part based on how they can generate preliminary data

Make it Pretty: Which Would You Rather Read?

Aim 3. Define the Role of MMP10 in Governing Macrophage Transcriptional Responses.

Rationale and Approach. We predict that the phenotypes (i.e., excess inflammation and reduced alveolar damage) seen in smoke-exposed *Mmp10*$^{-/-}$ mice are due to the lack of this proteinase in macrophages. We hypothesize that MMP10 functions to control the activation state of macrophages. In support of this idea, we found that M1 markers are generally upregulated in *Mmp10*$^{-/-}$ macrophages, whereas M2 markers–particularly *in vivo*–are downregulated. With respect to mechanism, we predict that MMP10 acts on an endogenous macrophage protein that, in turn, sets off specific signaling events in these cells controlling their activation status. In support of this hypothesis, we have found that MMP10 selectively affects the expression of immune and remodeling pathways in cigarette smoke-exposed lungs (Table 3). To test this idea and to focus specifically on gene expression in macrophages, we plan to undertake a systematic, computationally intensive search for MMP10-dependent pathways in specific subsets of macrophages. From these studies, we will build predictive maps to identify potential regulatory gene-product hubs, and the functional role of these proteins.

Preliminary Data. We performed gene expression studies on total RNA isolated from flushed lungs of smoke-exposed (6 mo) and air-breathing wildtype and *Mmp10*$^{-/-}$ mice (*n* = 4/genotype/condition). RNA integrity was confirmed with Agilent Bioanalyzer 2100, and 500 ng/sample was amplified, labeled, and hybridized to MouseRef-8 BeadChip whole-genome expression arrays (Illumina). Image analysis, background subtraction, and normalization (quantile method) were performed using BeadStudio software (Illumina).

Differential Expression of Immune Genes is Seen Only in Wildtype Mice. Using whole lung RNA, we identified 92 genes that were differentially expressed in wildtype mice (smoke-exposed vs. control, FDR <0.05) but not in *Mmp10*$^{-/-}$ animals (smoke-exposed vs. control, FDR <0.05). GO analysis (Table 3) of these 92 genes showed enrichment of several immune-mediated pathways in the lungs of smoke-exposed wildtype mice–pathways that were not affected in null mice. These findings indicate MMP10 plays an important role.

Genetic Network Map of Activated Pathways. Using published gene product relationships among the differentially expressed genes in smoke-exposed wildtype mice, we created a genetic interaction network (Fig. 10). It is important to note that while the existence of this relational network in our smoking model is theoretical, each depicted gene product interaction has been experimentally confirmed. Furthermore, we and others have demonstrated that the functional stability of such networks is critically dependent on highly connected nodes or "hubs".[139,148] An example of one such hub that is upregulated during smoking is IL1β. Over-expression of IL1β has been demonstrated to cause inflammation and emphysema in adult mice[149] and pulmonary dysplasia and impaired alveolar septation in infant mice.[150] Another upregulated node in the interactome is CD14 (LPS receptor), a key component of TLR4 signaling, and a pathway that also mediates inflammatory responses to cigarette smoke exposure.[151] We confirmed the differential expression of IL1β, CD14, and a few other network nodes by qRT-PCR. We determined differential gene expression (WT vs. *Mmp10*$^{-/-}$; control vs. smoke-exposed) using a Bayesian implementation of the *t*-test[143] followed by false discovery rate analysis (FDR cutoff ≤0.05).[144] Functional enrichment of differentially expressed genes was based on Gene Ontology (GO) annotation[145] and using Database for Annotation, Visualization, and Integrated Discovery (DAVID) software with correction for multiple hypothesis testing.[146] A gene product interaction network was constructed based on Ingenuity System's knowledge base[147] The interaction network, or "interactome", was built around genes with the highest connectivity (seeds) using an iterative algorithm that systematically connects additional nodes to the initial seed (Fig. 10). A powerful and important feature of our network approach is its ability to incorporate–in an unbiased manner–genes that were highly connected with many members of the network but were not themselves differentially expressed.[152] Although these hubs may not be transcriptionally regulated, they are captured by our network-based analysis due to their connectivity with other differentially expressed nodes. The network-generating algorithm added four such MMP10-related hubs to our interactome: TNFα, TGFβ1, NFκB and AP1 (shown in yellow, Fig. 10). Interestingly, a recent report has identified a functionally active AP1 binding site on the proximal region of the MMP10 promoter.[153] The central role played by these growth factors and transcriptional regulators in influencing inflammation is well documented. In particular, macrophage-derived TGFβ1 is an important immuno-suppressive factor that affects macrophage and T cell activation.[34,154] Even though total TGFβ1 levels did not differ between infected wildtype and *Mmp10*$^{-/-}$ lungs, these data suggest that MMP10 influences TGFβ1-dependent pathways. Together, these analyses demonstrate that MMP10 dramatically influences the lung's immunological responses and orchestrates this response via specific and experimentally testable gene product interactions. Because we used total lung RNA, our analysis did not differentiate between epithelial-derived and macrophage-derived MMP10 or its role in specific subsets of macrophages. In published studies,[92] we compared the transcriptional responses of wildtype, *Mmp7*$^{-/-}$, and *Mmp10*$^{-/-}$ airway ALI cultures to *P. aeruginosa* infection and found that MMP10 has broad influences on epithelial gene expression, affecting apoptotic and proliferation pathways. However, a relatively small number of genes involved in immune and remodeling responses were differentially expressed between *Mmp10*$^{-/-}$ and wildtype cells compared to the marked differences we determined in analysis of whole lung gene expression. As most MMP10 is produced by infiltrated macrophage, we predict that these preliminary gene expression .

Proposed Studies. Alveolar and tissue macrophages will be isolated from smoke-exposed and control wildtype and *Mmp10*$^{-/-}$ mice at different times, and subpopulations of activated macrophages will be sorted. We will also compare M1 and M2 macrophages differentiated from wildtype and *Mmp10*$^{-/-}$ BMDMs. Total RNA will be amplified, labeled, and hybridized to Illumina arrays. Because each BeadChip contains 8 identical arrays, the samples will be randomized to each platform to eliminate bias. For each time point/condition, 6 microarray experiments will be performed.

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To test this idea and to focus specifically on gene expression in macrophages, we plan to undertake a systematic, computationally intensive search for MMP10-dependent pathways in specific subsets of macrophages. From these studies, we will build predictive maps to identify potential regulatory gene-product hubs, and the functional role of these proteins will be validated by various approaches to manipulate their production or activity.

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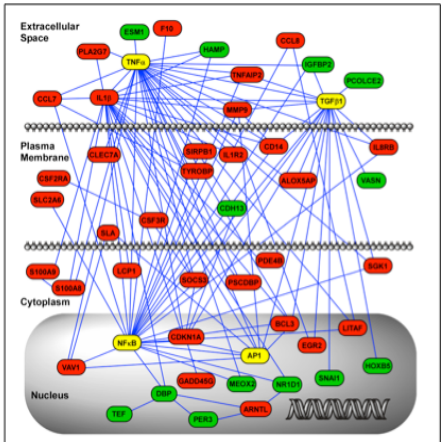

Fig. 10. Interactome of Differentially Expressed MMP10-dependent Genes.

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Table 3. Enriched Biological Modules during Smoking

Functional Category	Fold Enrichment	P-Value	FDR
Response to Stimulus	8.4	6.4×10^{-11}	3.3×10^{-7}
Chemotaxis	21.7	6.2×10^{-10}	1.6×10^{-6}
Response to Wounding	8.5	4.8×10^{-7}	4.9×10^{-4}
Inflammatory Response	9.7	3.2×10^{-6}	2.1×10^{-3}
Immune System Process	4.3	5.5×10^{-6}	3.2×10^{-3}
Response to Stress	4.1	2.2×10^{-5}	1.1×10^{-2}
Defense Response	4.4	2.6×10^{-5}	1.2×10^{-2}
Cytokine	9.4	8.9×10^{-5}	1.5×10^{-2}
Leukocyte Chemotaxis	42.7	1.0×10^{-4}	4.0×10^{-2}

Summary

- Choose mentors/advisors carefully
- Read the instructions
- Start early
- Write the Specific Aims page first
  - Get it “right” before writing the grant
- Include preliminary data
  - Be creative about preliminary data