Preparing Conference Abstracts and Grant Applications

ANNA M. EIRING, PHD
FACULTY DEVELOPMENT COURSE
FEBRUARY 15, 2019
A little about me…

- Involved in biomedical research since 2001
- Co-authored 59 conference abstracts that were accepted for oral or poster presentation
- Published 35 scientific papers or review articles, with first author papers in Cell, Blood, and Leukemia
- NIH-funded researcher having secured nearly $1,000,000 in funding from both federal and private agencies
Learning Objectives

After this session, learners will be able to:

1) Identify effective strategies for developing successful abstracts and grant proposals.

   a. Prepare competitive abstracts for submission to scientific conferences.

   b. Draft successful grant applications for submission to federal and private funding agencies.
Conference Abstracts

- An abstract is a brief summary of a research project you want to present at an academic conference.

- The reviewers will not only assess the quality and originality of your work, but will also read between the lines.
  1) How enthusiastic are you about your topic?
  2) How professional are you?
  3) Are you showing respect for the conference you are applying to?
Verify that your topic fits the call for abstracts

Limit yourself to the word count

Follow all instructions on formatting

Include all additional information

  e.g. Personal data, keywords, subsection where you will submit the abstract

Correct English syntax and spelling

Keep to the deadline

If you are working with other people, get their permission before you submit
Conference Abstracts - BASIC FORMULA

- TOPIC
- TITLE
- MOTIVATION
- PROBLEM STATEMENT
- APPROACH
- RESULTS
- CONCLUSIONS

CONFERENCE ABSTRACT
Conference Abstracts - OVERVIEW

Paragraph 1: What is the problem and why should people care?

Paragraph 2: What approach did you use to study the problem and what are your results?

Paragraph 3: What are your conclusions, and why should people listen to you? What are the overall implications of your findings, both inside and outside of your field?
Eiring Lab AACR Abstract

AACR Abstract

Title: Mechanisms of Tyrosine Kinase Inhibitor Resistance in Chronic Myeloid Leukemia

Short title: Mechanisms of TKI resistance in CML

Authors: Anna M. Eiring, Rebecca Ellwood, Carme Ripoll Fiol, Robert K. Hills, George Ntelisopoulos, Alistair Reid, Dragana Milojkovic, Jane Apperley, Jamshid Sorouii-Khorashad

Tyrosine kinase inhibitors (TKIs) targeting BCR-ABL1 have turned chronic myeloid leukemia (CML) from a fatal to a chronic disease. Despite improved survival, resistance is a clinical problem, and TKIs do not target the quiescent CML leukemic stem cell (LSC), meaning that patients must be treated for life at a high economic burden and sometimes with significant side effects. TKI resistance is frequently characterized by mutations in the BCR-ABL1 kinase domain, but they explain only ~50% of clinical TKI failure. The remaining patients have BCR-ABL1-independent resistance, defined as survival despite BCR-ABL1 inhibition. We have previously reported a gene expression signature predictive of TKI failure in CD34+ cells from chronic phase CML patients (McWeeny et al. 2015). This gene expression signature demonstrated significant overlap with signatures of blast phase CML (Zheng et al. 2006), suggesting that similar biological processes may be driving TKI resistance and disease progression. We expanded our analysis, and also found significant overlap between the expression profiles of TKI resistance and quiescent CML LSCs reported by Graham et al. (p=4x10^-11) and Cramer Morales et al. (p=7x10^-1). These data suggest that there is a core of genes whose expression is consistently associated with multiple scenarios of BCR-ABL1-independent resistance.

Our previous work has shown that BCR-ABL1-independent resistance is largely driven by STAT3, and that targeting STAT3 in combination with TKIs restores sensitivity in TKI-resistant CML stem and progenitor cells (Eiring et al. Leukemia 2015). Unexpectedly, gene set enrichment analysis revealed that our gene expression signature predictive of TKI failure (McWeeny et al. 2015) does not reveal a STAT3 transcriptional signature. Similarly, RNA sequencing data on TKI-resistant K562-R cells, which are resistant to TKIs but lack BCR-ABL1 kinase domain mutations, revealed that, while TKI resistance was not associated with a STAT3 transcriptional signature (p=1.0), it was correlated with a signature reminiscent of TNFα signaling via NFκB (p=0.024). Nucloceplasmic fractionation revealed higher levels of total- and phospho-NFκB in the nucleus of K562-R versus TKI-sensitive K562-S controls, and in CD34+ progenitors from TKI-resistant CML patients (n=3) compared to TKI responders (n=2) and normal individuals (n=2). These data indicate that NFκB may be driving the gene expression signature associated with BCR-ABL1-independent resistance, and suggest non-canonical functions for STAT3 that go beyond its traditional role as a transcription factor.
MSA43: A New Player in Leukemic Stem Cell Survival in Chronic Myeloid Leukemia

Anna M. Eiring1, Jamshid S. Khorasani2, Amiya Prasad3, Clinton C. Mason1, Russell Bell1, Anna Smail1, Anthony D. Pomier1, Fan Yin1, Hamzah M. Redvina1, Ambro D. Bowles1, Philipp M. Chiel1, Sinirayi Tantarchali1, Shincon R. McMenemey1, Istvan J. Donker1, Derek L. Stowers2, Vivian G. Oehler1, Thomas D’Orazi1,5, Michael W. Dustin1,5

Background: We have previously demonstrated that the transcriptional profile of diagnostic CD34+ cells from chronic phase chronic myeloid leukemia (CP-CML) patients exhibits primary tyrosine resistance to imatinib overlaps with that of patients with myeloid blast phase CML (BP-CML) (McMenemey et al. Blood 2010). These data suggest that primary resistance to imatinib in CML is associated with a common genetic alteration. The hematopoietic cell cycle regulator, MSA43, was identified as a principal component of the gene expression classifier predicting response to imatinib. Low MSA43 correlated not only with primary TKI resistance, but also with shorter overall survival in CP-CML (n=55). Consistently, microarrays (n=19 CP-CML, n=16 BP-CML), qRT-PCR (n=22 CP-CML, n=17 BP-CML), and immunoblot (n=3 CP-CML, n=3 BP-CML) analyses demonstrated that MSA43 mRNA and protein levels are reduced in CP-CML progenitor cells from BP-CML versus CP-CML patients, with no difference between CP-CML and normal CD34 progenitor cells (n=5) (Eiring et al. ASH 2015 #14). These data suggest that MSA43 may play a role in both primary TKI resistance and blast transformation of CML.

Results: To assess the functional role of MSA43 in CP-CML and TKI response, we used ectopic MSA43 expression and mRNA-mediated MSA43 knockdown in CD34+ cells from BP-CML and CP-CML patients, respectively. Ectopic expression of MSA43 in BP-CML CD34+ progenitors (n=5) markedly reduced colony formation in the presence and absence of imatinib, consistent with a tumor suppressor role for MSA43 in CML. While reexpression of MSA43 alone did not increase apoptosis compared to empty vector-expressing controls, imatinib-induced apoptosis in BP-CML CD34+ cells was increased by 62%, with no effect on normal CD34+ cord blood cells (n=2). Conversely, shRNA-mediated MSA43 knockdown (shMSA43) in CP-CML CD34+ cells (n=7) reduced the effects of imatinib in colony formation and apoptosis assays, with no effect on normal CD34+ progenitors (n=4). In contrast to a previous report (Donada JL, et al. J Clin Invest 2002), we detected no change in cell cycle status of CML or normal CD34+ cells upon MSA43 ectopic expression or knockdown (n=5). Altogether, these data suggest that MSA43 positively regulates patient survival and imatinib response in CML progenitor cells.

To evaluate MSA43 in the leukemic stem cell compartment, we performed qRT-PCR on primary CP-CML cells (n=5) and observed that MSA43 mRNA levels are 22±6% higher in committed CD34+38 progenitors compared to more primitive CD34+38 stem cells, suggesting a role for MSA43 in differentiation. Consistently, qRT-PCR, immunoblot, and flow cytometry demonstrated that MSA43 mRNA and protein were upregulated in CP-CML CD34+ cells upon G-CSF treatment (n=3). Flow cytometry also revealed that shMSA43 in CP-CML CD34+ cells resulted in a reduction of CD138+ cells by 45% in the presence of G-CSF (n=3). To assess the function of MSA43 in CML stem cells, we performed long-term culture-initiating cell (LTC-IC) assays and xenografts into NSF mice upon MSA43 knockdown in CP-CML (n=3). MSA43 increased Ph+ LTC-IC colony formation in the absence, and even more so in the presence of imatinib, with no effect on Ph+ LTC-ICs. Consistent with these data, shMSA43 enhanced engraftment of CD34+ CD45+ GFP+ cells into the bone marrow of NSF recipient mice. Preliminary data in primary TKI-resistant and BP-CML CD34+ cells suggests regulation of the gene by promoter hypermethylation.

Conclusions: Together, these data suggest that MSA43 plays a key role in imatinib response of 1) patients with primary TKI resistance, 2) patients with BP-CML, and 3) the CML stem cell compartment. Since the effects of MSA43 activity in CML do not involve changes to the cell cycle, experiments are underway to identify the mechanism by which MSA43 improves imatinib resistance and survival in CML.
Graduate Lab ASH Abstract

Suppression of RISC-Independent Decay and RISC-mediated miRNA Base-Pairing Activities of MicroRNA-328 is Required for Differentiation-Arrest and Enhanced Survival of Blast Crisis CML Progenitors

Anna M. Eiring, Ph.D.1, Jason G. Harb, Ph.D.1, Paolo Nevinian, B.Sc.1, Jedina Daku, B.S.1, Shujin Liu, Ph.D.1, Sebastian Schwind, M.D.1,1, Ramamytri Santatharan, Ph.D.1, Hao He, Beckett1, Jason C. Chandran1, Rashid Afkimos, Ph.D.1, Stephen A. Libshuter, Ph.D.1, Michael Caligiani, M.D.1, Ph.D.1, Guido Marzocchi, M.D.1, Raminou Guzen, M.D.1, Carlo M. Croce, M.D.1, George A. Calif, M.D.1, Ph.D.1, Danilo Pierotti, MD.1, Ph.D.1

MicroRNAs (miRs) and heterogeneous ribonucleoproteins (hRNP) are post-transcriptional gene regulators that bind miRNA in a sequence-specific manner. We have reported that a) hRNP-F is strongly suppressed, CEBPβ mRNA translation and inhibits myeloid maturation of bone marrow (BM) progenitors from chronic myelogenous leukemia patients in myeloid blast crisis (CML-BC);2,3 Pierotti et al. [Nat Genet 2002]; and b) miR-328 expression is lost in myeloid CML-BC1,4 progenitors (p<0.01) and its reduced expression at physiological levels rescues granulocytic differentiation and represses clonogenic potential of primary CBL-ABL blasts (Eiring et al. ASH 2007). Here we show by Northern blot, real-time PCR, and cell-based analyses that miR-328 levels increase during granulocytic differentiation of normal human CD34+ and mice BM BM progenitors, but during differentiation towards erythroid, macrophagic/megakaryocyte or monocytic lineages, CEBPβ binds to the same microRNA (miRNA) sites in hRNP-F and repressed cellular signaling pathways to suppress CEBPβ and miR-328 expression in CBL-ABL1 cells. In fact, functional CEBPβ binding sites are present in the CEBPβ promoter region and CEBPβ interacts in vivo with these regulatory elements to enhance miRNA transcription. Importantly, we also show that reduced maturation of CEBPβ and CEBPβ mRNA is mimicked in vivo by miR-328 expression in normal human CD34+ and mice BM BM progenitors. Although it requires direct interaction of hRNP-F to the CEBPβ regions of miR-328. Indeed, RNA immunoprecipitation (RIP) experiments demonstrated that miR-328 directly binds to hRNP-F independent of the RNA-induced silencing complex (RISC). Furthermore, despite miR-328, but no hRNP-F, is essential to in vivo binding of hRNP-F to the CEBPβ regions of miR-328, thereby releasing CEBPβ from hRNP-F-mediated miRNA transcription inhibition and rescuing CEBPβ-driven neutrophil maturation (adults activity). Differentiation of miR-328-expressing CML-BC1,4 blasts was rescued in part by levels of CEBPβ mRNA and hRNP-F protein levels remained unaltered. The existence of a direct miR-328/hRNP-F interaction in vivo and in vitro using RIP-directed translation assays and in vivo using the 11.1% increase of 328 miR-328-CBL-ABL1 cells that do not express endogenous (CEBPβ) mRNA and repress endogenous (CBL-ABL1) CEBPβ for differentiation. Addition of miR-328, but not miR-330, to hRNP-F-containing RLI reactions increased newly synthesized (CBL-ABL1) CEBPβ levels by 100%. Likewise, restored CEBPβ expression is mimicked in decreased hRNP-F binding to CEBPβ miRNA, induction of CEBPβ protein but not miRNA and rescued granulocytic differentiation of 618-norMOLT-CEBPβ but not vector-transduced 618 cells. While hRNP-F was not found in complex with hRNP components (Fibrinogen, PIP2 and ApoA2, we were unable to detect miR-328 associated to Rai and ApoA2 in miR-328-expressing cells, suggesting that it also acts through canonical RISC-dependent base-pairing with miRNA targets. Indeed, we identified the CEBPβ-regulated PIM1 gene as a bona fide miR-328 target in CBL-ABL1 cells. Further, miR-328 suppressed PIM1 protein but not mRNA levels, and this effect required integrity of the miR-328 binding site proximal to the PIM1 3′UTR. Forced expression of a wild-type but not kinase-deficient PIM1 lacking the TUTR in miR-328-expressing cells fully rescued CEBPβ expression and clonogenicity, suggesting that miR-328-reduced PIM1 suppression accounts for reduced survival of miR-328 infected CBL-ABL1 blasts. To show that miR-328 acts on PIM1 in a RISC-dependent manner, we mimicked the miR-328 seed sequence (miR-328-5′) but retaining its CEBPβ character. Similar to wild-type miR-328, miR-328-5′ efficiently interacted with hRNP-F, restored CEBPβ protein expression and rescued granulocytic differentiation. Surprisingly, we were unable to silence PIM1 in 328-DIO-ABL1 cells indicating that the CEBPβ character of miR-328 is essential for in vivo actions and, while it is a specific sequence, a factor necessary for RISC-dependent pairing in miRNA targets. The discovery of dual anti-sense for miR-328 does not only add a new layer of complexity to the mechanisms regulating CML disease progression, but also highlights the ability of miRNAs to alter RNA metabolism by acting as molecular deans for RNA binding proteins.
It is not easy to squeeze the research of an entire project into a few lines.

You will need to focus on one specific angle, answering 4 specific questions:

1) What is the problem you are addressing?
2) What method(s) did you use to research the problem?
3) What data have you been able to produce?
4) What finding will you be able to discuss?
Be bold in your opening paragraph.

Don’t get too bogged down in detail.

Draft and revise, revise, revise.

Ask colleagues if they have successfully submitted abstracts to the same conference before. Are they willing to share?

Check for published abstracts online.
When preparing a conference abstract, **TAKE YOUR TIME**!!!!

A good abstract is not written in just a few minutes. Even experienced researchers go through one abstract several different times!!

Have others read your abstract for clarity and provide input.

Ask for feedback and give yourself **time for revisions.**
Moving on to grant applications...
Grant Applications - FUNDING SOURCES

- Industry
- State
- Private Foundations
- Internal
- Research Funding
- Federal Agencies
Grant Applications – CHOOSE AN AGENCY

▶ Make sure that your research fits the mission of the funding agency!!!

▶ Read the Grant Proposal Guidelines CAREFULLY!!!

▶ Your chosen agency should NOT be the sole source of funding. Funding from other agencies gives credibility to your work!!!
Grant Applications - INSTRUCTIONS

- Be sure to follow the instructions.

- A common reviewer’s interpretation:

  “If the PI cannot follow instructions for the proposal, how can they be trusted to perform elaborate and accurate research?”
Grant Applications – PROPOSAL GUIDELINES

- Page limit
- Word limit
- Budget limit
- Abstract format
- Reference format
- PI and Co-PI eligibility
- Submission method (e.g. online vs. hard copies)

- Font and font size
- Image resolution
- Table of contents
- Research objectives
- Tables/Figures/Legends
Grant Applications – WHAT TO CONVEY

Your proposal should convey the following attitudes:

1) You have identified an important problem, and you are the right person to do the work.

2) You will get the job done and find answers to the problem discussed.

3) You are aware of previous relevant studies.
Grant Applications – KNOW YOUR FIELD

- What is the current state-of-the-art?
- What are the top ten researchers in the field doing now?
- What are the available sources of funding?
- What are the key research issues?
- Who would likely review your proposal?
Grant Applications – GENERAL OUTLINE

I. Abstract: Written in more general terms, readable by non-experts.

II. Background & Significance: Demonstrate that you know the field thoroughly and that there is a problem you intend to solve.

III. Specific Aims: 1-2 sentences on each point that you intend to investigate

IV. Experimental Plan: Outline how you plan to investigate the problem and any preliminary data demonstrating you are capable of performing the work.

V. Resources: Explain the resources available and required to complete the work. These can be at your institution or through established collaborations.

VI. References: List all cited references. Be sure to exercise any limitations, as some agencies only allow a specified number of references or pages.
Grant Applications – THE BASICS

Tips for successful grant applications:

1) Keep the audience in mind
2) Start preparing the application EARLY
3) Follow the instructions and application guidelines carefully
4) Be brief, concise, and clear
5) Be organized and logical
6) Be careful in the use of appendices
7) Carefully proofread and application
8) Learn how to navigate the online submission forms
Grant Applications – STATE YOUR OBJECTIVE

Make clear in the **FIRST PARAGRAPH** exactly what your proposal is about:

1) What is the subject of your proposal?
2) State the problem or gap of knowledge.
3) State why the issue is significant.
4) What is your hypothesis?
5) State what you are going to do.
6) Explain how you will carry out the proposed work.
Grant Applications – COMMON ERRORS

- Doesn’t fit the agencies mission(s).
- Violates one or more proposal guidelines.
- Proposal is beyond the capabilities of the PI, the trainees, or the institution.
- Over ambitious!!!!
- Lack of proofing. Blatant grammar, spelling, or other errors will kill an otherwise great application.

If the PI cannot follow instructions for the proposal, how can they be trusted to perform elaborate and accurate research?
Grant Applications – COMMON ERRORS

- Missing pages, figures, tables, or signatures
- Unfocused
- Poorly organized
- Not enough people to do the work
- Low impact - results will not be publishable
Grant Applications – THINK ABOUT REVIEWERS

Reviewers want to know:

1) What is your research about? What is the objective of the work?
2) How will you do it? What is the methodology?
3) Can you do it? Do you have the facilities and people to do the work?
4) Is the work worth doing?
5) Are there any secondary objectives relevant to the agency, such as educating students or promoting minorities in science/medicine?
6) What are the broader implications of your work?
Grant Applications – REVIEW CRITERIA

1) Significance

- Does the study address an important problem?
- If the aims of the application are achieved, how will they advance our current scientific knowledge?
- What will be the effect of the proposed study on concepts, methods, or treatment approaches driving the field?
2) Approach

- Are the design/methods/analyses adequately developed?
- Are they appropriate to the aims of the proposal?
- Does the applicant acknowledge potential problems and alternative approaches?
3) **Innovation**

- Does the project employ novel concepts, approaches, or methods?
- Are the aims original and innovative?
- Does the project challenge existing paradigms or develop novel methodologies or technologies?
4) **Investigator**

- Is the investigator appropriately trained and fit to carry out the work?
- Is the work proposed relevant to the experience level of the PI?
- Is there a track record of success (i.e. previous clinical trials, publications, or grant funding)?
5) Environment

- Will the scientific environment contribute to success of the work?
- Do the proposed experiments take advantage of unique features offered by the environment?
- Is there evidence of institutional support?
- Are adequate collaborators/mentors available for guidance?
REVIEWERS ARE TIRED!!!!

They want to find a reason to skip your proposal.

Wording that completely fills the space, without line spaces or indentations, affords a repulsive aspect for a tired reviewer.

Wording that is separated by line spaces and contains words like **Hypothesis** and **Specific Aims** in bold catches the eye and has a positive impact on the reviewer.
Magnesium (Mg) deficiency may play an important role in the pathogenesis of enhanced vascular reactivity in hypertension. The overall hypothesis to be evaluated is that Mg deficiency caused by glucose intolerance, insulin resistance, or other factors in hypertensives leads to increased vasomotor tone via altered release of vasoactive cyclooxygenase lipooxygenase products of arachidonic acid and enhanced angiotensin II (AII) action. To evaluate the effects of Mg deficiency in normal subjects we will induce the condition by administration of low Mg diet. Vascular and adrenal sensitivity to AII, platelet aggregation, and eicosanoid levels will be studied prior to and after Mg deficiency is established. Since evidence suggests that Mg deficiency can modulate insulin action, the effect of this deficiency on glucose tolerance will also be studied. In another project the effect of insulin on intracellular Mg levels will be studied using a new fura 2 Mg dye technique. These studies will be performed in groups of subjects with varied blood pressure and insulin levels. Also the effects of acute intravenous and chronic oral Mg loading on the above parameters will be studied in similar subject groups. We will directly study the effect of Mg on AII, insulin, and insulin-like growth factor action in isolated and cultured adrenal glomerulosa cells. Concentration of Mg will be varied and signal transduction and steroidogenic effects will be evaluated. These studies will provide insight into mechanisms important to the pathogenesis of altered vascular reactivity of subjects with hypertension or hyperinsulinemia.

Specific Aims: (1) Determine the effects of low Mg on vascular and adrenal sensitivity to AII (platelet aggregation and eicosanoid levels, and glucose tolerance). (2) Determine the effect of insulin on intracellular Mg levels (fura 2 Mg dye technique). These studies will be performed in subjects with varied blood pressure and insulin levels. (3) Determine the effects of acute intravenous and chronic oral Mg loading on the above parameters. (4) Determine the signal transduction and steroidogenic effects of Mg on AII, insulin, and insulin-like growth factor action in isolated and cultured adrenal glomerulosa cells.

Significance: These studies will provide insight into mechanisms important to the pathogenesis of altered vascular reactivity of subjects with hypertension or hyperinsulinemia.
NIH Grant/Career Timeline

Career Stage:
- Student
- Postdoc
- Junior Faculty
- Senior Faculty

Training:
- F30
- F31
- F32
- K Awards (career dev)

Research:
- R01, R03, R21
- P01
NIH Loan Repayment Program
NIH Loan Repayment Program

Research Outside NIH (Extramural)

- Clinical Research
  - For clinical investigators interacting with human patients in an inpatient or outpatient setting.
  - Show Details

- Health Disparities Research
  - For investigators conducting research that focuses on one or more of the minority health disparity populations defined by NHGRI and the Agency for Healthcare Research and Quality.
  - Show Details

- Clinical Research for Individuals from Disadvantaged Backgrounds
  - For clinical investigators coming from an environment that may hinder the individual from obtaining the knowledge, skill, and ability required to enroll in and graduate from a health professional school, or from a family with an annual income below low-income thresholds.
  - Show Details

Research Inside NIH (Intramural)

- Pediatric Research
  - For investigators conducting research strictly related to diseases, disorders, and other conditions in children.
  - Show Details

- Contraception and Infertility Research
  - For investigators conducting research in conditions that impact on the ability of couples to either conceive or bear young.
  - Show Details
“It takes a village”

The Department of Biomedical Sciences has research efforts in 4 areas:

1) Cancer biology
2) Diabetes and metabolism
3) Infectious diseases
4) Neurosciences

~85% research effort

Research collaborations can help with scholarly activity for clinicians

