National Institute for Public Health
and the Environment
Ministry of Health, Welfare and Sport

Educational material for addressing Safe-by-Design in biotechnology: *Cases and guidance*

Case 4: Genetically modified bacteriophages as therapeutic agents

Content of the case:

4.1 General information

Biotechnology domain and focus

- medical microbiology;
- clinical research;
- bacteriophage therapy.

Learning objectives

- understand potential hazards, risks, and applicable risk reduction measures;
- awareness of risks when applying genetically-modified microorganisms (GMMs) to patients;
- form an opinion on the dilemmas (e.g. moral and ethical), and how these may relate to safety.

Case specific knowledge required

- knowledge of bacteriophages, lytic and lysogenic lifecycle;
- knowledge of genetic modification.

A virulent bacteriophage is selected from the mixture and, if needed, genetically modified.

Bacteriophage

DNA

Due to a continuous re-infection process, the harmful bacteria are killed.

Genetically modified bacteriophages

4.2 Case description

Medical doctors, in collaboration with molecular virologists, designed a bacteriophage cocktail to treat a lethal bacterial infection in a young girl suffering from cystic fibrosis. The bacterium *Mycobacterium abscessus* had caused a lung infection and treatment with antibiotics had resulted in serious resistance development. The application of bacteriophages that could lyse the bacteria and diminish the infection was a last resort.

In developing the bacteriophage treatment, the scientists, with the help of many students in microbiology, selected several phages. The most virulent phage unfortunately showed a lysogenic lifecycle. Lysogenic phages, which do not kill the host bacteria, cannot be used for antimicrobial therapy. The researchers, therefore, deleted a regulatory gene in the particular phage genome and as a result the phage now exhibited a lytic lifecycle. A three-phage cocktail containing this genetically modified (GM) bacteriophage and two other selected wildtype phages, was applied to the patient.

This is the first therapeutic use of phages to treat a human mycobacterial infection, and the first use of GM phages.

Origin of case and background information

This case is based on a scientific paper[1,2], describing the development and application of a GM bacteriophage cocktail for the treatment of a patient suffering from an infection caused by *Mycobacterium abscessus*.

Because of the discovery of antibiotics in the early twentieth century, Europe and USA have neglected the possibilities of using phage applications for the treatment of bacterial infections. The use of bacteriophages for medical treatment, or diminishing food and feed contaminations, is however common in Russia, Georgia and Poland. In the last few years, bacteriophages have regained attention because of the resistance development of bacterial pathogens to antibiotics. Personalised treatments with phage cocktails are promising. Nevertheless, there are many challenges before phage therapies can be applied as a standard therapeutic (see RIVM report in the additional information), like GMP (Good Manufacturing Practice)-qualified production processes and regulatory aspects of medical products.

[1] https://www.nature.com/articles/s41591-019-0437-z

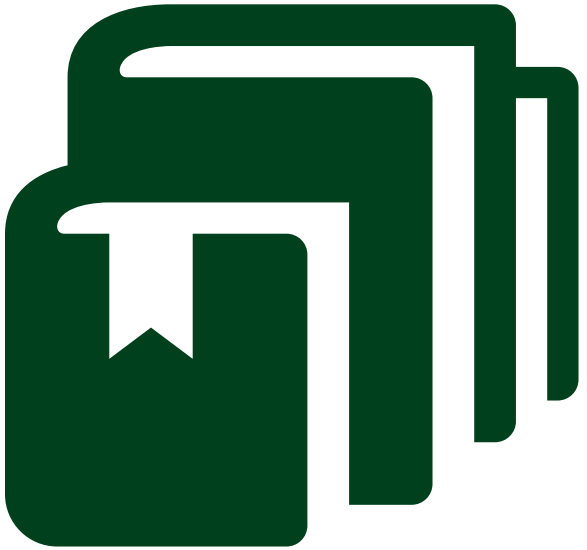
[2] https://static-content.springer.com/esm/art%3A10.1038%2Fs41591-019-0437-z/MediaObjects/41591_2019_437_MOESM1_ESM.pdf

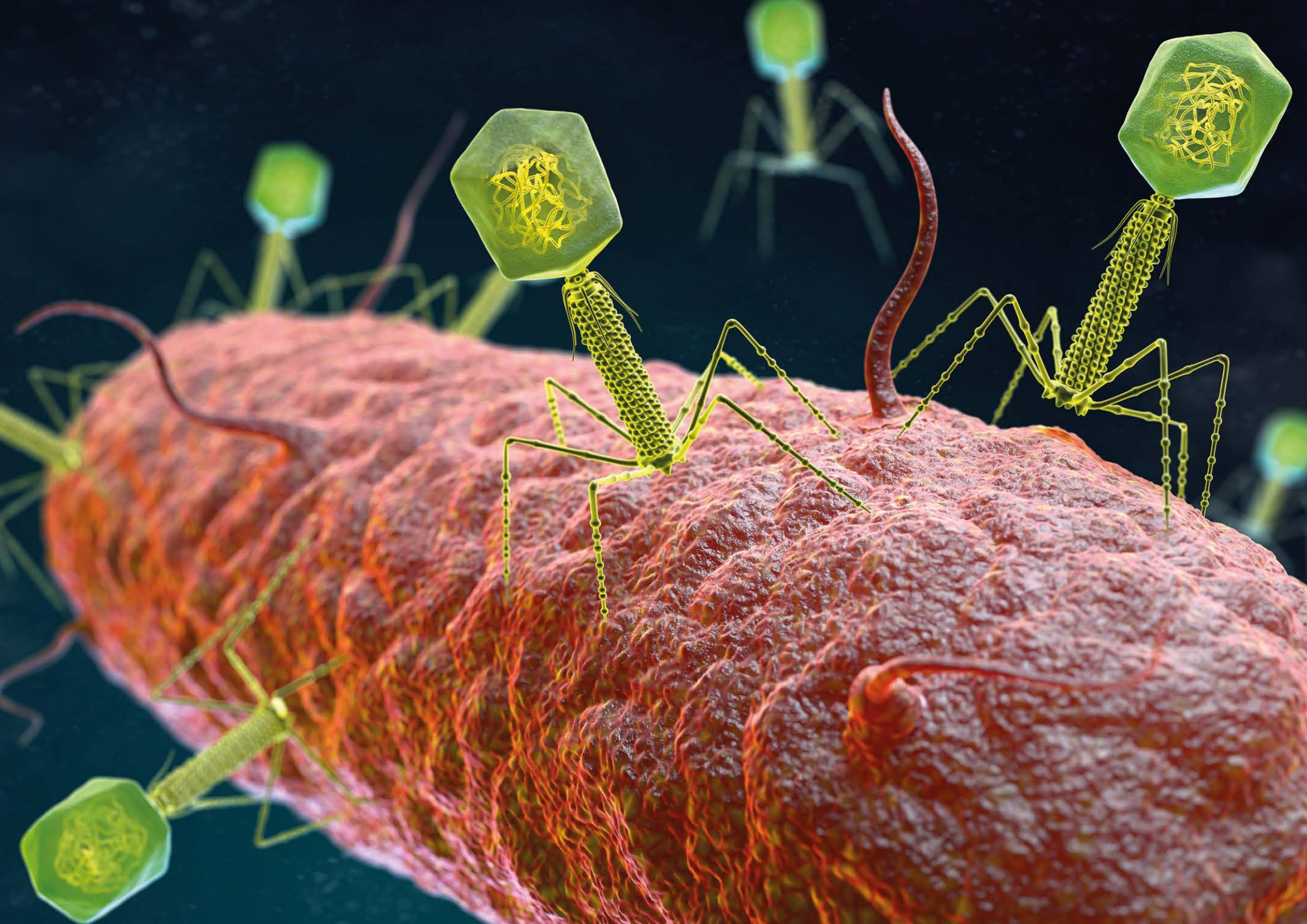
Additional sources

RIVM rapport on bacteriophages (Dutch with English abstract):
https://www.rivm.nl/publicaties/bacteriofagen-huidige-kennis-onderzoek-en-toepassingen

Opinion on the use of genetically modified bacteriophages for therapeutics:
https://www.nature.com/articles/s41591-019-0506-3

Information on the pathogenicity class of pathogens and working procedures:
https://health.canada.ca/en/epathogen





4.3 The group discussion

Theme 1: Safety concerns with regard to human health and the environment

Background information for discussion leader
In the described case, the patient was chronically infected with *Pseudomonas aeruginosa* and *Mycobacterium abscessus* subspecies *massiliense*. The latter strain (designated GD01) grew from sputum, showed resistance to a wide spectrum of antibiotics and was very clinically worrisome.

With the help of students on a Mycobacterial Genetics Course, the researchers searched a collection of >10,000 phages which were isolated using *Mycobacterium smegmatis* for their infecting ability towards GD01. In an additional search, over 100 environmental samples were tested on strain GD01 to select useful phages. A small set of phages resulted while showing variable lytic properties (upon different phage and bacterial cell concentrations). To improve the killing ability of a temperate phage the researchers removed a repressor gene by means of genetic modification. This GM phage, combined with two wildtype phages, resulted in the anti-*M. abscessus GD01* cocktail.

Application of this cocktail to GD01 showed no bacterial survival in vitro. After challenging a larger GD01 culture with the cocktail, some survivors were recovered and shown to be resistant to two phages, but at least partially sensitive to the third one.

The authors tested the effects of their phages on a small set of bacterial hosts and found no evidence of non-specific killing. However, this was only a limited test of the range of hosts for the phages and their impact, so one could not definitively conclude that these engineered phages have a restricted host range. Such a conclusion would require a rigorous and comprehensive set of tests conducted against more diverse bacterial hosts. While phages cannot directly infect humans, plants or animals, they may adversely affect these organisms by altering their associated microbiota. In this respect, the use of GM phages needs to be scrutinised very carefully, as they may influence bacterial community dynamics, genome evolution and ecosystem biogeochemistry.

Suggestions for the group discussion
The following issues are suggested for discussion:

Selection and genetic modification of phages
Cultures of the bacterial pathogen *M. absessus* are required for the selection of phages. What do you know about safe microbial practices regarding the use of human pathogens in the lab? What safety requirements have to be in place? Where and how would you look for the information?

The paper described the help of students for screening many phage isolates. How do you envisage this possibility as a learning experience? And how do you view it from a safety perspective?

Risks to human health and the environment
As the phage cocktail contains GM phages, the risks presented by these phages, and in particular the risks to human health (i.e. excluding the patient) and the environment, need to be taken into account. What kind of risks need to be considered? What kind of experiments are possible to collect data about the envisaged risks?

Risk reduction measures
What kind of risk reduction measures, e.g. to prevent the GM phages from being released into the environment, would you propose? Take into account the patient, the medical staff, and all the material and equipment that has been in contact with the phages. Suggestion: check Figs 1E and F in the publication, which shows patients' samples from different origins which contain phages.

Theme 2: Expanding the safety perspectives

Background information (for discussion leader)
The group is asked to act as a critical thinker and go beyond the safety aspects discussed under Theme 1. Suggestions for discussion, including some background (for the discussion leader), follows:

Suggestions for the group discussion

Safety to the patient
According to the publication: Possible risks to the patient were considered including anaphylaxis and immune responses to bacterial lysis, and these were discussed with the GOSH Drugs and Therapeutic Committee, the Ethics Committee, and the clinical team. Mitigating actions were implemented, including an initial test of topical (applied on the skin) test doses, an initial low intravenous dose prior to administration of the full dose, a therapeutic plan for the treatment of anaphylaxis or immune reactions, and the immediate availability of a bed in the intensive care unit if needed.
As such, there is no a *priori* knowledge about the success or failure of this medical treatment. Discuss with the students whether the choices made by the researchers are satisfactory and/or can be justified.

Personalized treatment
The authors mention that the three-phage cocktail is not a generalisable treatment for infections caused by *M. abscessus*: the phages are specifically selected for the particular *M. abscessus* strain. A second patient suffering from a *M. abscessus* infection may require another selection of phages. This makes this medical treatment highly personalised. What do you think about the availability of expensive (in money and in time) treatments? Do they ask for patients to be selected in a way that treatment will result in healthier perspectives? How would this development of personalised treatment influence safety?
NB. In the paper the authors report the urgent clinical status of the patient (15 yrs. old) on a palliative care pathway for which other treatments had failed, and that other lung transplant patients with disseminated M. abscessus infections had high mortality.

Publishing science successes
Cited from the publication:

> 'Phage treatment was associated with clinical improvement (over a period of 6 months), although we cannot exclude the possibility that patient gains would have occurred without phage treatment. However, we note that patients with similar clinical conditions typically have high morbidity and mortality, that improvement was not temporally associated with cessation or initiation of other drug administrations, and we show evidence to support *in vivo* phage replication. There were no adverse reactions to phage administration. We note that there is substantial variation in *M. abscessus* phage susceptibilities, and phage treatment of similar patients will require expansion of our understanding of phage infection of these strains.'

With this, the authors are critical of their own results. In non-scientific journals and newspapers, the results are often reported as new, very promising and the treatment of the future. What do you think about this way of reporting on preliminary data to inform the public of new developments and can you relate this to safety?

4.4 Wrap up

This case illustrates the successful treatment of a patient with a three-phage cocktail as a plausible alternative treatment to antibiotics. The cocktail also contained a bacteriophage that was genetically modified to improve the lytic activities of the cocktail. On the other hand, the newness of the therapeutic raises questions about its safety and effectiveness, and the moral and ethical issues.

By taking into account the learning objectives (see Section 'Knowing') you may summarise the collective view on the safety aspects of this therapeutic and the additional issues that were discussed in Theme 2.

Options for enriching the learning experience

- Gain insight into public opinion by compiling a patient information booklet on phage therapy.
- Find out at your university whether it is possible to include science data collection on a student's course. Or think of a research topic that could be investigated as a citizen's science project.

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