**Figures/Results Questions**

**Engineered bacteriophages for treatment of a patient with a disseminated drug-resistant *Mycobacterium abscessus***

***Dedrick et al., 2019***

Research papers contain lots of scientific jargon and can be difficult to understand, especially for emerging scientists.  Moreover, for any discipline of study, there are often thousands of previously published papers so it can be challenging to decide whether it is worth the investment of time to read a particular research paper.  The abstract section of a research paper is a useful tool for determining the general gist of the paper and can assist you in determining whether the research is interesting and relevant to your own study.

**Abstract**

Read the abstract of the paper and answer the following questions to the best of your ability:

1. Why did the authors undertake the research presented in the paper?  In other words, why did the researchers feel compelled to pursue this line of research?
2. After reading the abstract, can you list 1-3 main outcomes of the research study?  If so, please list the outcomes.  If not, why do you think the authors published the paper?
3. Would this paper be of interest to all phage researchers, or is the paper geared to a specific subset of researchers?
4. Scientists communicate for many different reasons.  After reading the abstract, do you think the authors are trying to:  (Be sure to support your answer with a brief explanation.)

:

1. *Share novel findings and the excitement of science*
2. *Increase an appreciation for science as a way to understand the modern world*
3. *Increase knowledge and understanding of science related to a specific issue*
4. *Influence people’s opinion, behavior, and policy preferences*
5. *Engage diverse groups in a dialogue to seek solutions to societal problems*

The Dedrick research paper is a form of research paper known as a Case Report.  Case Reports are generally shorter in length than a traditional research paper and therefore do not contain the separate sections that you find in a research paper (Introduction, Results with figures, Materials and Methods, Discussion).  Rather, the information usually reserved for these separate sections is included in the paper in a series of paragraphs that generally follow the sequence of the traditional sections of a research paper.  Likewise, you will see that the paper contains few figures but each multiple has multiple parts. Scientists will combine figures that all support a specific scientific question to show that multiple lines of evidence support the same conclusion.  Scientists will also sometimes combine figures in an effort to save space in the published article.

Both figures are broken into multiple segments. Each figure can be addressed as a collection of smaller figures with a common theme.

**Figure 1**

This figure shows how the patient responded to the bacteriophage cocktail using several measures of patient health.

Ideas that SEA-PHAGES students should recognize in this figure:

* Plaque assays (Fig. 1e)

Ideas that SEA-PHAGES students will probably need background on:

* How lung function assays are performed and what improvements are expected for CF patients with and without lung transplants.
* How PET-CT scans measure bacterial infections by measuring fluorodeoxyglucose (FDG) activity.
* How significant are the changes to the skin lesions (what are the remaining blackish spots?)
* How PCR works. How digital PCR works and why it was used to quantify the bacteriophage numbers.

***Lung function***

The authors tested the function of the patient’s new lungs by measuring the forced expiratory volume in 1s (FEV1) and forced vital capacity (FVC). Both measurements are made with a spirometer (https://www.verywellhealth.com/asthma-and-spirometry-200531) which measures the volume of air that a patient can exhale in one second (FEV1) or in one complete breath (FVC). The results are normalized based on height and converted to a percentage of “normal” function. Values below 40% are caused by severe obstruction of the lungs.

1. What was the patient’s lung function prior to lung transplant?

2. How much did lung function improve after the transplant? After the bacteriophage treatment?

3. Can lung function in CF patients improve without a lung transplant?

***PET-CT scan***

PET scans monitor an ingested radioactive molecule. In this paper, the authors use fluorodeoxyglucose (a modified sugar) to monitor metabolic activity. Since bacteria grow and divide much faster than human cells, the tracer concentrates at sites of bacterial infections.

4. How does the PET-CT scan detect bacterial infections?

5. What changes are observed in PET-CT scans after bacteriophage treatment?

6. Why do the authors perform a whole body and cross-section scan?

7. What are the unlabeled black areas in Fig 1b?

8. How do the patient’s skin lesions change after bacteriophage treatment?

9. In what other ways did the patient’s health improve after bacteriophage infection?

***Bacteriophage therapy***

The patient received intravenous injections of 109 bacteriophages every 12 hours for 32 weeks (when this paper was published). The authors wanted to know if bacteriophage replication in the patient is needed for them to be effective therapy. They did not test this directly and instead asked a simpler question: do the bacteriophages replicate once they were inside the patient? They tested for the presence of phages using plaque assays and polymerase chain reaction (PCR). The PCR was performed with oligonucleotides specific to each phage (see Table S5) and allowed the researchers to test for the presence of each phage. They used a methods called digital PCR (dPCR or ddPCR) that allowed them to quantify low amounts of phage genomic DNA. See <https://www.youtube.com/watch?v=WU3qKhIUc54> or <https://www.youtube.com/watch?v=5f8_L8nv_Nw> for a detailed explanation.

10. The researchers believe that the data in Figures 1e and f suggest that the bacteriophage is replicating in the patient. Why?

11. Describe how the plaque assays were performed on the patient samples.

12. Why are there differences in bacteriophage concentration when calculated by plaque assay compared to dPCR?

13. Even though the phage cocktail was administered every 12 hours for 32 weeks, the bacteriophages are undetectable after ~1 week of treatment. What happened to them? Do you think the researchers could have stopped the daily dosing with bacteriophages?

14. The absence of phages is usually an indication that there is insufficient host to infect. Were all the host bacteria cleared after the phage treatment?

15. Why is there a disconnect between the bacteriophage number measured by plaque assay and dPCR?

16. How might the authors have tested if bacteriophage replication is required for the therapy to be effective? Why didn’t the authors do this?

17. Why are the bacteriophages undetectable after one week?

18. Will the patient fully recover?

**Figure 2**

***Engineered bacteriophages***

A new concept in this figure is the testing of mutant phages that were engineered in the laboratory.

19. Use phagesdb.org to determine whether phages BPs and Zoe J are temperate or lytic.

20. Why would it be of concern to use a temperate phage in a phage therapy treatment protocol?

***Immunity repressor***

An immunity repressor gene is a gene found in phage genomes that indicates a phage may be temperate.  In the life cycle of a temperate phage, the immunity repressor protein binds to specific DNA sequences (stoperators) found throughout the phage genome.  The binding of the phage immunity repressor protein to stoperator sites in the phage genome prevents active transcription of lytic genes and thereby allows the phage genome to remain as a prophage.

***Genetic nomenclature***

Another new concept in Figure 2 is the naming of the phages and the engineered mutant phages that were included in the study.  You may see the names of phages with a small Δ symbol in the name.  The Δ symbol indicates a deletion.  For example, the name ZoeJ refers to the original, wildtype phage but ZoeJΔ45 is the name of a phage that was engineered in the lab using original ZoeJ DNA but gene 45, the repressor protein gene, has been deleted.

The following is a brief list of some of the technical nomenclature for phage and bacterial strains important for the figure.  Strains are biological entities of the same species (or viral taxonomic group) that have distinct genotypes. There is no formal system for naming strains.  There is no need to read all of these names and descriptions now, but feel free to refer back to this list when reviewing the figure.

* + ZoeJΔ45 - phage ZoeJ with gene 45 (immunity repressor) deleted. A nice figure of this made with Phamerator can be found in figure 4 from this paper: <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6452893/>
  + BPs33ΔHTH - phage BPs with a non-functioning immunity repressor (gene 33 in BPs). This mutation is slightly different from the full gene deletion for ZoeJ - in BPs only the HTH DNA binding motif is deleted - but the result is essentially the same: there is no repressor protein that can bind the lytic gene operator sequences.
  + BPs33ΔHTH-HRM10 - BPs33ΔHTH was not effective at killing the clinical bacteria on its own, so the lab isolated mutants of this phage that were better at infecting hosts other than *M. smeg*, including (as is clear from the figure) the clinical strain. The method they used for this isolation is not detailed in the paper.
  + *M. smegmatis* mc2155 - The strain of *M. smeg* used for SEA-PHAGES isolation.
  + *M. abscessus* ATCC19977 - The stain of *M. abscessus* from the American Type Culture Collection (ATCC) one of the largest repositories for bacterial isolates.
  + *M. abscessus* GD01 - The stain of *M. abscessus* isolated from the patient.
  + *M. abscessus* GD02, GD03, GD04, GD05 - Strains of *M. abscessus* isolated from other patients with *M. abscessus* infections.

**Infectivity of bacteriophages on *M. smegmatis* and *M. abscessus* strain GD01**

Figure **2A** shows the results of assays where serially diluted bacteriophages were spotted on lawns of either *M. smegmatis* or *M. abscessus* GD01. The authors tested several bacteriophages isolated through SEA-PHAGES and similar programs: Isca, ZoeJ, BPs and Muddy, as well as engineered mutants of these phages.

21. The images for Figure 2 all show data related to the 3 phages selected for the therapeutic cocktail used to treat the *M. abscessus* infection, Muddy, ZoeJ, and BPs. The researchers used a spot test to determine the effectiveness of the phages isolated on *M. smeg* infecting *M. abscessus*.  Why did the researchers perform this experiment?

22. Why did the researchers perform 10-fold serial dilutions of the phages when performing this experiment?

23. Which phages showed similar infectivity on *M. smeg* and *M. abscessus*? Which phages had different levels of infectivity?

24. The names ZoeJΔ45, BPsΔ33 and BPsΔ33-HRM10 indicate that these phages contain engineered mutations that the researchers designed. What are these mutations specifically? Why were these mutations made?

25. Using data from figure 2A, explain why the researchers may have decided to further mutate BPsΔ33 to BPsΔ33-HRM10.

26. What did the researchers learn after completing this work?

**Details of therapeutic bacteriophages used to tread *M. abscessus* GD01**

Figure **2B** and **2C** show important supporting information about the phages used in the study.

27. The image in **2B** shows electron micrographs of the wild-type (unmutated) strains of the 9 phages used in the therapeutic cocktail. What family do these phages belong to?

28. The table in **2C** shows detailed information about each phage used in the study. Explain what data is indicated under each of the following column headings: **Cluster**, **Accession**, Δ **coordinates**, **Other mutations**, and **Lifestyle**.

29. Bonus question: there is a typo in table **2C** - see if you can catch it.

**Infectivity of therapeutic phages on other clinically isolated stains of *M. abscessus***

Figure **2D** shows the results of an experiment performed just like the one shown in **2A**.

30. Briefly review from your discussion of **2A** how the experiment was done.

31. How are the host bacteria *M. abscessus* GD02, GD03, GD04, GD05 and ATCC19977 different from *M. abscessus* GD01?

32. How do the infectivity of the phages tested differ between the different host bacteria? What implications would this result have for treating other *M. abscessus* infections using phage therapy?

**Phage Killing of *Mycobacterium abscessus* GD01**

Figure **2E** shows the results of phage killing assays to determine the effectiveness of the 3 phages selected for the therapeutic cocktail in killing the GD01 bacteria.

33. Compare figure 2E with figure 2A and 2D. Discuss the similarities and differences between the 2 experimental procedures. Point out on both figures where bacteria is visible and where it is absent.

34. Which of the 3 individual phages was most effective at killing *M. abscessus* GD01? Justify your answer from the data.

35. Which of the 3 individual phages was least effective at killing *M. abscessus* GD01? Justify your answer from the data.

36. Is the 3-phage cocktail more, less or equally effective at killing *M. abscessus* GD01 than the phages individually?