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 5: Combining genetic parts

Content of the case:

5.1 General information

Biotechnology domain and focus

- contained use;
- molecular virology;
- risk (biosafety and biosecurity) of combining genetic elements.

Learning objectives

- awareness of risk when combining different genetic elements of viral origin;
- an understanding of what is meant by ‘Gain-of-Function’;
- outreach to stakeholders (BVF, BGGO) to collect knowledge about the options available to reduce risks.

Case specific knowledge required

- knowledge of molecular virology;
- knowledge of genetic modification;
- knowledge of viral diseases and ways of infection.

The Adenovirus spreads by aerosols, e.g. coughing and sneezing.

The Hepatitis B virus spreads by blood, but is unable to infect mice.

Adeno virus

Hepatitis virus

And what if these viruses are combined?

The Hepatitis virus unable to infect mice can now with the help of the Adenovirus be studied in mice.

Risks of a hybrid virus

5.2 Case description

This case has its origin in the fundamental research of Hepatitis B virus (HBV). To study HBV *in vivo* animal models are necessary. Mice, the most commonly-used research animals, are not naturally susceptible to HBV infection. To enable mice to be infected with HBV in order to investigate viral replication, the researchers came up with the idea of creating a hybrid virus. This hybrid virus consisted of the HBV genome integrated into the adenovirus (AdV[1]) genome, which resulted in AdHBV. The adenovirus efficiently infects the mice cells and delivers the HBV genome into them. Both AdV and HBV can be safely handled in ML-II / BSL-II labs. The researchers would therefore like to perform their experiments in an ML-II / BSL-II lab as well.

Origin of case and background information

This case describes a research project conducted at a university. A project leader asked permission to construct a hybrid virus in an ML-II (BSL-2) laboratory. Biosafety officer, GMO office and Commissie Genetische Modificatie were consulted to determine the risk reduction measures.

Hepatitis B virus (HBV) infection is a major global health problem. The virus infects the liver and can cause both acute and chronic disease. The WHO estimates that, in 2015, 257 million people were living with chronic hepatitis B infection. In 2015, hepatitis B resulted in an estimated 887,000 deaths, mostly from liver cirrhosis and hepatocellular carcinoma (i.e. primary liver cancer). Hepatitis B can be prevented by vaccination. An effective and safe vaccine that offers a 98-100% protection against hepatitis B is available.[2] However, more understanding on the infection and replication of the virus remains important.

Additional sources

Safety data sheet on HBV:
https://www.canada.ca/en/public-health/services/laboratory-biosafety-biosecurity/pathogen-safety-data-sheets-risk-assessment/hepatitis-b-virus.html

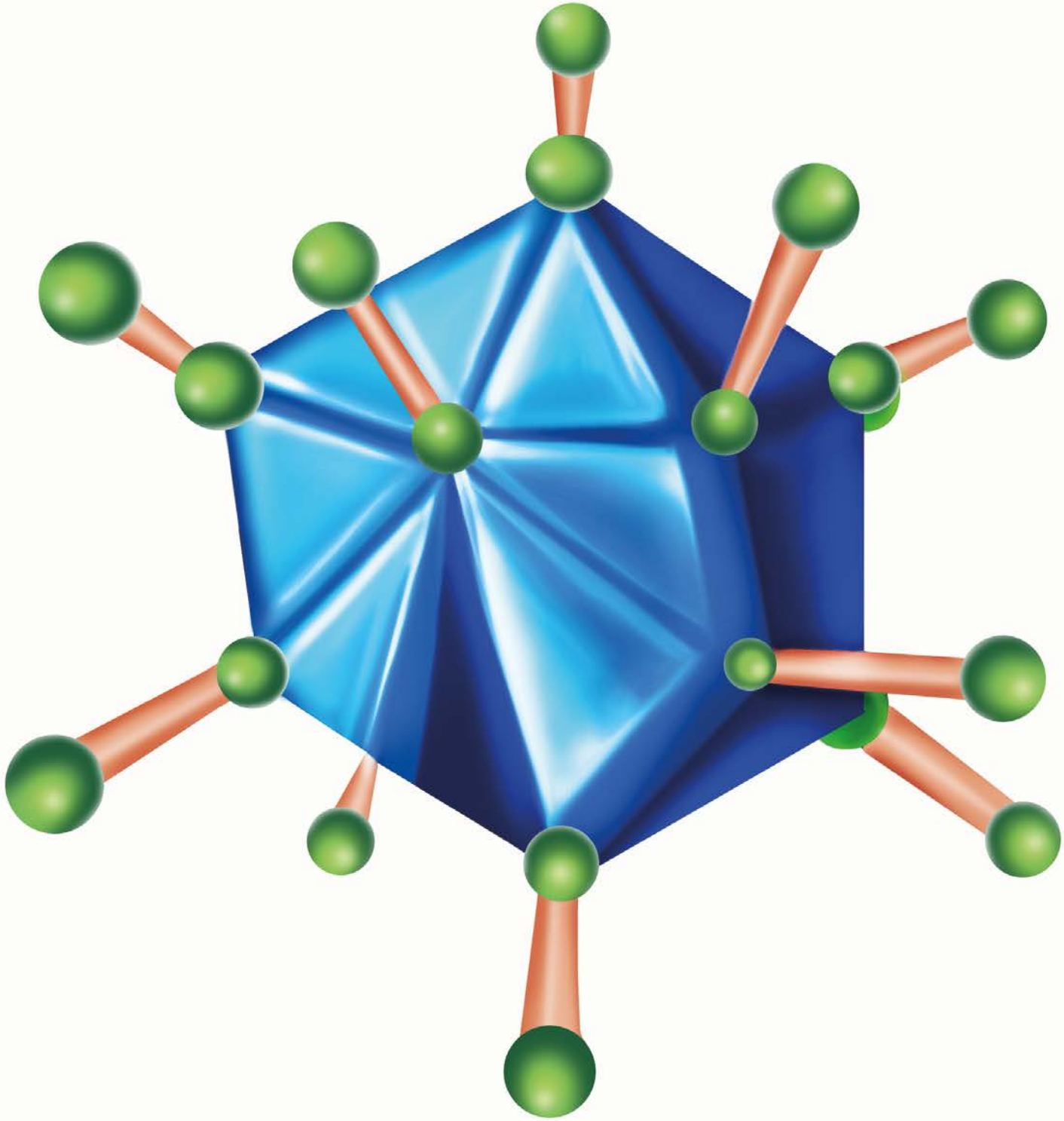
Safety data sheet on AdV:
https://www.canada.ca/en/public-health/services/laboratory-biosafety-biosecurity/pathogen-safety-data-sheets-risk-assessment/adenovirus-types-1-2-3-4-5-7-pathogen-safety-data-sheet.html

Advice from the Commissie Genetische Modificatie (COGEM) related to this case:
2014: https://cogem.net/publicatie/replicatiedefectieve-adenovirale-vector-met-het-complete-genoom-van-hepatitis-b-virus/
2018: https://cogem.net/publicatie/inschaling-van-werkzaamheden-met-adenovirale-vector-met-gemuteerde-genoomsequentie-van-hepatitis-b-virus/

Information on the Gain-of-Function research
https://www.knaw.nl/nl/actueel/publicaties/debate-on-gain-of-function-research

[1] https://www.addgene.org/guides/adenovirus/

[2] Source and further reading: https://www.who.int/news-room/fact-sheets/detail/hepatitis-b



5.3 The group discussion

Theme 1: Potential hazards and risks of a hybrid virus

Background information for discussion leader
HBV is determined as a Class 2 pathogen based on the fact that the virus does not spread easily throughout the population as blood-blood contact is needed. Furthermore, a vaccine is available which prevents disease development. Working with a Class 2 pathogen is allowed in an ML-II / BSL-II laboratory.
AdV is a virus that easily spreads by aerosols, but causes only mild symptoms in humans. Because of these qualities, the virus is also classified as a Class 2 pathogen.
By constructing the hybrid virus AdHBV, the characteristics of both viruses are combined: the adenoviral part enables the infection of mice cells with HBV. Expression of the genetic information of HBV carried by the adenovirus will result in HBV replication and pathogenicity. HBV, as part of AdHBV, has acquired the potential to spread like an airborne virus, thereby causing a more serious exposure to humans.
While working with the hybrid virus in the laboratory, the probability of it being unintentionally spread outside the laboratory because of aerosol formation, or spread by contaminated material or by the infection of a technician is more likely than it is the case for wildtype HBV that does not spread by aerosols. As the harm caused by an HBV infection is more serious than an AdV infection, more stringent risk reduction measures should be put in place to prevent risk to human health from AdHBV.
To reduce the risk of spreading AdHBV by aerosol formation, the use of a biosafety cabinet is required and protection of the environment by the standard measures of a BSL-III / ML-III laboratory (e.g. Hepa filters and an air lock) is required.

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

Hazards
The airborne spread of AdHBV is identified as a hazard in addition to the pathogenicity of HBV. Discuss this hazard to gain a shared understanding of this type of hazard.

Risks
What do you expect from the hybrid virus AdHBV in terms of its spreading potential and ability to cause disease?
How would you assess the probability (e.g. none, low, medium or high) of AdHBV spreading (by accident) from the laboratory and causing a risk to human health? And what about the probability of it spreading during experiments with AdHBV infected mice?

Risk reduction measures
What kind of risk reduction measures do you think have to be in place to make the risk (of spreading AdHBV from the lab) negligible?

Theme 2: Expanding the safety perspectives

Background information (for discussion leader)
The group is asked to act as critical thinkers and go beyond the safety aspects discussed under Theme 1. The COGEM advice of 2018 (see additional information) can be used as preparatory material for a discussion on a safer combination of genetic parts. As a scientist you have also the option of consulting your Biosafety Officer, the RIVM/GMO office regarding biosafety issues and the RIVM/Biosecurity Office regarding biosecurity issues such as dual use or gain-of-function research.

Suggestions for the group discussion

- The options offered by the construction of the hybrid virus AdHBV for use in future experiments are welcomed enthusiastically by members of your research group. There are also voices raising concerns about the spread and pathogenicity of the hybrid viruses.
  - What do you think of risks and benefits of this type of research? Do you think this kind of research is worth the risk?
  - Who can help you to find out about biosafety issues?
  - How will you deal with the different voices within your research group?
- The research group has acquired the GMO permit to work with AdHBV.
  - Do you still consider it worthwhile to perform the experiments in order to demonstrate the potential of AdHBV to spread by aerosols?
  - Is there any reason to rethink the construction of AdHBV and determine other ways to enable the infection of mice cells by HBV?
- In light of biosecurity issues, gain-of-function research seems to be applicable to the construction of AdHBV.
  - Discuss whether you consider this research to be gain-of-function research.
  - What are the possible consequences of identifying this research as gain-of-function research?

5.4 Wrap up

This case describes research that makes use of two different viruses. By combining the genetic information of these viruses, the resulting hybrid virus acquires the characteristics of both. This combination of viral parts should be carefully evaluated to ascertain whether there are any additional risks.
The broader scope of the case, with discussions on responsibility and design and need for alternative strategies, provides good illustrations of the aspects of Safe-by-Design.

By taking into account the learning objectives (see Section 1) you can summarise the collective view on the safety aspects of the experiments on the hybrid virus and the additional issues that were discussed in Theme 2.

Options for enriching the learning experience

- Gain experience in acting proactively by reaching out to stakeholders (e.g. Biosafety Officer, GMO Office) to identify hazards and risks;
- Gain insight into the relevant viral and cellular functionalities, and the ways of infection, by drawing a cartoon.

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