



Cornell University

2017 Responsible Conduct of Research (RCR) Symposium: Rigor and Reproducibility January 23, 2017

INTRODUCTION

The goal of this symposium is to promote discussion of rigor and responsibility in research. It is not essential that each group discuss all of the cases or all of the scenarios in each case. For some of the questions that may come up, clear ethical and professional considerations may be apparent that should lead one to a straightforward answer. In other cases variations in practices may occur across disciplines, or a spectrum of answers may be acceptable, depending on the circumstances, and therefore no one “right” or “wrong” answer is obvious.

In discussing the cases, focus on why an action is acceptable or unacceptable:

- Who has a stake in the action?
- What might be the consequences of the action?
- What might be the obligations of the protagonist?
- What professional norms and values might give rise to those obligations?

At the end of the 90 minute discussion period, each group should send in responses to as many questions posed at the end of the cases as they have been able to explore. It is not necessary to respond to all the questions or to have complete answers. All answers will be discussed at the end, in a general session.

After the symposium, each of the graduate student or postdoc participants should lead or facilitate a discussion in his/her own research group, similar to the one in which the student or postdoc participated in the symposium. The Principal Investigator for the research group, and Director of Graduate Studies (or someone designated by the DGS), may want to record which research groups carried out such discussions, and may want to collect feedback on how successful this exercise turns out to be. Such data may be useful for NIH Training Grant applications.

CASE STUDIES

Case 1: The elusive P-value

Alex is a second year graduate student in Professor Wells's lab. The research involves testing the drug Wegrotuximab, a novel potential inhibitor of epidermal growth factor signaling (EGF). EGF signaling appears to contribute to tumor growth in several cancers by increasing cell division and survival.

There is preliminary evidence from previous experiments in Professor Wells's lab and from other labs that this inhibitor will result in slower cell proliferation, thus making it a candidate anti-cancer drug.

Alex wants to make sure that she has everything in order so that she does not waste any trials; she cannot afford to make any mistakes because Wegrotuximab is not only very expensive but also is difficult to obtain; it might take several weeks before more will be available. Alex carried out experiments with two plates of cultured human cells growing in parallel in a solution that is used to keep such cells alive. One plate of cells included the drug Wegrotuximab ("drug treated"), and the other plate ("control") had the same conditions but with no drug. She counted the number of cells in each plate after 24 hours. Alex repeated this experiment three times. After the 24-hour treatment, the drug treated plates showed a reduction in the number of the cells compared to the control. A t-test to see if the magnitude of cell proliferation was significantly different between the drug-treated and control wells showed a pvalue = 0.1. Professor Wells had stated that in order for experimental results to be publishable, a t-test must show a minimum p-value of 0.05. The lab has only enough Wegrotuximab to test the number of cells in two more plates.

Alex mentions the results and her dilemma to her colleague Pam. Pam tells her that this is a common problem but that there is an easy solution. She suggests that Alex add to the data that she has already collected by doing the experiments with the remaining drug, in order to see if the p-value drops below 0.1. Alex reminds Pam that she doesn't have enough compound to repeat her three experiments. Pam clarifies that he is not suggesting she repeat the entire set of experiments, but rather that she add to her existing data. Alex doesn't recall coming across this method in her data management class, and wonders if this approach is on the "up and up"! Pam tells her that this is totally fine because it is not as if she is making up data or falsifying it; she is just working with the constraints that she is under and making the most judicious use of the limited resources to get the best results. This makes sense to Alex and it seems smart to do only as many experiments as necessary. If she grows two more plates of cells and treats them with Wegrotuximab, and also grows two more plates of cells as controls without the drug and the results prove to be similar to the previous experiments, she might have enough data to bring the p-value down to 0.05. If the p-value drops below 0.05, Alex thinks that she will have the results she needs to present to Professor Wells. If she doesn't see a reduction in the p-value, she can continue to do more experiments to add to her data. Given the results to date and the evidence from previous studies in the lab, Alex thinks that it is just a matter of time before she will get the p-value that will satisfy Professor Wells.

Discussion Questions:

- What are your thoughts on the approach of “checking as you go and stopping when you get the desired result” in research? Do you agree with this approach? Why or why not?
- Should Alex have set up her experiment differently from the outset? If so, how?
- What would you advise Alex to do now?

Case 2: Have blood, will clot

Sam is testing the hypothesis that Ancrod, a snake venom, will help reduce blood clotting in a model of Polycythemia vera, a disease characterized by having too many red blood cells in which patients suffer from blood clots and poor circulation. In order to test this hypothesis, he uses a mouse model and designs an experiment that requires multiple steps (Figure 1):

- To induce the disease, mice are given a bone marrow transplant from transgenic donor mice that have a mutated JAK2 gene that, when expressed, will cause the overproduction of red blood cells.
- These animals then receive an intraperitoneal (IP) injection of tamoxifen, which triggers the production of the mutated JAK2 protein. This triggers the onset of the disease and the disease progresses over the course of two weeks, during which time the animals overproduce red blood cells. This, in turn, leads to increased clotting and related symptoms.
- The animals are then treated for three weeks with a single IP injection of Ancrod, or of a control solution.
- At the end of the treatment period, the mice are sacrificed and the blood is collected from the heart for use in clotting assays that can report how long it takes for blood to clot.



Figure 1. Experimental timeline

Sam's PI, Dr. Morse, was able to get 20 mice from other labs at the university for free. The documentation that came with these mice suggested that they were 9-10 weeks old and about half were male. Sam began his experiments right away.

**** NOTE: Experimental details and results are described in Tables 1 and 2.**

Twenty mice received the bone marrow transplant. Unfortunately, three died during recovery from the procedure. Sam injected all of the surviving animals with tamoxifen. After two weeks, he injected 9 of the remaining animals with Ancrod and the remainder with a control solution. Over the next two weeks, some animals died and a couple of the treated animals looked sick; they weren't moving and their breathing was labored, so rather than risk them dying before he could get the data, Sam took their blood right away and ran the clotting assay. The remainder of the animals made it to the end of the planned treatment period. When Sam took blood from one of the treated animals, the needle seemed to clog and he just couldn't get the blood out, so he could not get any data from this mouse. Once he had all the blood from all the mice that he could get, Sam ran his analysis and was thrilled to find that while the average clotting time for the control group was only 133s, the clotting time for the treatment group was 162s with a p-value of 0.05 (Figure 2). He drew the tentative conclusion from these data that Ancrod does help with clotting problems in Polycythemia.

When Sam presented his results at the next lab meeting, Nina, a post-doc in the lab asked about the high rate of animal death and the animals that look sick and wondered if the Ancrod itself

might be compromising mouse health. She asked if Sam had taken into account the data from the sick mice. Sam said that he had and that his calculations showed that if he excluded data from those mice, the p value would rise from 0.05 to 0.13.

Dr. Morse asked Sam about the treated animal with the failed blood harvesting, wondering if there was a reason he couldn't get the blood out. "Do you think you put the needle in the wrong place", she asked. Sam was pretty sure that he hadn't. "What if the treatment did not work on this animal so that the blood still clotted quickly and that was the reason the blood extraction failed? Then we would have eliminated an animal that suggests the opposite conclusion. Perhaps Ancrod does not always help with clotting in this disease?"

As if that weren't enough, Ming, his fellow student, said that she recently read a paper suggesting that clotting can depend on sex and asked him if he had taken the sex of the animals into account. Sam hadn't. He was crestfallen; his victory was starting to sound like a defeat. He went back and considered the sex of the samples and found that most of the control animals were female.

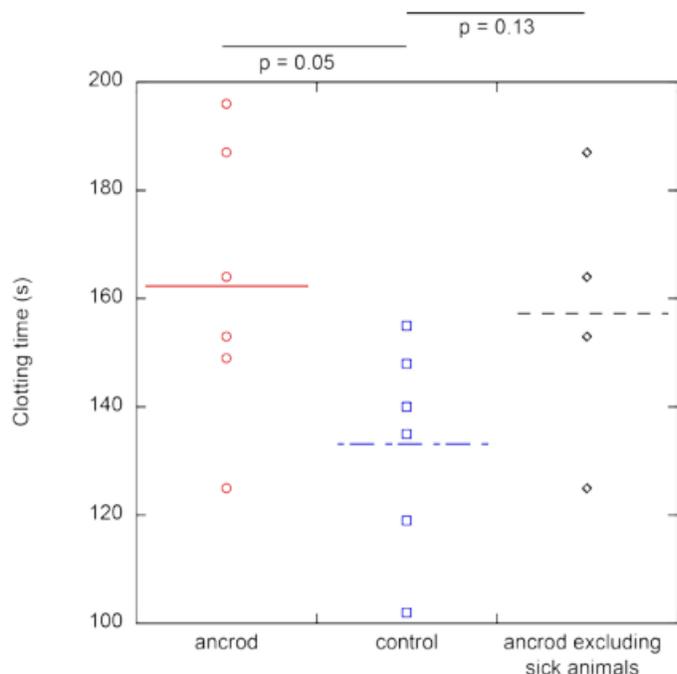


Figure 2. Clotting times for treated group and the control group. Also shows the treated group with the sick animals excluded. Lines indicate means. Each dot is a measurement from one mouse.

Table 1. Treatment and outcomes by mouse

Mouse ID	Sex	Day 0: Bone Marrow Transplant	Week 1-6: recovery	Week 7-8: JAK2 expression	Week 9, Day 1: Treatment	Week 9-11: Observation	Week 12, Day 1: Measurement of Clotting time (seconds)
1	m	Successful		Injected with Tamoxifen	Drug (Ancrod)		125
2	m	Successful	died	NA	NA		NA
3	f	Successful		Injected with Tamoxifen	Drug (Ancrod)		153
4	f	Successful		Injected with Tamoxifen	Drug (Ancrod)	Died	NA
5	m	Successful		Injected with Tamoxifen	Drug (Ancrod)	Died	NA
6	m	Successful		Injected with Tamoxifen	Drug (Ancrod)	Looked sick so took blood on Week 10	149
7	f	Successful		Injected with Tamoxifen	Drug (Ancrod)	Looked sick so took blood on Week 10	196
8	f	Successful	died	NA	NA		NA
9	m	Successful		Injected with Tamoxifen	Drug (Ancrod)		164
10	m	Successful		Injected with Tamoxifen	Drug (Ancrod)	needle clogged so could not get sample	NA
11	m	Successful		Injected with Tamoxifen	Drug (Ancrod)		187
12	f	Successful		Injected with Tamoxifen	Control		140
13	f	Successful	died	NA	NA		NA
14	f	Successful		Injected with Tamoxifen	Control		155
15	m	Successful		Injected with Tamoxifen	Control	Died	NA
16	f	Successful		Injected with Tamoxifen	Control		119
17	m	Successful		Injected with Tamoxifen	Control		102
18	f	Successful		Injected with Tamoxifen	Control	Died	NA
19	f	Successful		Injected with Tamoxifen	Control		135
20	f	Successful		Injected with Tamoxifen	Control		148

Table 2: Summary of Results

	Treated (all animals)	Treated (excluding sick)	Control
average time (s)	162	157	133
standard deviation (s)	21	26	20
number of animals	6	4	6
male	4	2	1
female	2	2	5

Discussion Questions:

- Consider the variables in this experiment. Has Sam incorporated appropriate variable control methods in the experiments?
- What are the implications of the dead animals, two sick animals and needle clogging? How would you decide whether this information is useful, or if it helps to support or invalidate the hypothesis?
- What would you advise Sam to do at this point?