Supplemental Materials

for

Reading, Writing, and Presenting Original Scientific Research: A Nine-Week Course in Scientific Communication for High School Students

Elizabeth S. Danka\textsuperscript{1,2,*} and Brian M. Malpede\textsuperscript{1,3}

\textsuperscript{1}Young Scientist Program, Washington University in St. Louis, St. Louis, MO 63110, \textsuperscript{2}Department of Pediatrics, Washington University in St. Louis, St. Louis, MO 63110, \textsuperscript{3}Department of Microbiology, Washington University in St. Louis, St. Louis, MO 63110

Table of Contents

(Total pages 61)

Appendix 1: Pre-assessment questions
Appendix 2: Writing pre-assessment
Appendix 3: Student syllabus
Appendix 4: Homework assignments and worksheets
Appendix 5: Presentation assessment rubric
Appendix 6: Post-assessment questions
Appendix 7: Sample student writing pre-assessment responses and corresponding final papers (2)
Appendix 8: Sample student final presentations (2)

\textsuperscript{*}Corresponding author. Mailing address: 125 Mason Farm Rd., 6101 Marsico Hall, Campus Box 7290, Chapel Hill, NC, 27599. Phone: (919) 966-1060. E-mail: esdanka@email.unc.edu.

©2015 Author(s). Published by the American Society for Microbiology. This is an Open Access article distributed under the terms of the Creative Commons Attribution-Noncommercial-NoDerivatives 4.0 International license (https://creativecommons.org/licenses/by-nc-nd/4.0/ and https://creativecommons.org/licenses/by-nc-nd/4.0/legalcode), which grants the public the nonexclusive right to copy, distribute, or display the published work.
Appendix 1: Pre-assessment questions.

Name:          Number:
Please read each question and circle the answer that describes your feeling most accurately.
1) Have you ever read a scientific paper?
   Yes          No          Not sure

2) Can you name the parts of a scientific paper?
   Yes          No          Not sure

3) Have you ever interpreted basic scientific results?
   Yes          No          Not sure

4) I feel comfortable interpreting basic scientific results.
   Strongly Disagree    Disagree    Neutral    Agree    Strongly agree

5) I feel confident in my writing skills.
   Strongly Disagree    Disagree    Neutral    Agree    Strongly agree

6) Have you ever written a scientific paper before?
   Yes          No          Not sure

7) I feel comfortable writing a scientific paper.
   Strongly Disagree    Disagree    Neutral    Agree    Strongly agree

8) I feel confident in my oral presentation skills.
   Strongly Disagree    Disagree    Neutral    Agree    Strongly agree

9) I have strong critical thinking skills.
   Strongly Disagree    Disagree    Neutral    Agree    Strongly agree

10) I can review and critique a peer's work effectively.
    Strongly Disagree    Disagree    Neutral    Agree    Strongly agree
Appendix 2: Writing pre-assessment.

Writing Course Name: ________________________
Pre-course free write

You have 15 minutes to create a response to the following:

Think of a time when you read an article (magazine, newspaper, online source) about a science or health topic. What did you learn from this article? Did you consider it to be a credible source at the time? Why or why not? What do you think of this article now? If you have changed your mind about the article, why did you change your mind?
Appendix 3: Student syllabus.

Writing Course Syllabus

Course Instructors:

<table>
<thead>
<tr>
<th>Name</th>
<th>Phone number</th>
<th>Email address</th>
</tr>
</thead>
<tbody>
<tr>
<td>Name 1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Name 2</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Goals:
While taking this course, you will:

- Recognize the style, format, and content of scientific papers.
- Understand your research and present this research to an audience.
- Understand and discuss others’ research at a basic level.
- Develop your ability to review others’ work.
- Appreciate the importance of scientific writing, speaking, and other ways communication.

Expectations:
Skills in writing, oral presentations, and peer review are required for your success in college, with further education, and in building and maintaining a career. This writing course is designed to teach you about these life skills, but exactly how much you get out of the class will depend on how much time and effort you put into it. Attendance is mandatory (unless you have a valid reason for missing, and you inform the course instructors ahead of time). We expect that you will arrive on time each week and be ready to participate in class, as your participation will help you engage and actively think about the writing/editing process. The assignments for each week will help you prepare for what we will go over in class, as well as reinforce in-class topics, so please be thorough and thoughtful when completing them. You will receive written comments each week on your assignments from the instructors. We expect you to use these comments to improve your writing throughout the summer. You will thank yourself later for the effort spent on writing and editing this summer, as the paper will come in handy when the time comes to talk about your research, provide admissions committees with a written summary, or compete in a science competition in the future.

We hope that you have a wonderful summer experience! There is a lot to learn, but we want you to enjoy the experience. If you have any problems with the writing course, your labs, or any other aspects of the summer, DO NOT HESITATE to inform your instructors, the liaisons, or any of the Summer Focus leaders.

Assignments:
The assignments for each class session are listed in your course syllabus and outline. For the first few weeks, you will learn how to read scientific articles and their basic structural format. In addition to reading these papers, you will learn to discuss them with other students in the format of a journal club. In addition, you will learn to present your project to an audience early on in the summer, so you have to become familiar with your project’s main question,
background research, hypothesis and methods in the first few weeks. Starting in July, you will begin writing your paper. We will guide you through each section, and each week you will write a specific section of your paper and update any previously written sections. For example, for class on July 3, you will write your Materials & Methods section. On July 11, you will turn in your first draft of your Results section and improve your Materials & Methods section. You will also be asked to discuss your project in a small group (other students and a volunteer) by explaining your summer project and the progress you have made for that week. The instructors will provide more detailed information on these assignments in class.

**Progress charts**: You will be asked to fill in a progress chart each week. In this chart, please list the experiments you performed for the week, why you did them, and what your results from these experiments show. Write whether you worked independently, with the help of your mentor, or whether you watched your mentor do the experiment. We will review the charts to ensure that you are receiving the maximum amount of time at the bench and the best experience possible! Progress charts have been given to you and are in your binder.

**Final paper**: A scientific paper on your research project will be due at the end of summer. Your paper should be at least five pages long, double-spaced, with 12-point Times New Roman font. Formal requirements for formatting your paper will be distributed later. We encourage you to write a longer paper if you would like to use the paper for science competitions. A rough draft of your entire paper is due on July 18. We realize that some sections may not be complete because you may still be working on the experiments. However, you should still have at least begun to draft all of the sections of the paper. You will edit and improve this draft until the end of the program. The final draft is due August 1 by 5:00pm. Throughout the editing process, we encourage you to save each draft as a separate document (e.g. SF Paper-Draft 1, SF Paper-Draft 2) to at least two different hard drives (e.g. Desktop & USB) in case your computer crashes at the last minute (it happens!!).

**Final presentation**: You will present your research to an audience of all SF students, their family and friends, and SF volunteers at an end of SF Symposium on Friday, August 2. This presentation will be 5-7 minutes long and will demonstrate why you did your research, what your project was, and the main results and conclusions from your work. Your presentation will have an outline similar to that of your final paper. We will help you prepare this presentation and give you lots of feedback as you practice it over and over! Like your final paper, we encourage you to save your final presentation to at least two different sources so that you do not lose your hard work unexpectedly.

**Writing Course: Detailed Course Outline**

<table>
<thead>
<tr>
<th>Date</th>
<th>In-class</th>
<th>Due today</th>
<th>Due next week</th>
</tr>
</thead>
<tbody>
<tr>
<td>June 5th</td>
<td>-6 C’s of writing and plagiarism</td>
<td>-summary of lab/project overview</td>
<td>-read example paper and fill out critical</td>
</tr>
<tr>
<td>(Wed.)</td>
<td>-types of science writing</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Bootstrap Camp</td>
<td>-how to do database searches, practice sections of a scientific paper</td>
<td>(main Q, hypo, methods)</td>
</tr>
<tr>
<td>----------------</td>
<td>----------------</td>
<td>---------------------------------------------------------------------</td>
<td>-------------------------</td>
</tr>
<tr>
<td>June 13 (Thurs.)</td>
<td>-critical reading skills example paper -discuss progress of project</td>
<td>-critical reading prep worksheet</td>
<td>-find and read 2 papers for project, summarize -prepare 5 min PPT presentation</td>
</tr>
<tr>
<td>June 20 (Thurs.)</td>
<td>-5 min project presentations (background, main Q, hypo, methods)</td>
<td>-summaries of 2 papers related to your project -5 min presentation</td>
<td>-read example M&amp;M, fill out M&amp;M worksheet -prepare 5 min PPT with group about M&amp;M</td>
</tr>
<tr>
<td>June 27 (Thurs.)</td>
<td>-format and content of Materials and Methods section -discuss progress of project</td>
<td>-M&amp;M worksheet -presentation for Journal Club discussion of format and content of M&amp;M</td>
<td>-write M&amp;M for your project -read example Results, fill out Results worksheet -prepare 5 min PPT with group about Results</td>
</tr>
<tr>
<td>July 3 (Wed.)</td>
<td>-format and content of Results section -discuss progress of project -schedule one-on-one meetings</td>
<td>-M&amp;M -Results worksheet -presentation for Journal Club discussion of format and content of Results</td>
<td>-read example Abs/Intro/Disc, fill out worksheet -write Results for your project -chalk talk outline</td>
</tr>
<tr>
<td>July 8 (Mon.)</td>
<td><strong>ONE-ON-ONE MEETINGS (9AM-12PM Rooms TBA)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>July 11 (Thurs.)</td>
<td>-format and content of Abstract/Intro/Discussion sections -2 min project chalk talk</td>
<td>-Draft #1 (edited M&amp;M, Results) -Abs/Intro/Disc worksheet -chalk talk outline</td>
<td>-Draft #2 (edited M&amp;M and Results, add in Abstract, Introduction and Discussion)</td>
</tr>
<tr>
<td></td>
<td>(continued on next page)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Date</td>
<td>Activity</td>
<td></td>
<td></td>
</tr>
<tr>
<td>-----------</td>
<td>---------------------------------------------------------------------------</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
| July 18 (Thurs.) (July 18 cont’d) | - presentation skills  
- how to do Citations and in-text references  
- schedule one-on-one meetings  
- Draft #2 (edited Draft #1 plus Abstract, Intro and Discussion)  
- 5-10min practice PPT presentation |
| July 22 (Mon.) | **ONE-ON-ONE MEETINGS (9AM-12PM Rooms TBA)** |
| July 25 (Thurs.) | - project presentation to class (5-10 minutes)  
- practice presentation  
- final presentation and final paper |
| July 29-August 2 | - practice final project presentation every morning at 9 AM  
- final presentation  
- continue editing paper and presentation |
| August 1 (Thurs.) | **FINAL PAPER DUE BY 5 PM** |
| August 2 (Friday) | **SYMPOSIUM: PRESENTATIONS AND BANQUET at 6 PM** |
Appendix 4: Homework assignments and worksheets.

Lab summary and project overview

Name of PI:

General lab interest:

Project topic:
  - why is this interesting?

Main question of project:
  - why is this worth investigating?

Hypothesis:

Approach (what experimental methods will you be using to test your hypothesis? add more if needed):
  - Experiment 1:

  - Experiment 2:

  - Experiment 3:
6 C’s of writing notes

Effective communication of ideas in written form is an incredibly valuable life skill. The 6 C’s of writing provide basic guidelines to successfully present your ideas in a written document. The 6 C’s are clarity, conciseness, credibility, completeness, consistency, and coherence. They are not presented in a specific order. Each C is essential to the formation of a well-written composition. The 6 C’s are presented below.

1) Clarity: The goal of writing is to convey your ideas, thoughts, and conclusions to the reader. Thus, your writing should be clear, easily understandable, and without errors that will hinder a reader’s comprehension of your thoughts. To achieve clarity, the following are essential:
   • Do the simple things right: check spelling and grammar! Proofread extensively.
   • Always use citations where necessary.
   • Be clear about what is fact versus what you hypothesize or present as a potential model (especially important for scientific writing).
   • Proper sentence structure is essential. Proofread each sentence individually to yourself to assess how well the words flow and the sentence relates your intended meaning.
   • Use active tense when writing (Jen is counting cells) and avoid passive tense when possible (The cells are being counted by Jen).
   • Pay attention to verb tenses: past tense for what you did, present tense for what is currently being done, future tense for what you plan on doing.

2) Conciseness: Your writing should be succinct but comprehensive. The following suggestions will help maintain concise writing:
   • Get to the point! Make sure that you are only adding important details.
   • Make statements in simple terms.
   • Remove any unnecessary sentences and words that do not enhance the reader’s understanding of your thoughts.
   • A sentence should not be longer than 3 lines. However, do not make sentences incomplete when trying to be concise.
   • Conciseness also applies to paragraphs and the paper as a whole: avoid being redundant at all times. Proofread to ensure that you do not repeat yourself.

3) Credibility: Your writing must be convincing and trustworthy.
   • How does your reader know that you didn’t just make this up?
   • Detail and context are important. Convince your audience!
   • Cite proper sources to provide the reader with all of your sources.

4) Completeness: Your writing should contain all necessary and appropriate information. To make sure that your writing is complete, follow these guidelines:
   • Introduce new information in all appropriate situations to avoid confusing the reader.
   • Finish each thought or phrase in a sentence.
   • Write in paragraphs that continue the flow of your writing and lead to your conclusions.
• Give reasoning for statements and justify why those statements are important and true. Convince your audience!

5) Consistency: A writing composition should be uniform. Maintaining consistency will ensure that the writing is understandable and does not confuse the reader.
  • Check to make sure that everything in your document matches, including: noun/verb, verb tenses, pronouns, etc.
  • Introduce the reader to your topic and ideas.
  • Ensure that your sentences and paragraphs flow effectively towards your conclusions.

6) Coherence: Your writing should have a logical, orderly, and consistent flow that will please the reader. In addition, the sentences and paragraphs should be logically connected. Logical connections and proper flow will make your writing understandable and easy to read. When completing your document, assess the following aspects of the writing:
  • Do things follow a logical order, or are you jumping around between topics and ideas?
  • Does your work as a whole make sense? Reading out loud can help with this. You shouldn’t need to backtrack and reread any sentence.
  • Check that you have followed the other 6 C’s.
  • Proofreading is a critical aspect of writing. Editing should be performed throughout the writing process and multiple times before a document is considered complete.
Sources of Scientific Information worksheet

There are many different ways for the average person to find out scientific information. You are probably most familiar with popular science sources and textbooks right now, but there are other sources of scientific information available. The credibility of these sources varies, and so it is always important to evaluate the source and where the information in that source came from. A list of some of these other sources is below.

-Pamphlets/Informative Brochures: Brochures for medications, pamphlets on disease
- Newspaper Articles/News Shows: Articles in the St. Louis Post Dispatch, segments on recent developments in the evening news
- Documentaries: National Geographic documentaries, Discovery Channel documentaries, NOVA programs
- References or Text Books: The Merck Manual, “Genes VII” by Benjamin Lewin
- Science Fiction Books or Movies: “Frankenstein” by Mary Shelley, Jurassic Park, Contagion
- Special Pieces in Non-Science Magazines: An article about reproductive health in a women’s magazine
- Online: CDC website, TED talks, science blogs, Wikipedia, “The Onion” science page
- Experts in the field (often oral sources of information): Your PI, collaborators, your doctor

As you may know, scientific articles are the way that most scientists communicate new findings and results within the field. In order to make sure that scientific papers are credible and worthwhile, they are subjected to the process of peer review.

What is peer review?

Why is peer review important in science? Why is it important in writing?
What are the guidelines for peer review?

Sample paper for practice peer review:
-use the brief paper below to fill out the table on the following page

Introduction
Blind people have cataracts. The majority of cataracts are age-related. Cataracts occur when crystallins clump together, making the lens cloudy. This causes the light hitting by the lens to be scattered. Cataracts can occur at birth, in childhood, or as older adults. There is increasing evidence that age-related cataract has a strong genetic component (Hammond et al., 2000). People with cataracts that appear at birth or in childhood usually have a mutation in one gene. Some scientists think that studying families with early-onset cataracts will help us understand age-related cataracts.

Methods
Our lab collected DNA from a family with an autosomal dominant inherited posterior polar cataract. They used linkage analysis to map the family’s disease to chromosome 1p36. I looked at the gene Q9UKB5 because it codes for hypothetical protein MOT8. MOT8 may be a member of the aldehyde dehydrogenase family of enzymes. ALDH is similar to omega-crystallin (Piatigorsky). We think that this gene is important for cataracts because it is similar to a protein found in scallop’s eyes.

Results
I sequenced Q9UKB5. There are 5 exons. We did this using wild type DNA and DNA from two affected individuals. I used the polymerase-chain reaction (PCR) to amplify the exons. I used 2 microliters of DNA and 1 microliter of primer. A gel was run to look at the polymerase chain reaction product. It was sequenced with the ABI machine using the method. I printed out the data and looked at it to see if there were any mutations. No one had two peaks. I screened 3 exons.

Conclusions
There aren’t any mutations. This project isn’t finished because we need to sequence the rest of the exons.
References:

<table>
<thead>
<tr>
<th>Problem</th>
<th>6 C</th>
<th>Comment</th>
<th>Solution</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Plagiarism worksheet

Database searches and material given to you by your lab will provide you with lots of background information about your project. You will likely refer back to this information as you start to put together your paper and your presentation. When you do this, it will be important to avoid plagiarism.

Discuss: What is plagiarism? How do we avoid plagiarizing?

When do we use citations? What sources need to be cited? Is there ever a time when we do not need to cite information?

Like you have probably learned in your English classes, citation methods vary. There are different formatting guidelines for citations in general writing (such as MLA, APA, CMS and IEEE) and in science writing. Most peer-reviewed journals have specific guidelines for citations. When we discuss how to do citations, we will give you a format to use.

Why have we gone over this? It is important for you to document your sources for when you start to write your papers, so save papers that you need (either hard copy or electronically) or write down details about the paper (authors, journal, title, etc.) on your notes so that you can find the paper later!
Peer Review Worksheet

Fill out this worksheet as you read through your HW article. It was written by a previous student! Be sure to read slowly enough to identify the important parts that are outlined here. Also, as you read, think about things that the author does well and things that the author could have done better. We will discuss this paper in class on Thursday.

Title:

Just from the title, what do you think the paper will be about?

After reading the Introduction, what did you learn is the topic of the paper?

What is known about the subject? (list at least 3 things)

What is not known about the subject?
What is the main question that the author is trying to answer?

What is the significance of the question? Are you convinced that this is an important question?

What is the author’s hypothesis?

How does the author plan on testing the hypothesis? (In other words, what methods will the author use during their experiments?)
What are the main results of the paper?

List 3 strengths of this paper.

List 3 weaknesses of this paper.

What 5 things do you think are the most important for you to consider when you are writing your own paper?
Materials and Methods
The Materials and Methods section of a scientific paper can give you lots of information that is not available in any of the other sections of a paper. As its name implies, this section tells you about the materials (chemicals, buffers, cells, etc.) that the authors used to do their experiments and the methods (protocols, etc.) that the authors used to do their experiments. You will likely find some things that you are familiar with in this section, as well as some things that you have not yet learned about.

For next week, each group will make a presentation to give to the class in which they go through the provided Materials and Methods section and discuss any points that they think are particularly important. This presentation should be about 5 minutes long. A couple ideas of things to consider discussing when making your presentations:

- What is the purpose of this section?
- What is the format of this section?
- What voice is used when writing the M&M?
- How much detail do the authors include?
- How does this section relate to the other sections of a paper?

This is not a complete list. Please be thorough when analyzing what goes into an effective Materials and Methods section! Think about what is helpful and what the author has done well, as well as what could be improved. Remember, you will be writing the M&M section for your own paper soon!
Results section homework handout
You will analyze the Results section from the old SF paper that you read last week. As you read the Results section from that paper, please answer the questions below. Then fill out the final question for your summer project.

What is the purpose of the Results section? What is the format of this section? Did the author of your paper adhere to this format?

Name at least 3 things that should be included in the TEXT of the Results section.

Name at least 5 things that should be included in the FIGURES or FIGURE LEGENDS. Distinguish between things that belong in the figure or in the figure legend.

What did the author do well in their Results section? What could they have done better?

Write out one or two subheadings that will be included in the Results section of your paper. What kinds of figures will these subheadings describe?
Abstract/Introduction/Discussion worksheet

Although the Abstract, Introduction and Discussion sections of a paper each have a different purpose, they can give the reader similar pieces of information, so we will learn about these three sections of the paper together.

Writing the Introduction:

Writing the Discussion:

Writing the Abstract:
It is often easiest to write the Abstract last, since it is a short summary of the entire paper. However, you should be very thoughtful when writing your abstract as you have a lot of information to convey to your reader in a short space. Readers will often use abstracts to judge whether or not a paper is worthwhile, so your abstract should be convincing!

Where in the paper is the abstract placed? Is this important? What is the purpose of the abstract?

How many sentences of the abstract should be devoted to each of the sections of your paper?
1. Introduction:

2. Materials and Methods:

3. Results:

4. Discussion:

5. Citations:

Write your abstract below. Remember to include all of the parts of the paper. If you want to leave out the discussion/conclusion sentence for now, that is fine.
Extras!
- your title should be specific and informative to give any potential readers an idea of what the paper describes
  - can be fairly long, are usually almost a complete sentence
  - important to mention your specific topic (protein or gene of interest, model system, general method used)
  - the major conclusion can be included in the title
- if you choose to include an acknowledgements section, put it at the end of your paper, before your citations
  - generally these include people that helped with experimental design, brainstorming ideas/conclusions, or paper editing, and funding sources
**Summer Project Chalk Talk worksheet**

A chalk talk is a quick, informal way of presenting your research to an audience. Instead of using a formal, lengthy Powerpoint presentation, you will instead give a much shorter presentation, and you will use the white board to draw out concepts, models and/or important points. This presentation will only be **3 minutes** long! This presentation will include a brief introduction to your project’s main question and hypothesis, followed by some brief results that you have obtained, and then a summary of what these results mean and how they fit into the big picture. You do not have to write everything you are going to say on the board. Fill out the following outline to help you prepare for your chalk talk (and use the back of this page to practice drawing the models or diagrams that you will show your audience!):

**Introduction**
- General topic of research with brief background (relevancy, short previous work):

- Main question:

- Hypothesis:

**Results** (can describe as many as wanted, probably little time for more than 3)
- Experiment 1:
  - Methods:
  - Results:

- Experiment 2:
  - Methods:
  - Results:

**Summary**
- Conclusion based on results:

- Big picture implications:
Appendix 5: Presentation assessment rubric.

Directions: Please use this rubric to rate these 7 parts of the students presentation (4 categories focused on the parts of the presentation and the organization on the first two pages, 3 categories focused on the presentation style on the third page). Indicate your numerical choice for each section on the accompanying reporting sheet. You may use in-between numbers and you can write notes to clarify decisions (or as you are listening) on the reporting sheet.

The students will not see or receive these reporting sheets, so please be honest and as critical as you would be with any other scientific presentation.

Thank you for your help with this!!

Parts of the presentation categories (4):

<table>
<thead>
<tr>
<th>Category</th>
<th>POOR (0 pts)</th>
<th>FAIR (1 pt)</th>
<th>GOOD (2 pts)</th>
<th>EXCELLENT (3 pts)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Backgrounf section</strong></td>
<td>-Section (or key parts) missing</td>
<td>-Section present but has far too much or far too little information</td>
<td>-Section present with close to the appropriate amount of material (1 extra or too few slide)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>-Main Q and hypothesis not presented or nonsensical</td>
<td>-Text presented as paragraphs, no citations</td>
<td>-Main Q and hypo</td>
<td></td>
</tr>
<tr>
<td></td>
<td>-Excessive use of clipart/unrelated pictures</td>
<td>-Extra figures/clipart are added when not needed</td>
<td>-Clear main Q and hypo</td>
<td></td>
</tr>
<tr>
<td><strong>Methods section</strong></td>
<td>-Section missing</td>
<td>-Section present but too many experiments described/too much detail included</td>
<td>-Text presented as bullet points or complete sentences</td>
<td>-Most figures are of value to the audience</td>
</tr>
<tr>
<td></td>
<td>-Experiments not described or missing key points</td>
<td>-Too much text on slides, mostly paragraphs, no citations</td>
<td>-Appropriate citations included</td>
<td></td>
</tr>
<tr>
<td></td>
<td>-Excessive text</td>
<td>-No figures to clarify or figures do not aid in description</td>
<td>-Relevant figures used to illustrate key data (from other articles or constructed</td>
<td></td>
</tr>
<tr>
<td></td>
<td>-Too little explanation</td>
<td>-Student present minimal understanding of methods</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>-Student does not understand methods/cannot explain properly</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
| Results section | -Section missing  
-Figures presented without labels (axis, samples, units)  
-No accompanying text with figures  
-Significance not indicated | -Section present with some figures that are mostly appropriate for the data they are displaying  
-Too many/too few figures  
-Many labels are missing  
-Figures are complicated or require a lot of interpretation  
-No flow/consistency between figures  
-Accompanying text not sufficient or significance missing | -Section present with appropriate figures to display data  
-Figures are labeled (may be missing 1 or 2)  
-Figures are generally easy to read and interpret  
-Figure style is mostly consistent  
-Accompanying text is brief, but includes significance | -Section present with appropriate figures chosen  
-Figures used to present data including labels, titles, units and keys  
-Figures are easy to read (size, coloring) and style is consistent through talk  
-Accompanying text highlights key findings  
-Significance is indicated |
| --- | --- | --- | --- |
| Discussion section (includes conclusions and acknowledgements) | -Section (or key parts) missing  
-Results repeated, no conclusions or analysis  
-No future directions  
-No acknowledgements  
-Student did not understand the purpose of their research | -Section present, but missing one key part (no conclusions or no future directions or no acknowledgements)  
-Results repeated without stated conclusions  
-No future directions or no acknowledgements  
-Student demonstrates minimal understanding of the broad purpose of their research | -Section present with appropriate summary of conclusions and significance/interpretation  
-Results are needlessly repeated  
-At least one future experiment described  
-Lab/mentor are acknowledged  
-Student demonstrates solid understanding of their research as whole | -Section present with appropriate amount of information  
-A summary is given with the main conclusions and significance  
-Model presented for clarity  
-Future directions are described  
-Lab/mentor are thanked  
-Student demonstrates complete understanding of their research project |
### Presentation style categories (3):

<table>
<thead>
<tr>
<th>Category</th>
<th>POOR (0 pts)</th>
<th>FAIR (1 pt)</th>
<th>GOOD (2 pts)</th>
<th>EXCELLENT (3 pts)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>General slide design</strong></td>
<td>- Font size is too large or small</td>
<td>- Font size mostly okay</td>
<td>- Font size is appropriate</td>
<td>- Font size is appropriate</td>
</tr>
<tr>
<td></td>
<td>- Font is hard to read</td>
<td>- Appropriate font chosen</td>
<td>- Font is easy to read</td>
<td>- Easy to read font chosen</td>
</tr>
<tr>
<td></td>
<td>- Color scheme is distracting or hard to read, lots of distracting animation</td>
<td>- Color scheme not consistent at times, animation occasionally detracts from presentation</td>
<td>- Color scheme is easy to read and consistent, some animation included in presentation</td>
<td>- Color scheme makes presentation easy to follow and is consistent, animations are used to emphasize points</td>
</tr>
<tr>
<td></td>
<td>- Too many words and little use of visual cues/support on most slides</td>
<td>- Extensive use of words on most slides</td>
<td>- Predominantly appropriate mix of words and images on most slides</td>
<td>- Appropriate mix of words and images on every slide</td>
</tr>
<tr>
<td><strong>Verbal delivery</strong></td>
<td>- Student did not plan out what they wanted to say</td>
<td>- Student had rough outline for what they wanted to say, used notes whole time</td>
<td>- Student planned out what they wanted to say, occasionally glanced at notes</td>
<td>- Student planned out what they wanted to say, notes were not used</td>
</tr>
<tr>
<td></td>
<td>- Student was not able to convey purpose of presentation to audience, may have been confused about the conclusions</td>
<td>- While presenting the student was easily distracted and prone to tangents, hard to follow along to definite conclusion</td>
<td>- Presentation rarely strayed from topic, audience understood the conclusion</td>
<td>- Presentation was focused and the audience understood the conclusion</td>
</tr>
<tr>
<td></td>
<td>- Many fillers used</td>
<td>- Fillers used often</td>
<td>- Fillers used a few times</td>
<td>- Minimal filler</td>
</tr>
<tr>
<td></td>
<td>- Thoughts left unfinished</td>
<td>- Some complete thoughts, but some trailing off speech</td>
<td>- Most statements are complete thoughts</td>
<td>- Statements delivered as complete sentences</td>
</tr>
<tr>
<td></td>
<td>- Not loud enough for audience to heard, trails off while speaking, minimal enunciation</td>
<td>- Pitch, rate and emphasis are occasionally varied</td>
<td>- Voice is clear and strong with good enunciation and pronunciation</td>
<td>- Strong, loud, clear speaking voice with enunciation and correct pronunciation</td>
</tr>
<tr>
<td></td>
<td>- Pitch, rate and emphasis not varied</td>
<td>- Correct pronunciation used most of the time, some pronunciation</td>
<td>- Audience is engaged by some changes in pitch, rate and emphasis</td>
<td>- Pitch, rate and emphasis varied to engage audience</td>
</tr>
<tr>
<td><strong>Nonverbal delivery</strong></td>
<td>- Back mostly to the audience, or head down to avoid audience</td>
<td>- Occasionally turns back to the audience while speaking</td>
<td>- Faces the audience for the majority of the talk, some eye contact</td>
<td>- Faces the audience and “interacts” via eye contact</td>
</tr>
<tr>
<td></td>
<td>- Reads from the board and/or points to every word</td>
<td>- Pointer not used or used ineffectively</td>
<td>- Pointer occasionally used effectively</td>
<td>- Use of pointer to emphasize material</td>
</tr>
<tr>
<td></td>
<td>- Gestures are distracting or inappropriate</td>
<td>- Gestures are distracting or inappropriate</td>
<td>- Appropriate gestures used</td>
<td>- Appropriate gestures while speaking</td>
</tr>
</tbody>
</table>
Appendix 6: Post-assessment questions.

Name:
Please read each question and circle the answer that you feel is most accurate.

1) Could you name the parts of a scientific article before this summer?
   Yes       No

2) Can you name the parts of a scientific paper now?
   Yes       No

3) I was about to interpret basic scientific results at the beginning of the summer.
   Strongly Disagree      Disagree      Neutral      Agree      Strongly Agree

4) I feel comfortable interpreting basic scientific results now.
   Strongly Disagree      Disagree      Neutral      Agree      Strongly agree

5) I felt comfortable writing a scientific paper before Summer Focus.
   Strongly Disagree      Disagree      Neutral      Agree      Strongly agree

6) I feel comfortable writing a scientific paper after completing Summer Focus.
   Strongly Disagree      Disagree      Neutral      Agree      Strongly agree

7) I am more confident in my writing now that I have completed the Writing Course.
   Strongly Disagree      Disagree      Neutral      Agree      Strongly agree

8) I am more confident in my oral presentation skills now that I have completed the Writing Course.
   Strongly Disagree      Disagree      Neutral      Agree      Strongly agree

9) I feel that I have developed critical thinking skills.
   Strongly Disagree      Disagree      Neutral      Agree      Strongly agree

10) I feel that I can review and critique a peer’s work effectively.
    Strongly Disagree      Disagree      Neutral      Agree      Strongly agree
Appendix 7: Sample student writing pre-assessment responses and corresponding final papers (2).

Student responses to the writing pre-assessment prompt. These responses were directly transcribed and spelling and grammar mistakes were not edited.

Student 1:
I once read an article in 17 magazine about how people are too afraid to tan and are vitamin D deficient because of all the statistics about skin cancer. Although 17 isn’t a scientific journal, they referenced some government funded websites which added to their credibility. Therefore I assumed that at least their basic facts may be correct but further investigation was warranted. Later, I saw a segment on NBC about the same topic and their facts were similar. I think that while not everything may have been correct or may have been diluted down for the general public in the article, it communicated a basic level message effectively.

Student 2:
I read an article on how an old building downtown contained chemicals bad for our health. I considered it a credible source because it was published in the newspaper. I still feel the same about it because it had facts in it.
Tamoxifen Resistance Induced by EpCAM Control of Extracellular Signal-Regulated Kinase Activation

Student 1

Abstract
While Tamoxifen (TAM) is a popular breast cancer drug, its mechanisms of action and methods of resistance are not completely known. Previous experiments have linked TAM to an unknown protein that has been characterized as a cell cycle regulator. The epithelial cell adhesion molecule (EpCAM), which is overexpressed in most epithelial cancers, communicates with the mitogen-activated protein kinase pathway and is a logical intermediate in TAM induced apoptosis. To test whether EpCAM plays a role in TAM resistance, EpCAM negative and positive cell lines were treated with TAM and apoptosis was quantified via a trypan blue exclusion assay and Titer Glo assay. EpCAM negative cells showed an increase in cell death and sensitivity in response to TAM treatment, indicating that EpCAM overexpression may lead to TAM resistance in breast epithelial cells. Finally, ERK was shown to bind to the EpCAM promoter and serve as a marker for EpCAM overexpression. A better understanding of the EpCAM regulated pathways of TAM resistance may provide insight into additional treatments and increase breast cancer survival rates.

Introduction
Breast cancer prevalence in women is second only to skin cancers and is also the leading cause of cancer induced death. Tamoxifen (TAM) has been used for over 30 years and is still the most commonly used treatment for estrogen receptor (ER) positive breast cancers, but nearly 40% of individuals treated with adjuvant TAM eventually die from their disease. Many chemotherapy drugs are ineffective in treating these cancers due to the overexpression of oncogenes. These oncogenes often force cells to become resistant to drug treatment. Such might be the case with TAM, although the responsible oncogenes have not yet been identified. TAM was originally thought to work only by competitively binding to ERs in ER positive breast cancers; however, high concentrations are also effective in killing ER negative cells. Recently, TAM was shown to cause apoptosis through several different mitogen-activated protein kinase (MAPK) pathways in ER negative cells, specifically ERK and JNK. It is likely that similar pathways exist as a secondary means of inducing apoptosis in ER positive cells; however, specific pathways have yet to be confirmed. Not only has TAM been linked to inducing apoptosis, but it has also been shown to cause anti-proliferative effects. TAM has been shown to arrest cells in G0/G1 phases. Interestingly, ERK interacts with cell cycle arrest in many different types of cells including human breast cancer cells, further linking TAM and ERK pathways. This cytostatic action of TAM indicates an interaction with a cell cycle checkpoint regulating protein that has yet to be identified. Previous research has shown that the epithelial cell adhesion molecule (EpCAM) controls the function of many genes and is essential for normal cell growth and differentiation. EpCAM overexpression-which is common in breast cancer-has been shown to induce tumor progression and metastasis. EpCAM is a protein shown to possibly interact...
Figure 1: EpCAM negative cells show increased TAM-induced apoptosis. Trypan blue staining is evidence for EpCAM’s role in cell’s apoptotic signaling in response to TAM treatment. (A) EpCAM knock down increases the amount of cell death in MCF-7 cells. (B) EpCAM knock down increases the amount of cell death in T47D cells.

with many cell cycle checkpoints including cyclins D, A, and E. Further, studies have shown a marked down regulation of MAPK pathways in response to EpCAM knockdown, suggesting a large role for EpCAM in cell cycle progression.

The aim of this study is to determine whether EpCAM has a role in TAM resistance and briefly find an interaction with a signaling or apoptotic pathway in each cell line. Additionally, we wanted to determine what other proteins can serve as markers for EpCAM overexpression and therefore TAM resistance. EpCAM’s interaction with MAPK pathways and implications as a regulatory protein led us to examine its role in TAM resistance in two model breast cancer cell lines- MCF7 and T47D. We hypothesized that EpCAM regulates ERK and that overexpression of EpCAM causes TAM resistance. We also believe that ERK may regulate EpCAM expression by binding to the EpCAM promoter. Indeed, we found the presence of EpCAM increased TAM resistance via a trypan blue exclusion assay and Titer Glo assay. Western blotting of treated cells showed EpCAM presence reduced the amount of apoptosis as well as the amount of activated ERK in MCF7 cells.

T47D cell death was instead executed via apoptotic pathways. Chromatin immunoprecipitation of ERK showed binding to the EpCAM promoter. Therefore, low ERK levels and ERK mutations may lead to EpCAM overexpression and thus, TAM resistance.

Materials and Methods

Materials
Tamoxifen (TAM) (Sigma-Aldrich, St. Louis) was prepared in Dimethyl Sulfoxide (DMSO)(Sigma-Aldrich, St. Louis) and stock was stored at -20°C until needed.

Cell Culture
MCF7 and T47D cell lines were purchased from ATCC and maintained in Dulbecco’s modified medium (DMEM, Gibco) with 10% Fetal Bovine Serum (FBS)(Gibco) at 37°C in a humidified atmosphere at 5% CO₂ in multi-well plates (Sigma-Aldrich, St. Louis). Growth medium was replaced with serum-free medium for 24 hours before experiments were performed.

Lentiviral RNAi
ShRNA target sequences specific for EpCAM (5’-CTACAAGCTGGCCGTAAC-
3’ and 5’-ACTGGCTTTACCAATCTTG-3’)
and a scrambled control (SCR) were cloned
into a lentiviral vector previously. Lentiviral
shRNA and packaging constructs were
transfected into HEK-293T cells, and viral
supernatant was added to MCF7 and T47D
cell lines. Stable EpCAM shRNA cells were
selected in puromycin and confirmed for
EpCAM knockdown.

Trypan Blue Exclusion Assay
We aspirated serum-free medium and
added back 0.5mL of serum-free medium.
Cells were allowed to incubate for 2 hours and
were subsequently washed with 1X Dulbecco’s Phosphate Buffered Saline
(DPBS)( Gibco). Serum free medium was
added back to each well and 10µm TAM was
added to cells. Cells were placed in the
incubator for 1 hour. Subsequently, we washed
all cells twice with DPBS. For staining, we
added 0.3mL of 0.4% Trypan Blue Dye
(Sigma-Aldrich, St. Louis) directly to each
well on the plate. After 3 minutes of
incubation time, we washed cells twice with
DPBS. For viewing, 0.5mL DPBS was added
back to each well. Cell count was performed
using an EVOS microscope under 10x
magnification. Total number of cells and
number of blue cells were counted to quantify
percentage of death. Specifically, three fields
of view were captured per well and data from
six 250µm² squares from each field of view
were collected. The number of dead cells
divided by the total number of cells was used
to determine the death rate.

TiterGlo Luciferase Assay
Amount of apoptosis was confirmed
using the Cell Titer Glo assay (Promega,
Madison, WI). Cells were seeded at a density
of 5,000 cells per well in a 96-well plate.
Forty-eight hours after plating, cells were
treated with TAM in phenol red-free, serum-
free medium for the indicated time and doses.
MCF7 cells were treated with 1µm TAM and
T47D cells were treated with 2µm TAM for
the time dependent assay. All cells were
treated for 24 hours for the dose dependent
assay. Assay was carried out following
manufacturer’s instructions. Briefly, the assay
uses a reagent-luminometer system which
quantifies the amount of ATP present. The
lack of signal indicates a higher amount of cell
death.

Western Blotting
Cells were treated with 10µm TAM for
the indicated time points or vehicle (DMSO)
in 2mL serum free media (for 40 minutes).
Then we washed the cells with PBS and added
100µl lysis buffer (187.5 mM Tris-HCl, 6%
(w/v) SDS, 30% glycerol and 0.03% (w/v)
bromophenol blue). Lysate was scraped down
and placed in micro centrifuge tubes. We then
sonicated the samples for 30 seconds to reduce
their viscosity. Samples were boiled for 5
minutes at 98°C for 5 minutes and
subsequently replaced on ice. Proteins were
separated by electrophoresis on 10% SDS-
polyacrylamide gels (PAGEs, Novex) and
transferred to nitrocellulose membranes
(Millipore). Membranes were incubated in
blocking buffer (5% non-fat milk in Tris
Buffered Saline-0.05% Tween (TBS-T)) for 30
minutes. For the primary antibody, blots were
incubated for 2 hours at RT with either rabbit
anti-ERK1/2 (Cell Signaling) diluted in
blocking buffer at 1:1000, rabbit anti-phospho-
ERK (Cell Signaling) diluted in blocking
buffer at 1:2000, mouse anti-phospho-
JNK(Cell Signaling) diluted in blocking buffer
at 1:1000, mouse anti-EpCAM(Santa Cruz
Biotechnology) diluted in blocking buffer
at 1:1000, or rabbit anti-p38 MAPK(Cell
Signaling) diluted in blocking buffer
at 1:1000 to their
respective primaries. After secondary antibody incubation, the membranes were washed three times with 0.05% TBS-T. Mouse β-Actin HRP (Santa Cruz Biotechnology) was used as an internal loading control and applied for 15 minutes diluted in blocking buffer at 1:500. Chemiluminescent Substrate (Thermo Scientific) was used to visualize bands on autoradiography film (Midsci).

**Crystal Violet Staining**
Cells were treated with 1nm TAM in serum-free medium for one week with two media changes. After incubation time, we washed cells twice with chilled PBS. We then fixed and stained with 0.5% Crystal Violet (Sigma-Aldrich, St. Louis) for 10 minutes. Then cells were washed until no violet stain remained. Pictures were captured using an EVOS microscope under 4x magnification with a 498nm filter.

**Chromatin immunoprecipitation assay**
MCF-7 cell were processed for chromatin immunoprecipitation assay at 8 h using the Millipore chromatin immunoprecipitation kit. Briefly, confluent MCF-7 cells were fixed with 1% formaldehyde and lysed. After sonication, ERK-bound DNA was immunoprecipitated with an ERK antibody, washed, and eluted in 1% SDS, 0.1 mol/L NaHCO3. Reverse cross-linked DNA was purified using DNA columns and analyzed by PCR analysis using EpCAM promoter-specific primer sequences.

**Results**

**EpCAM expression induces TAM resistance**
To examine the rate of EpCAM negative cell death in response to TAM treatment, a trypan blue exclusion assay was performed. Several treatment points were used, optimized for each cell line. The difference in MCF7 cell death was clearly shown at 20 minutes TAM treatment (Fig. 1A). T47D cells were treated for longer time points to show a cast difference in EpCAM negative and SCR cells (Fig. 1B). Both cell lines showed an increased amount of cell death when EpCAM was knocked down and TAM was added in a does dependent manner. These results demonstrate EpCAM’s protection of cells from TAM treatment.

**Ablation of EpCAM increases sensitivity to TAM**
These results prompted an investigation of the amount of TAM that could elicit a different response in EpCAM negative and SCR cells. Cells were treated either concentration dependently (at a fixed time of 24hrs) or time dependently (at a fixed concentration of 1µm TAM for MCF7 and 2µm TAM for T47D) for the indicated time/concentration values (Fig. 2). Raw data are plotted along with a linear best fit line. Generally, EpCAM negative cells had lower luciferase counts, indicating higher rates of cell death, which supported the trypan blue assay findings. Even short time points and low concentrations were enough to provoke a difference in EpCAM negative and SCR MCF7 cells (Fig. 2A-B). As with the trypan blue assay, MCF7 cells were more susceptible to TAM than T47D cells. After 24hrs, the difference between MCF7 EpCAM negative and control cells’ survival rate was over 70%. T47D appeared to require 1µm TAM for a true difference to be visible; however, little change was seen in the time dependent assay (Fig. 2C-D). While MCF7 showed a clear difference in cell death between EpCAM negative and control cells, T47D’s differences may not be as significant. This may be due to different mutations which distinguish each cell line.

**EpCAM shRNA and TAM treatment increased ERK1/2 Phosphorylation**
EpCAM levels show efficiency of shRNA and verigy knockdown of EpCAM (Fig. 3A). MCF7 cells treated with TAM expressed phospho-ERK dose dependently
Figure 2: Removal of EpCAM increases sensitivity to TAM. Luciferase assay quantifying the level of ATP (and therefore cell survival) was higher in EpCAM expressing cells than those with the knockdown gene. (A-D) ATP decreased in a time (A&C) or dose (B&D) dependent manner in MCF-7 (A-B) or T47D (C-D) cell populations.

Figure 3: EpCAM knockdown increases activation of ERK in MCF-7 and apoptotic pathways in T47D following TAM treatment. SCR and SH1 EpCAM knockdown cells were treated with 10µm TAM for indicated time points. Cells treated with DMSO were used as controls (Veh). Proteins from cell lysate were resolved via SDS-PAGE electrophoresis. (A) EpCAM levels are evidence for efficiency of shRNA. (B) Total ERK is not changed during TAM treatment. (C) Phospho-ERK increases in MCF-7 but not T47D EpCAM knockdown cells after TAM treatment. (D) Equal actin levels serve as an internal loading control. (E) Phospho-JNK levels were not detectable in MCF-7 cells following TAM treatment. Phospho-JNK is present in T47D cells following TAM treatment and is increased in EpCAM negative cells. (F) p38 levels were not detectable in MCF-7 cells. p38 is present in T47D cells following TAM treatment and is increased in EpCAM negative cells.
Figure 4: EpCAM knockdown increases the cytostatic effect of TAM. Treatment of 1nm of TAM for one week reduced the total number of cells and therefore proliferation rate. Cell populations with EpCAM knockdown and treated with TAM had fewer cells than SCR cell populations treated with TAM.

(Fig. 3B). MCF7 EpCAM negative cells showed an increase in phospho-ERK expression indicating that EpCAM plays a role in limiting ERK phosphorylation following TAM treatment (Fig. 3B). All cells express the same level of total ERK (Fig. 3C). Actin levels serve an internal loading control (Fig. 3D). T47D cells show phosphorylation of JNK in a dose dependent manner with an increased amount in EpCAM negative cells (Fig. 3E). Similarly, T47D EpCAM knockdown cells showed elevated levels of p38 compared to control cells (Fig. 3F). ERK activation along with previous data indicating a higher amount of apoptosis suggests that over phosphorylation of ERK is the method of apoptosis in MCF7 cells. Conversely, T47D cells show the activation of traditional apoptotic pathways and have no relation to ERK activation. Lack of activation of these pathways in cells that express EpCAM indicates that EpCAM contributes to TAM resistance in both ERK activation driven apoptosis and traditional apoptosis.

EpCAM expression induces resistance to cytostatic effects of TAM

To examine the cytostatic effect of TAM treatment on EpCAM negative cells, a crystal violet assay was used. When SCR cells were treated with TAM, some cytostatic effects became evident but cells without EpCAM had much more pronounced cytostatic effects (Fig. 4). There were fewer cells in EpCAM negative cell lines treated with TAM when compared to TAM treatment of SCR cells (Fig. 4). This indicates that EpCAM plays a vital role in resisting TAM arresting cells. Cells expressing EpCAM proliferate at a higher rate than those without EpCAM when treated with TAM. This suggests that EpCAM expressing cells are not only resistant to apoptosis, but are resistant to the cytostatic effects of TAM.

**ERK2 binds to the EpCAM promoter**

A chromatin immunoprecipitation assay revealed that ERK binds to the EpCAM promoter (Fig. 5). EpCAM specific primers were used to ensure that DNA was amplified only if it was the target sequence. A whole cell extract served as the 5% input to normalize the data. Pulldown with antibodies to IgG served as a negative control. There was over 200% more DNA detected with SYBR green in the DNA pulled down with Flag-ERK than the non-specific IgG (Fig. 5). This data confirms that ERK binds to the EpCAM promoter.

**Discussion**

These results are evidence that EpCAM plays an ERK dependent role in TAM
induced apoptosis and that ERK regulates EpCAM expression. Kinetic studies of tamoxifen action reveal T47D to be more resistant to TAM than MCF7. T47D may be naturally more resistant to apoptosis due to mutations which distinguish the cell lines. EpCAM negative cells decrease the minimum amount of TAM needed to induce apoptosis in both cell lines. Because EpCAM knockdown cells are more susceptible to apoptosis following TAM treatment, it is likely that cells which overexpress EpCAM may be protected against TAM induced apoptosis. This protection from death translates to TAM treatment resistance in EpCAM positive or overexpressing cells. The presence of phospho-ERK in response to TAM treatment indicates a regulatory role for EpCAM in ERK phosphorylation, but the mechanism linking the two is not defined. Since EpCAM is not a kinase, this deactivation must be indirect. Over-activation of ERK has been shown to be necessary in inducing apoptosis of cancer cell lines. Therefore, the increase in ERK indicates a higher rate of cell death in EpCAM negative cells. It also suggests that without EpCAM, ERK is not effectively regulated. This loss of regulation may cause the over activation of ERK which induces apoptosis in EpCAM negative cells. The over-activation of ERK induced apoptosis only in MCF7 cells which was not the case for T47D cells. Instead, T47D cells carried out apoptosis through traditional apoptotic pathways. While other papers have indicated p38 and JNK as being altered with TAM treatment, our results only showed activation in T47D. This might be due to the use of different cell lines and treatment times or doses.

EpCAM not only protects against TAM’s apoptosis-inducing characteristics but also hinders the cytostatic action of TAM. EpCAM expressing cells are then able to proliferate and become invasive faster than those that do not express EpCAM. Finally, the finding that ERK binds to the EpCAM promoter explains the previously documented inverse relationship between ERK and EpCAM expression. It also suggests that increased expression of ERK may reduce transcription of EpCAM but further experiments are needed to determine how ERK acts in binding to the promoter. Low levels of ERK then may serve as a marker for EpCAM overexpression and indicate cells that resist tamoxifen treatment. Understanding EpCAM’s role in tamoxifen induced apoptosis may allow for double pronged treatment methods which could inhibit EpCAM expression, possibly via upregulation of ERK expression for patients suffering from epithelial cancers which overexpress EpCAM.
Ultimately, these findings will aid in decreasing tamoxifen resistance in patients suffering from breast cancer.

Acknowledgements
The utmost thanks go out to Dr. Gillanders and Dr. Fleming for offering this opportunity. The author thanks her mentor, Dr. Sankpal, and tutor, Elizabeth Clipperton, as well as everyone involved in The Young Scientist Program 2012.

References
Developing a Screening Test for Thrombotic Thombocytopenic Purpura Using a TrxVWF82 Fusion Protein

Student 2

Abstract
Thrombotic Thombocytopenic Purpura (TTP) is a rare disorder that can be life threatening if it is untreated. It has many symptoms, which makes it easy to confuse with other diseases. Using a TrxVWF82 fusion protein, we plan to develop an assay to test patients’ ADAMTS13 activity to determine whether they have TTP or another disease. Using SDS-PAGE gels and Western blots, we analyzed the patients’ ADAMTS13 activity. We found that patients who do not have TTP have smaller fragments of VWF protein, while those who have it have the large fragment of TrxVWF82 protein. This study could lead to faster identification of TTP patients and help decrease morality rates.

Introduction
Thrombotic thrombocytopenic purpura is very rare, with an incidence of 3 per 10 million residents per year in the United States, but it is also deadly. Its symptoms include thrombocytopenia, microangiopathic hemolytic anemia, neurological symptoms, and renal dysfunction. TTP causes a patient's blood to clot in smaller arterioles and capillaries. The von Willebrand factor (VWF), a protein that mediates platelet adhesion, is the causative agent.

In healthy people, VWF is what prevents excessive bleeding. When there is an open wound, it forms multimers that adhere to platelets to seal the wound. But in patients with TTP, the VWF forms large multimers that are not necessary, which circulate through the blood and keep getting bigger until they get stuck in small capillaries. This happens because patients with TTP have an ADAMTS13 deficiency. ADAMTS13 is a metalloprotease, which cleaves ultra-large VWF multimers. It cleaves VWF at the Tyrosine1605-Methionine1606 bond within the A2 domain of the VWF protein. Without ADAMTS13, VWF forms large multimers without being cleaved, which causes blood clots.

It is very important that TTP patients get immediate treatment. Those who go without treatment have a 10-15% chance of surviving, but when treated, their chances improve to 80-85%. Due to the lack of an easy way to test patients in hospitals, doctors have to wait for results from specialized research labs, which are not very common. This can cause doctors to give an inaccurate diagnosis. We plan to develop an assay for clinical institutions to test patients and have results as soon as possible, without the use of a research lab.

In order to test patients, we developed an ADAMTS13 assay which uses a TrxVWF82 fusion protein to test the activity of the ADAMTS13 in patient plasma. We hypothesize that if we use a western blot, patients with TTP will have a 27kDa TrxVWF82 protein, instead of two smaller fragments, one 20.2kDa and the other 6.9kDa. We have seen that some patients have a protein fragment slightly larger than the 5kDa marker, and some do not. The patients with no small fragment likely have TTP, and can begin treatment for it.

Materials and Methods

Media and Plates
We used Luria-Bertani (LB) (Sigma) to make our media and plates. We used 500ml of LB media with Agar (Invitrogen) with an ampicillin concentration of 100ug/ml and made 1L of the media, with no ampicillin added.

VWF82 Cloning
To clone the VWF82 peptide, we used a Polymerase Chain Reaction (PCR) Kit (QIAGEN). We used the protocol and normal PCR cycles. We analyzed PCR product on 1% agarose gel with ethidium bromide. We purified the PCR product using the purification kit (QIAGEN), analyzed the product on an agarose gel, and extracted the DNA using gel extraction
kit (QIAGEN). After we isolated the DNA, we treated the VWF82 with T4 DNA Polymerase and incubated with a pET-32 vector (Novagen). When the annealing process was complete, the vector was transformed into GigaSingles cells (Novagen). We grew the cells overnight on LB plates, and the cells that grew were inoculated in LB media with 100ug/ml ampicillin and incubated overnight. Then, after plasmid purification with the QIAprep Spin Miniprep Kit, the plasmid size was confirmed using a 1% agarose gel. The plasmid sequence was verified with DNA sequencing.

**TrxVWF82 Protein Expression**

To perform protein expression, we transformed the pET-32 plasmid with the correct DNA sequence to BL21 (DE3) cells. The cells were grown in LB media with ampicillin and induced with IPTG. The induction lasted for 2h and 30min, and samples were taken each 30 min. We centrifuged the cells to remove the supernatant, and used the Bacteria Protein Extraction Reagent protocol (B-PER) (Pierce).

**TrxVWF82 Protein Purification**

To purify the protein, we used a His-tag column with sepharose matrix. We washed the column with the binding buffer (10mM imidazole), and then applied the TrxVWF82 protein from expression. We washed with binding buffer, and collected samples from the flow through and wash. Then we eluted the column with elution buffer (300mM imidazole). We used a spectrophotometer to determine the OD of the protein elution fractions and combined the samples with highest concentration and lowest concentration in two different tubes. We analyzed samples from each step of the purification on SDS Page gel and analyzed by western blot. In our second assay, we used 10ul of patient plasma and normal plasma. We then added 600ug of desalted TrxVWF82 protein diluted 1:20 with the same buffer to each sample. The samples were then incubated at room temperature for 4 hours and moved to a 37°C water bath for one hour. They were analyzed by western blot. In our third assay, we used 600ug of desalted TrxVWF82 protein in HEPES buffer in 10ul of patient plasma, with the addition of protease inhibiting cocktail. The samples were incubated at 37°C for 5 hours and analyzed by western blot.

**Western Blot**

After the gel was transferred to a PVDF membrane, it was washed for 5 min using Phosphate Buffered Saline (PBS) made in the lab. We then washed the blot for 1 hour with casein blocker, and sealed the blot in a package with 1ul of A2 VWF antibody and 10ml of casein blocker. This was stored overnight at 4°C on a shaker. The blot was washed 4 times for 10 minutes with PBS .001% tween. After that we added 10ml of casein blocker and 1ul of goat anti-mouse antibody and put on shaker for 2 hours. Then we washed the blot again, and used ECL kit (Invitrogen) for illumination.

**Results**

**Creation of TrxVWF82 Fusion Protein**

Amplification of theVWF82 was performed and inserted into the Novagen pET32 Xa/LIC vector. We confirmed the insertion on an agarose gel (Figure 1) and sent functioning properly as a substrate. We incubated the TrxVWF82 protein with ADAMTS13 (used stock or 1:20 dilution) at 37°C for 1 hour. The proteins were then analyzed on a SDS Page gel to check for protein cleavage.

**Patient Plasma Screening**

To test the activity of the ADAMTS13 within the patient plasma, we gathered 6 patient plasma samples, and one normal plasma sample and incubated them with purified TrxVWF82 for 5 hours in a 37°C water bath. The samples were run on a SDS-PAGE gel and analyzed by western blot. In our second assay, we used 10ul of patient plasma and normal plasma. We then added 600ug of desalted TrxVWF82 protein diluted 1:20 with the same buffer to each sample. The samples were then incubated at room temperature for 4 hours and moved to a 37°C water bath for one hour. They were analyzed by western blot. In our third assay, we used 600ug of desalted TrxVWF82 protein in HEPES buffer in 10ul of patient plasma, with the addition of protease inhibiting cocktail. The samples were incubated at 37°C for 5 hours and analyzed by western blot.

**ADAMTS13 Assay**

We used the ADAMTS13 assay in order to check if the TrxVWF82 fusion protein was...
Figure 1: PCR and Insertion to Vector. The PCR (A) produced the expected size DNA, around 246 bp. The insertion of the VWF82 DNA (B) also produced expected band sizes, around 6000 bp.

Figure 2: The BL21 (DE3) cells were induced using 1mM IPTG (final concentration). Samples taken after each 30 minutes show the increase in the amount of VWF82 protein around 27 kDa, the size of our protein.

Figure 3: Purification (A): The supernatant (1) had many contaminating proteins. A large quantity of the VWF protein was bound to the column, as you can tell from its absence from the flow through (2) around the 25 kDa marker. Contaminating proteins were mostly cleaned out in the first 20ml wash (3), and nearly completely purified in the second 30ml wash (4). The elution (5) had a large quantity of the cleaner TrxVWF82 protein. HPLC purification (B-C): The VWF protein was able to be purified further through High Performance Liquid Chromatography. In the first run (B), the protein was still slightly contaminated, but after the second run (C), it became much more pure.
the plasmid for sequencing. We were able to induce expression of the VWF82 protein in the BL21 (DE3) cells using IPTG (Figure 2). The protein was successfully purified with a His-tag column and even further purified using the HPLC (Figure 3).

**ADAMTS13 assay**

The ADAMTS13 assay showed that our VWF substrate was functioning properly. The cleaving site in the TrxVWF82 protein split the protein into one 6.9kDa fragment and another 22.2kDa fragment (figure 4), as expected. The digestion worked as well with stock ADAMTS13 as with a 1:20 dilution of ADAMTS13.

![Figure 4: ADAMTS13 Assay.](image)

In the third assay, the TrxVWF82 was cleaved and the 2 fragments were more visible than in the first assay (Figure 5).

**Discussion**

The purpose of this project was to develop a simpler way to screen patients for TTP. We hypothesized that if our TrxVWF82 protein was incubated with patient plasma sample, TTP patients would have no cleavage of the TrxVWF82 fusion protein. Our VWF peptide was successfully expressed, isolated, and used in *in vitro* assays. The first assay showed signs of cleavage, but because the TrxVWF82 protein has a Xa cleavage site, it could have been cleaved by another protein in the plasma (Muia, J., personal communication). This means that even though there was cleavage, we cannot say for sure it was the ADAMTS13. The second assay brought cleaner results, but with no cleavage. We think this happened because the samples were left at room temperature for their incubation period, whereas they are normally incubated at 37°C. This could have slowed the process down, resulting in no cleavage.

The final assay brought more definite results. We could see the small and large TrxVWF82 fragments much more clearly, and we’re sure that only the ADAMTS13 cleaved it because of the protease inhibiting cocktail we added. From the results of this assay, we can say that we have successfully developed an assay that is capable of diagnosing a patient for TTP.

In other research labs, there are similar ideas for developing a screening assay for TTP, but in comparison, ours seems to be the least complex. The first generation assays incubated the patient plasma and VWF protein for 24 hours, while our TrxVWF82 assay reduced the time to 5 hours. The assay developed by Zhou and Tsai was made not only to identify whether the patient has TTP, but if they are a carrier for the ADAMTS13 mutation as well. This could help identify patients who have a chance of developing TTP, because the data suggested that people who carry the mutation only have about half as much ADAMTS13 activity. This

**Patient Screening**

In our first assay, a smaller fragment of TrxVWF82, a sign of cleavage, was detected through western blot. The fragment was around the 5kDa marker, but there were no inhibitors added in this assay. In the second assay, there were no signs of cleavage in any plasma samples, including the normal plasma samples.
Figure 5: Patient screening. We used a western blot to compare patient plasma to normal plasma samples. We can then determine whether the patient has TTP. In western blot A we used a control that should cleave, and also used normal plasma samples. Some patient samples showed signs of TTP, such as lane 3 or 4. In western blot B, we saw that most patients that showed signs of TTP showed the same signs in this test, having little or no cleavage.

assay takes around 7 hours to perform.

The collagen binding assay is another test that can indirectly measure ADAMTS13 activity. Larger VWF proteins have a higher affinity to the collagen than smaller fragments, making ADAMTS13 activity measurable by the size and amount of VWF bound to the collagen. This experiment takes around 6 hours to perform, and does not directly test for ADAMTS13 activity. These are all helpful ways of identification, but the performance time limits the use of the assay clinically. Our assay provides a simple, more direct way of measuring ADAMTS13 that can be performed quickly, making it more useful for clinical applications.

Identifying TTP patients can be extremely difficult for doctors who have no way of testing the patient. There are many disease that are easily confused with TTP because of similar symptoms, such as hemolytic uremic syndrome, Evans syndrome, and HELLP syndrome. With a reliable assay available for testing the patients, better diagnoses can be made, which can save many lives. I believe that if we want to improve our assay, we will need to conduct a blind experiment in which we use more patient samples to test the accuracy of the assay. Such an experiment would provide strong support for the TrxVWF82 assay and be the first step for promoting its use in clinical settings.

References
8. Zhou, W. & Tsai, H. An enzyme immunoassay of ADAMTS13 distinguishes patients with thrombotic thrombocytopenic purpura from normal individuals and carriers of


Autoantibodies against vWF-binding TSP8 domain of ADAMTS13 in TTP patients

Sadler’s Lab
Student 1
August 2, 2013
Thrombotic Thrombocytopenic Purpura (TTP)

- Clots form in vessels cutting off blood supply to other parts of the body causing vessels to burst open
- 95% mortality rate of untreated cases
- Two causes of TTP:
  - genetic mutation in ADAMTS13
  - autoantibodies against ADAMTS13.
Von Willebrand Factor & ADAMTS13

- Von Willebrand factor (vWF) is a protein that helps with clot formation to repair vascular injury.
- ADAMTS13 is an enzyme that cleaves vWF.
- vWF binds at the TSP8 domain.
ADAMTS13 & Von Willebrand Factor

http://www.drugswell.com/winow/+%20b19/20Antibody-Mediated%20Thrombotic%20Disorders.htm

http://stroke.ahajournals.org/content/43/2/599/F1.expansion.html
Main Q & Hypothesis

- **Main Q:** How does the C-terminal domains regulate ADAMTS13 activity?

- **Hypothesis:** Autoantibodies produced in TTP patients block the TSP8 domain of ADAMTS13, making vWF unable to bind.
Cloning TSP8 domain of ADAMTS13

- PCR of TSP8 gene of ADAMTS13
- Ligation-independent cloning into plasmid vector
- Has a **his-tag** for protein purification

1.8% Agarose gel that shows TSP8 PCR product at 339bp
Expression and Purification of TSP8 protein

- Transformed Rosetta BL21(DE3) cells
- IPTG used to induce expression
- Purified TSP8 by metal ion affinity chromatography

4%-12% SDS-PAGE gel shows purified TSP8 at 32 kDa
Immunoprecipitation & blotting for TSP8

- Mixed monoclonal antibodies, protein G magnetic beads, and TSP8
- Western blot to see if antibodies bound TSP8
- Only anti-TSP8 captured TSP8*
Conclusion

- Our results support our hypothesis that antibodies can be detected by TSP8 domain of ADAMTS13
- We developed method to detect antibodies against TSP8
- Monoclonal antibodies were able to capture TSP8
Future Direction

- The method we developed can be used to screen autoantibodies in TTP patients.

- We will try to test if polyclonal antibodies can detect TSP8.
Acknowledgements

- Dr. J. Evan Sadler
- Sadler’s lab
- My mentor Joshua
- My tutor Annelise
- Elizabeth and Brian
- All SF volunteers
Alpha Beta Hydrolase Domain Protein 13 as a Regulator of Neuronal Protein Lipidation

Student 2
Blumer Lab
08/02/2013
Palmitoylation Regulates R7BP

Why is this important?

• Necessary for neuronal function
• It’s reversible, but mechanisms not well known
• Potential drug targets for neurological disorders
Question

* Does Alpha Beta Hydrolase Domain Protein 13 (ABHD13) depalmitoylate R7BP?
Outline

* Determine the Localization of R7BP with ABHD13 overexpression
* Label the palmitate attached to R7BP
* Determine Knockdown constructs of ABHD13
Overexpression of ABHD13 does not affect Localization of R7BP

RFP-R7BP  Merge

GFP

GFP-C-ABHD13

GFP-N-ABHD13

Localization of R7BP

Percentage

0% 10% 20% 30% 40% 50% 60% 70% 80% 90% 100%

Diffused

Nucleus Not Expressed

Nucleus Expressed

GFP  GFP  GFP
<table>
<thead>
<tr>
<th></th>
<th>0 hr</th>
<th>2 hr</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>GFP</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>ABHD13</strong></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Labeling of Palmitate

**Expected**

**Actual**

64 kDa
Knockdowns of ABHD13

<table>
<thead>
<tr>
<th></th>
<th>C</th>
<th>sh1</th>
<th>sh2</th>
<th>sh3</th>
<th>sh4</th>
<th>sh5</th>
</tr>
</thead>
</table>

- Knockdown constructs 3 and 4 reduce expression of ABHD13
No evidence supports that overexpression of ABHD13 enhances R7BP depalmitoylation

Effective Knockdowns of ABHD13 were established

Future Research: Effects of knockdowns on R7BP depalmitoylation
Acknowledgements

* My Mentor: Lixia Jia
* My Tutor: Kevin Flanagan
* Blumer Lab:
  * Kendall Blumer
  * Hao Zhou
  * Mohammad Maktabi
  * Kevin Kaltenbronn
  * Matthew Cain
* Michi Kanai
* Sakshi Uppal
* Stephanie Scherer
* Patrick Osei-owusu
* Young Scientist Program