A Brief Review of

Biothreats and Biodefense

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Summary

Growing concerns that the research on bioweapons is intensified again and recent developments in synthetic biology, genetic engineering and gain of function (GoF) research indicate the need for a review of biothreats and biodefense. After a brief review of the classical biological weapons and recent developments the potential of GoF research and possible defense strategies are presented. Finally, potential new biothreats are discussed. The aim of the paper is to discuss ways to enhance biodefense and biosecurity.

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1. Fundamentals

1.1 Introduction

There are growing concerns that the research on bioweapons is intensified again:

At the end of 2017, US researchers suspected Russia to work on biological weapons[1]. The Russian army has established *Science Squadrons*[2] in 2015. Staffing is done from leading universities such as Moscow, St.Petersburg, Novosibirsk, Rostov and Far East. Activity areas include amongst others aviation, laser technology, software research *and biotechnology*. The research will be mostly classified. The *Military Scientific Committee of the Armed Forces* has control which is affiliated to the *National Defense Management Center NDMC*. However, concrete evidence for this is missing.

However, Russia as well is alerted that United States would be doing the same. In 2017, this was triggered by the targeted collection of synovial fluid/RNA samples from Russians by the *US Air and Training Command* since July 2017, but it denied that this is done with bad intentions.

Officially, human poxviruses (also known as small pox or Variola) are safely stored at only two locations in the United States and Russia, but in reality United States are seriously concerned that this is not true: As a consequence, all military personnel on the Korean peninsula staying longer than 15 days and staff of the US Central Command must be vaccinated with smallpox vaccine, also known as the *MILVAX program*.

China expressed in 2013 serious concerns about the so-called **Gain of Function (GoF) research** where researchers enhance the properties of viruses with respect to contagiosity and damage to the infected organism by targeted mutations. However, on 19 Dec 2017, US lifted the ban of funding of GoF research by the National Institute of Health (NIH), accompanied by a regulatory framework.

Already in 2016, a researcher was able to re-create an extinct virus, the **horse pox** virus, by synthetic DNA and other measures[3]. While this was done with the good intention to have a new technology platform for safer smallpox vaccines for

[1] Because Sverdlovsk-19, a facility involved in an Anthrax accident in 1979, was renovated and expanded, Keim//Walker/Zilinskas 2017, p.43
[2] Gerden 2015, SCMagazine 2015
[3] DiEuliis/Berger/Gronvall 2017

humans, scientists are very concerned about the future consequences of this large technology advance in synthetic biology[4].

Taken together, this shows the need for a review of biothreats and biodefense. After a brief review of the classical biological weapons and recent developments, the potential of GoF research and possible defense strategies are presented. Finally, potential new biothreats are discussed.
The aim of the paper is to increase problem awareness and to discuss ways to enhance biodefense and biosecurity.

1.2 Background

1.2.1 Some introductory remarks

Biotechnology allows to change genes or to introduce new genes into organisms, which raised concerns that new dangerous organisms maybe created intentionally[5] or inadvertently. However, already since the 1980ies fears exist that the world as we know it will be overwhelmed by mutated superanimals, superplants, superviruses, superbacteria and so on.
The genetically manipulated male *Aedes Aegpytic* insects from the company *Oxitec* are used since years to cut down infectious mosquito populations, e.g. those bearing the Zika virus. When pairing these mutated males with females, reproduction fails by releasing a blocker protein. However, WHO and the US Drug Agency FDA do not consider this as a superanimal or similar, but as environmentally safe[6].

Apparently, the world was not overwhelmed by any kind of superorganism and the reasons for this are quite complex, but maybe some simple technical points may help to understand the difficulties:
There are three main strategies:

- **Mutation**, i.e. change of genetic code (Deoxyribonucleic acid DNA), resulting in altered protein products
- **Insertion** of new genes into the set of genes of an organism (genome), resulting in additional proteins and functions
- **Deletion** of genes, resulting in loss of functions of the organism. Deletions are e.g. done to make viruses less harmful (attenuation), which is quite useful for vaccines, but obviously not for bioweapon production.

[4] Kupferschmidt 2017
[5] The virus researcher Fouchier enhanced infectious properties of avian flu ('bird flu') virus to get a better understanding of the virus, Guterl 2012. Both US and China expressed serious concerns, see Guterl 2012, Zeng Guang 2013. Practical recommendations for defense against biological weapons were released by the European Medicines Agency EMA (former EMEA), refer to EMEA 2002 (updated 2007).
[6] For discussion refer to Lahrtz 2016

Insertions create a material and energetic burden to a cell. The synthesis and metabolism of genetic information requires relevant amounts of energy-rich triphosphates (known as adenosin triphosphate ATP), so adding genes is for smaller organisms not like downloading an App to a smartphone, but more like adding an additional operating system (bacteria and viruses are microscopic 'single cell'-entities only). As a consequence, the mutated organisms are often not able to compete with the natural form, also known as **wild type**. Sometimes, this has also to do with dependency from laboratory conditions that are not available in nature.

Also, for smaller organisms, mutations or insertions have impact on the structure of the organism. So e.g. viruses may become more infectious, but less harmful. Note that the DNA and the resulting proteins as well as other parts are three-dimensional structures which interact with each other in the organism and outside which adds a kind of 'fourth' dimension in the system.
Finally, whatever is genetically modified, the whole system needs to run in reality, the receptors, enzymes etc. still need to work. Sometimes, mutations are not stable, but are lost again, i.e. the cells get rid of the artificial and/or additional genetic burden.

In summary, there are **material, energetic, structural and functional hurdles** for genetic mutations which are practically much higher than in theory.
An approach to overcome this is to interpret organisms as mathematical problems; simply spoken viruses could be seen as long three-dimensional vector equations which need to be optimized along certain restrictions.
In research practice, United States researchers use computers which are able to present *partial* solutions of this problem for Human deficiency virus (HIV, also known as AIDS virus), to enhance the development of better AIDS therapies.

But the organism itself is one thing, the environment is another: feeding, temperature, storage, optimization of pH values and electrolyte balance etc. etc. requires a lot of experience.
These few initial considerations have major consequences for biosecurity, because the technical hurdles for biotechnological activities are multiple times higher than for cyber attacks[7].

[7] For background information on cyber warfare you may refer to the free Paper "Cyberwar –Methods and Practice" http://www.dirk-koentopp.com/downloads/saalbach-cyberwar-methods-and-practice.pdf, and the literature cited therein. Also it includes a section on cyber-biosecurity of medical devices and implants.

1.2.2 Biohacking and bioterrorism

In the last decade, a new phenomenon called **bio-hacking** was observed[8]. The typical biohacker works outside established research units or companies as **DYI (do it yourself, also DYIBIO)** researcher and tries as a kind of ethical hacking to modify genes to invent something useful, but due to biosecurity reasons the biohacking scene is closely observed by government authorities[9].
A recent overview on the biohacking scene has demonstrated substantial progress in creation of cheaper and easy-to-handle technical equipments for such experiments (such as thermocyclers for DNA replication), but no critical biohack incident was reported so far[10]. The example of tobacco plant fluorescence by a gene-gun shown by Trojok would have been technically possible already in the 1980ies[11]. However, for future problems refer to Section 4.2.

The other issue is **bio-terrorism**. The anthrax attacks in the aftermath of 9/11 were done by an insider who stole the relevant material from a state research unit.

The group *Aum Shirinkyo* was able to conduct terrorist attacks with chemical weapons, but failed with their plans to do bio-terrorist attacks, too. The repeated spraying of anthrax in Tokyo in June and July 1993 apparently caused no reports of diseases or anthrax-related symptoms[12].
In 2013, a man tried to mail letters containing the herbal toxin **ricin** to parliamentarians and to President Obama. This attempt was detected and failed; the attacker was very quickly identified and imprisoned[13].

The above described hurdles (equipment, experience, money, infectious agents, design and modifications etc.) make the concept of a genuine bio-terrorism *unlikely*. Currently, any larger attack with biologic agents would require support and funding by states, i.e. *large-scale bioterrorism could currently only be executed as state-backed activity*.

1.2.3 Attribution of biological attacks

No state should believe that a camouflage as ‘terrorism’ would help to avoid identification of the real attacker. The state would be guilty of an attack with

[8] Kunze 2013, p.19-20
[9] In US, the responsible authority for biosecurity is the **National Science Advisory Board for Biosecurity** NSABB, but the biohacker scene is also observed by the FBI, the CIA is also interested in this matter, Hofmann 2012, p.14.
[10] Trojok 2016
[11] Trojok 2016, p.131-132
[12] Dembek et al 2007, p.48
[13] SZ online 2013. In 2011, it was discovered that ricin blocks a cell protein named Gpr107, which maybe an important step forward to a treatment, IMAB 2011

weapons of mass destructions (WMDs) and would have to bear the consequences (as this would legally be an act of war).

Attribution methods include:

- **Facilities for biological weapons** have a certain structure and look which could already be identified in the 20th century by photographs from the sky, i.e. can now be detected by espionage satellites[14]. Note that peaceful and weapon usages can be distinguished during inspections.
- While after the collapse of the Soviet Union there was a risk that experts may look for other opportunities for whomsoever, nowadays people working in top-security environments cannot expect that a defection would be unattended. The United States have expanded the security clearance for biosecurity experts to an overall **integrity check**, i.e. evaluating whether the personality and psychological condition of such a person makes them eligible for research with dangerous biological material[15]. Also, potential vulnerabilities are checked to avoid ransom etc. It is plausible to assume that other states use similar systems to ensure a maximum security for such research positions.
- The order of respective materials including **research materials, bioreactors, feeding materials** etc. can give further hints; cyber intelligence may help to reconstruct such events or to track them.
- Microorganisms exist often in variants (**strains**) which are derived from precursor strains. The genetic code of an infectious agent may give hints to the original strains and the sources.
- Sometimes, microorganisms need a special preparation for use in attacks. This is particular true for anthrax, where negative loads on the surface have to be sealed on a microscopic level to improve inhalation. This is a high-tech method which creates a **molecular fingerprint**[16] and thus could identify the original production plant.
- Genetic modifications of bacteria or of proteins typically result in unique microscopic **variations of surface glycoproteins** which could be used for production plant attribution like a fingerprint.
- All distribution methods and carries systems have a **technology history** giving further hints to the attacker, i.e. the attack method is another trace.

[14] Sometimes even internet images shelp, see Keim//Walker/Zilinskas 2017, p.43

[15] For detailed guidance, see NSABB 2009. The bioweapon expert Bruce Ivins who was resposnsible for the post 9/11 anthrax attacks was reported to have suffered from a psychiatric disorder, so the above mentioned security measures show the consequences, Winkler 2008, Schaaf 2008

16 Thus, the **Ames strain** was identified to have caused the post 9/11 anthrax attacks and the attacker could be identified.

1.2.4 Dual Use Research as key problem

There are many occasions where research, equipments, materials, procedures etc. can be used for peaceful and for military purposes at the same time.
The key problem is that peaceful/civilian research could inadvertently open chances for research and development of bioweapons.
The US Government has released in 2012 policies with respect to *dual use research of concern (DURC)*. DURC is defined as "*life sciences research that, based on current understanding, can be reasonably anticipated to provide knowledge, information, products, or technologies that could be directly misapplied to pose a significant threat with broad potential consequences to public health and safety, agricultural crops and other plants, animals, the environment, material or national security*" [17].

- The synthesis of already extinct poxviruses for the good purpose of creating safe smallpox vaccines (Section 2.5) and the Gain of Function (GoF) research (Section 3) which aims to better understand nature and risks of influenza viruses are the most pressing examples how research which is done with good intention may inadvertently open the door for return of old or creation and release of new bioweapons.
- The CRISPR/Cas9 technology is causing a massive expansion of genome editing and available organisms and options. The surveillance of these activities will be quite challenging and probably require more capacities for the biosafety authorities.
- Another problem is the research communication. Experience shows that suppression of research communication may slow down progress (or leave this to the adversaries); however, there is a significant risk that information is helping the wrong actors.
- Note that a camouflage as peaceful research of a certain activity will very likely not be able to mislead bioweapon inspectors or experts.

2. Conventional bioweapons

2.1 Definitions and concepts

2.1.1 Definition

Theoretically any harmful biological agent that could be released with bad intentions is a 'biological weapon'.
On the other hand, not each biological product is easy enough to handle or effective enough to be used as weapon in practice.

[17] NIH 2012

Also, the understanding of bioweapons is globally a military one, i.e. the intoxication of single persons e.g. with harmful fungi is a matter of crime, but not seen as an act of biological warfare.
The US released the *Select Agent* rules in 2015, a list of harmful biological agents which are under particular surveillance and restrictions. [18]
In biosecurity, bioweapons are those agents which can cause harm to a larger number of people, i.e. are Weapons of Mass Destruction (WMD).

2.1.2 Criteria for bioweapons

The characterization of bioweapons as WMD has not only legal, but practical consequences: Viruses that are dependent on direct access to blood, are by far too slow to be WMDs. So HIV and Hepatitis C Virus HCV are undoubtedly very harmful viruses, but no bioweapons[19].

Factually, bioweapons are those agents which can be transmitted via the air (**airborne infection**).
The **delivery** can be done by aerosols or more targeted by missiles, maybe with bomblets, but the latter would require that the agents survive the explosion/opening of the missile in the target region, which is another high technical hurdle.
Further, a mass infection requires mass production. However, **scale-up** of such productions is quite complex and significantly increases the risk for detection, also this coincides with an increased risk of accidents (such as the above mentioned Sverdlovsk-19 incident from 1979).
If a particle is too large = heavy, it simply falls down to the earth. Many agents degrade quickly, with the important exception of anthrax where the spores may rest for years. Also, large particles may not be inhalable anymore. Practically, only those weapons with a few microns length are of military relevance.
A main obstacle which also may explain why bioweapons were not used during wars is **control of infection spread** which may hit the own soldiers or civilians as well if the situation gets out of control.
Finally, an attack with WMDs could be **retaliated** by the attacked state.

2.2 Classification

Bioweapons can be differentiated into **bacteria, viruses** and **toxins**.
Simply spoken, **bacteria** are single-cell organisms use the infected body for nutrition and replication, e.g. by creating layers (biofilms) on tissues or by

[18] NSABB 2006
[19] In the 1980ies, the Soviet Union spread rumours that HIV was created in US laboratories. Meanwhile, it is clear that HIV evolved from simian viruses and more importantly, the re-evluation of frozen blood samples form navy persons who died for unclear reasons, showed that already in the 1950ies people died from AIDS.

circulating in blood and other fluids. Sometimes, they secrete **toxins**; these are proteins which block important processes in cells which led to damage and death of the affected areas.
Viruses infect cells, use the cells for their replication and leave the cells again, which often destructs the infected cells. Some viruses do not destroy the cells, but leave copies of their genes in these cells which may later on reinfections and other problems.

The *Center for Disease Control and Prevention CDC* has released an internationally recognized *CDC Bioweapon Classification* with the three categories A (highest risk; easily spread or transmitted with high death rates and major public impact such as smallpox and anthrax), B (high risk; moderately easy to spread, moderate illness rates and low death rates such as *Coxiella burnetii* (Q-fever) and C (emerging pathogens with future risk).

Principal treatment strategies are:

- **Preventive**, i.e. avoiding contact to infectious agents and vaccination
- **Therapeutic,** i.e. treating the infectious agent directly, like antibiotics for bacteria, antivirals for viruses and antitoxins for toxins
- **Supportive/symptomatic**, i.e. treating the symptoms, e.g. fever, myalgia, headache, nausea etc. etc.

The following table gives a brief overview on practically important bioweapons. Further details on symptomatic treatment and general treatment strategies are given in Sections 2.4.2 and 2.4.3.

Table 1 Overview on classical bioweapons[1]

Type	Infectious agent	Disease caused	Treatments discussed in literature
Virus	Variola (Smallpox, human pox virus)	After oral infection, massive replication in lymphatic tissues with subsequent swelling; later on spread to mucosa and skin with multiple lesions which allows infection of others	Vaccination Vaccinia immune globulin, methiasazone[2] Cidofovir (antiviral), maybe also ribavirin (antiviral), but only limited data available
	Viral hemorrhagic fever VHF including Ebola Virus EBOV and Marburg Virus MARV	Ebola and other VHF viruses cause cytokine release and severe inner bleedings (e.g. apparent as spontaneous nose bleeding)	In 2017, various agents are tested. GS-5734 showed activity against both EBOV and MARV[6] ZMapp, a mix of three chimeric antibodies manufactured in tobacco plants (Nicotiana benthamiana) had statistically inconclusive results, [6] Ribavirin was shown to be effective in some VHF viruses[6]
Bacteria	Anthrax (Bacillus anthracis)	Spores that get in touch with respiratory tract are taken in by immune cells, the macrophages. One protein protects the spores from destruction, two others form a toxin that destroys the macrophages and results in hyperinflammation, which is a CRS-like syndrome.	Vaccination Ciprofloxacin (antibiotics) [5]
	Francisella tularensis	Tularaemia, a zoonosis which can be inhaled or transferred by contact and is causing severe fever and sepsis symptoms. Occurs naturally, e.g. contact with wild animals with inhalation of bacteria	Streptomycin (antibiotic) and gentamicin
	Yersinia pestis	Plague Most common form are transmissions by infected flea. Then bacteria cause a painful lymph node swelling, known as buboes. Then septic reactions follow, appearing as “black death”	Historically, streptomycin, limited data for the antibiotics doxycycline, preclinical data exist for gentamicin, fluoroquinolones. [4]
Toxin	Botulinus toxin Released by bacterium Clostridium botulinum	The toxin irreversibly blocks cholinergic synapses, causing neuromuscular blockade.	Equine botulinum antitoxin[3] The antitoxin is effective, but needs to be given quickly (often, the diagnosis is initially not considered due to diffuse neuromuscular symptoms)

[1]If not otherwise mentioned, presented information is derived from the respective EMA Guidance CPMP/4048/01 which was last updated 2007

[2]Cetaruk 2002b

[3]Jaeger 2002, Arnon 2001

[4]Brent 2002

[5]Cetaruk 2002a

[6] Bixler, SL, Duplantier, AJ, Bavari, S. 2017, in particular p.310

2.3 Key Regulations

The most important global regulation is the *United Nations Biological and Toxin Weapons Convention from 1972* which makes the development, production and stockpiling of biological and toxin weapons illegal. The **use** of biological weapons by nation states was already prohibited by the Geneva protocol which was ratified in 1928 as a consequence of World War One.

In response to 9/11 and to the subsequent anthrax incident, the USA released the *US Patriot Act* and the *Public Health Security and Bioterrorism Preparedness and Response Act 2002*. Based on the *USG Policy on Biosecurity in Life Sciences Research,* the *National Science Advisory Board for Biosecurity NSABB* was establihsed as key institution from 2004 on. The NSABB is managing the *Select Agent Rules* which were released in March 2005 to ensure control of potentially dangerous biological agents[20].
The federal regulation *18 USC175* clarifies e.g. the penalties and imprisonment of persons, who unlawfully try to gain or to work with bioweapons or try to gain too large portions of the poxvirus genome sequence.

Also, the NSABB analysed 2006 the documentation and security checks of clients who ask DNA synthesis firms for certain DNA fragments and emphasized the need for a through security check of anyone who requests such sequences[21].

Following the post-9/11 anthrax attacks, the *European Medicines Agency* EMEA (meanwhile EMA) released guidelines and recommendations ofr bioterrorism[22].
Then, the seven laboratories with the hightest Biosecurity-Level (BSL) P4, which are able to check and to treat samples of very dangerous agents like Poxvirsues and viruses causing haemorrhagic fever were linked with each other to ensure information exchange, excercises, trainings and education[23].
Already in 2003, the European Commission introduced strict surveillance rules for *Bacillus anthracis* (für Anthrax), *Franciscella tularensis* (für Tularemia), *Coxiella burnetii* (Q-Fever) and *Variola major* (smallpox) and added these rules to an EU list of critical infectious agents.

2.4 Biodefense strategies

2.4.1 Introduction

As mentioned above, **bacteria** use the infected body for nutrition and replication, e.g. by creating layers (biofilms) on tissues or by circulating in blood and other

[20] NSABB 2006
[21] Relman 2006
22 EMEA 2002 and EMEA 2003
[23] EU 2004

fluids. Sometimes, they secrete **toxins**, these are proteins which block important processes in cells and lead to destruction of cells or even larger tissues.
Note that Anthrax bacteria have a special storage form, the spores, which allow survival outside bodies for many years, until somebody inadvertently gets in contact with this particle. This makes Anthrax very problematic: once released for an attack, the contamination of the area may persist for years(!) while other bacteria are more or less diminished after a few days or weeks.
Viruses infect cells, use the cells for their replication and leave the cells again, which often destructs the infected cells. Some viruses do not destroy the cells, but leave copies of their genes in these cells which may later on reinfections and other problems.

However, certain aspects are particularly important for bioweapons as well:

Sometimes, the infectious agents are suppressing the immune system by blocking certain molecules in the immune system. This prevents counterreactions of the immune system and to override defense lines. The defense gaps are very specific (many viruses find ways to reduce Interferon levels which is the key cytokine for anti-virus actions[24]).
Some viruses, e.g. from the group of influenza ('flu') viruses, can even confuse the immune system communication, resulting in imbalanced and/or excessive release of cytokines (immune hormones used for internal communication) and/or enhance **secondary infections** (infections on top) with bacteria[25].
For one of worst pandemics in human history, the Spanish Flu from World War One, it seems that secondary infections by bacterial lung infection (pneumococcal pneumonia) massively contributed to large amount of deaths which could nowadays treated with antibiotics (this may explain why later influenza pandemics had much lower death rates).

However, there are situations where the infectious agents *overstimulate the immune system*. This includes e.g. for bacteria circulation in blood, too many bacteria or too many bacteria fragments after destruction by immune system. Bacteria may also have **superantigenes** which lead to a diffuse and untargeted massive activation of the immune system. Viruses can also lead to an overstimulation of the immune system for various reasons.

The overstimulation leads to an excessive communication between immune cells via hormones that are called **cytokines**. Cytokine subclasses are **interferons (IFNs), interleukins (ILs), tumor necrosis factors (TNFs)** and others.

[24] Haller 2009, p.57
[25] Kash et al 2011, Stegemann-Koniczewski 2012

A **cytokine storm (Cytokine Release Syndrome CRS)** is a life-threatening shock-like condition as described in the next section.

However, this has major consequences for biodefense.
In the Gain of Function (GoF)-Research, *the induction of cytokine storms is the key strategy to make viruses more aggressive*, the knowledge of this and the treatment is thus crucial for a proper biodefense.
Also, a post 9/11 biodefense analysis of the US Air Force from 2002 recommended boosting the immune system[26]. At that time, this was plausible and reasonable, but in the light of the new findings it would be counterproductive in case of a cytokine storm.

For an effective biodefense, it is important to know that e.g. Ebola infections drive the infection by an increased release of TNF-alpha and other cytokines[27], i.e. a kind of cytokine storm and that the variola genome carries homologues of human cytokines (known as **virokines**) and of cytokine-receptors (**viroreceptors**) which indicates that at least partially the Poxviruses damaged humans by a cytokine storm[28]. Also, Anthrax toxin leads to 'hyperinflammation' which is in modern terms a CRS-like reaction[29].

Note that the so-called Swine Flu H1N1 virus from 2009 caused an increase of the cytokine Interleukin-17 which mediated the acute lung injury in these patients.
For such viruses, corrective actions on immune system communication (such as cut-off of cytokine excess) by cortisone and other substances could be a new option to mitigate infections in addition to the established approaches of prevention by vaccines and antiviral medications[30].

So the *understanding and the treatment of the Cytokine Release Syndrome CRS could be a key element of biodefense, also it is likely to be the most important defense strategy in case of new GoF-based bioweapons and as the CRS is also important for classical biological weapons like Anthrax and Poxvirus (Variola)*.
In principle, the current symptomatic treatments address CRS-related syndromes already, but the deeper understanding of the pathophysiology of these infections may open additional and more standardized defense options.

[26] Ainscough 2002, p.24
[27] Becker 2010, p.577
[28] Schwantes, A, Süzer, Y., Sutter G. 2010, p.705
[29] Cetaruk 2002a
[30] See also Li et al. 2012/ Li, C., Yang P., Zhang Y., Sun Y., Wang W. et al 2012

2.4.2 The Cytokine Release Syndrome (CRS)

The excessive release of cytokines, known as **cytokine release syndrome** or 'cytokine storm' can result in potential fatal shock-like conditions (circulation failure, organ failure, blood clotting etc.).

Lee et al. 2014 suggested a practical Grading system for CRS, in short:

- Grade 1 fever and constitutional symptoms (e.g. myalgia, headache, nausea)
- Grade 2 Hypotension with need for fluid support or one low-dose vasopressor/hypoxia with need for oxygen supplementation/moderate organ toxicities
- Grade 3 Hypotension with need for multiple vasopressors or one high dose vasopressor/hypoxia with need for strong oxygen supplementation/severe organ toxicities
- Grade 4 Mechanical ventilation and life-threatening organ toxicities[31].

This literature reference is in line with many similar CRS articles. A widely accepted strategy is to treat the symptoms as shown in the grading system with the option to add corticosteroids (cortisone-like substances) and maybe with tocilizumab. This is a monoclonal antibody that blocks the cytokine interleukin-6 IL-6, which is key communication molecule in the immune system.

The current treatment of CRS is already elaborated, but for biosecurity purposes the capacity needs are problematic. So it *may* make sense to look for simplified approaches which would allow mass treatments as a kind of 'muddling through' until the infection is over.

2.4.3 Treatment strategies

There are three treatment strategies:

- **Preventive**, i.e. avoiding contact to infectious agents and vaccination
- **Therapeutic,** i.e. treating the infectious agent directly, like antibiotics for bacteria, antivirals for viruses and antitoxins for toxins
- **Supportive/symptomatic**, i.e. treating the symptoms, e.g. fever, myalgia, headache, nausea etc. etc.

For military prevention, the United States set up in 1998 the Military Vaccination Programs under the direction of the *Military Vaccine Agency*, also known as MILVAX program[32]. The program provides not only vaccination as such, but also

[31] For a detailed guidance and explanation please refer to Lee et al. 2014.
[32] Anderson 2008

consultation services, education, safety surveillance, research support and advocacy.

After 9/11, as part of the MILVAX program, all military personnel on the Korean peninsula staying longer than 15 days and US Central Command staff must be vaccinated with smallpox vaccine from 2002 on.
In October 2006, a new policy implemented mandatory anthrax vaccinations for the same groups. However, Anthrax vaccinations were already started earlier and from 1998 to 2008, 1.9 million military personnel received anthrax vaccinations already[33]. Further vaccination activities include general protection, e.g. against influenza.
For specific and known bioweapons, authorities (such as CDC in the US, EMA in the EU and others) have the experts and know-how available. The EMA recommendations were presented in abbreviated form in Section 2.4.1.

Based on the above presented findings, a *provisional biodefense concept* for an unknown weapon should at least include a combination of broadband antibiotics (to kill bacteria and also to prevent secondary infections!), ideally of different drug classes and medications that are able to treat CRS or CRS-related syndromes like e.g. shock and fever.

Unfortunately, while resistance against antibiotics gets global attention, see Section 4.3, the existence of *major gaps in antiviral treatment* is critical.
The influenza antiviral oseltamivir has been shown to mitigate the influenza infection, but this may be not enough in case of Gain of Function (GoF)-upgraded aggressive influenza virus infections, also resistancies may occur. Further antivirals would be needed to prevent such GoF-virus pandemics.

While a lot of research and success for antiviral treatment of herpes, hepatitis and AIDS patients was achieved, for Ebola and also for Variola only limited antiviral studies are available[34].

The antiviral drug gap is a biosecurity problem, because *viruses have a much larger potential for weaponization* than the complex and slower bacteria.

[33] Anderson 2008, silde 8 and 9
[34] The same is e.g. true for the wide-spread dangerous tick bite virus FSME, which causes potentially fatal encephalitis. Furthermore, the Zika virus epidemia had not seen an antiviral medication. Another important and wide-spread virus is e.g. the Ebstein Barr Virus EBV, as EBV-DNA is the driver of various immune cells cancers (lymphomas), so that a more effective antiviral EBV-treatment may save many lives and money for the health system as well.

2.5 Smallpox (Variola)

Smallpox (Variola) virus was officially declared to be extinct in 1980 after an intense global vaccination and surveillance campaign[35]. From that moment on, the vaccination against poxvirus (which historically was the first vaccination ever) was cancelled, because it appeared to be quite dangerous. There were a significant proportion of children who suffered from brain inflammation (encephalitis) or heart inflammation (perimyocarditis), both diseases with a high risk for long-term damage or even death.
On the other hand, this meant that from that moment on the world population was not protected against poxviruses anymore.
From the very beginning, there was a debate what would happen if poxviruses return. The mortality rates may not be as high as in the past as medicine made further progress, but a global pandemic could nevertheless still kill several hundred million people[36].

Officially, human poxviruses (also known as small pox or Variola) are safely stored at only two locations in the United States and Russia, this is the Center for Disease Control and Prevention CDC in Atlanta and the Center of Virology and Biotechnology VECTOR in Koltsovo, but e.g. in 2014 the United States NIH found forgotten frozen poxviruses in their own archive[37].
There are increasing reports of pox-like infections with monkey pox[38], in Germany some fatal pox infections were reported already in 1990 mainly in immunosuppressed patients where the cow pox virus was able to pass species barrier to cats and from there to the humans[39]. So, poxviruses may also naturally return.

Since decades, a milder (attenuated) poxvirus variant, the so-called **Vaccinia Virus (VV)** exists, which is further developed incrementally by cultivation and mutation to make it even milder (safer, attenuation) while keeping its efficacy (causing immunity against poxviruses). After 9/11, as part of the *Military Vaccine Agency (MILVAX)* program, all military personnel on the Korean peninsula staying longer than 15 days and US Central Command staff must be vaccinated with smallpox vaccine from 2002 on[40].

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[35] DiEuliis/Berger/Gronvall 2017
[36] This being said, it should be noted that total eradication scenarios like in the movie *12 Monkeys* are unrealistic. A certain proportion of humans will very likely survive an infection of whatever agent, due to accidental immunity by genetic disposition or genetic defects, by cross-immunity to other agents, by interference with other ongoing diseases, current medication intake or metabolic situations. This topic was partially discussed in another movie from the 1970ies, the *Andromeda strain*.
[37] DiEuliis/Berger/Gronvall 2017, p.2
[38] Shah 2014, p.27
[39] Scheubeck 2014, p.7
[40] Anderson 2008, slide 10

To decrease the still existing risk of perimyocarditis and other side effects, a new variant was cultivated, the **Modified Ankara Strain (MAV)** which lost the ability to self-replicate in human cells. This change led to positive results[41].

In a further step, it is planned to develop a **synthetic viral platform** for pox vaccine, by this overcoming the need for further VV cultivations. For them, the virus researcher Evans was able to re-create an extinct virus in 2016, the **horse pox viru**s, by synthetic DNA and other measures[42]. While this was done with the good intention to have a new technology platform for safer smallpox vaccines for humans, scientists are very concerned about the future consequences of this large technology advance in synthetic biology[43].
However, the *World Health Organization WHO* stated already in 2015 that such a development could be expected[44].

The details of this work were not published in 2017, but an analysis of the existing information showed that the combination of DNA fragments in the right order was not so easy to do[45], as this required e.g. the involvement of a helper virus system[46]. Furthermore, reporting duties of companies submitting DNA sequences and the fact that ownership of too long poxvirus DNA sequences is unlawful are further security measures.
Nevertheless, the question for the future will be how far this could stop adversaries of the United States, who have their own experts, no reporting requirements and no laws to stop synthesis if done by the own researchers?

Outside vaccination plans, it was mentioned earlier that the variola genome carries copies of human cytokines (known as **virokines**) which indicates that at least partially the poxviruses damage humans by a cytokine storm (CRS). This however means that poxvirus infections may probably be mitigated by medication used in CRS treatment[47]. In theory, the virokine 'IL-6 homolog' could be a promising target to block expansion phase in the lymphatic tissue.

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[41] Zitzmann-Roth et al. 2015
[42] DiEullis/Berger/Gronvall 2017
[43] Kupferschmidt 2017
[44] WHO 2015. Prior to the meeting of the WHO Independent Advisory Group on Public Health Implications of Synthetic Biology Technology Related to Smallpox, a Scientific Working Group (SWG) on Synthetic Biology and Variola Virus and Smallpox met in April 2015 and concluded that "*there is now the capability to recreate the variola virus, the causative agent of smallpox [….] by multiple institutions and persons, including those with malicious intent.*"
[45] Kupferschmidt 2017
[46] DiEuliis/Berger/Gronvall 2017 Also, the firm behind this, Tonix, is already closely working together with the US Department of Defense (DoD) in another study for posttraumatic stress (see their website from 2017), i.e. this work was done in a secure environment.
[47] Schwantes/Süzer/Sutter 2010, p.705

For **antivirals**, only very little data are available, which has to do with the fact that poxviruses were extinct, before the age of antivirals had really started[48]. Limited data are available for Cidofovir and ribavirin may cover small pox as well.
But it may possible that *in vitro* studies may identify promising candidates for a poxvirus treatment.
Interestingly, the pox virus (variola) is a large double-stranded DNA virus, so maybe modified **virophages** may open new treatment options.

3. Gain of Function Research

3.1 Concepts

In 1983, the Japanese researcher Kawaoka noted that a limited virus mutation in a local avian flu outbreak made the causing influenza virus much more aggressive. This was the starting point for a long-term research. In 2011, Kawaoka combined an avian flu and swine flu virus and showed that this was transmittable between ferrets (which are the animal model for humans due to similar reaction pattern) by droplet transmission[49]. This contributed significantly to the understanding how e.g. avian flu influenza could suddenly be harmful for human beings as well. Note that influenza viruses do such recombinations also in nature.
In parallel, the researcher Fouchier showed that an influenza virus could be much more contagious to ferrets after introduction of certain mutations. Initially, there was a misunderstanding that this virus would also be more aggressive, but later on it could be clarified that this was not the case[50].
The Influenza A virus (IAV) experiments in particular of the teams of Fouchier and Kawaoka have led to the DURC policies of the USA in 2012, which address seven aspects as potentially problematic, these including (abbreviated) enhancing the harmful consequences of an agent or toxin, disrupting immunity or effectiveness of an immunization, causing resistance to prophylaxis or therapies, increasing stability or transmissibility, altering the host range, enhancing the susceptibility of a target population or generating or reconstituting an eradicated or extinct agent[51]. The publications of the studies were postponed for some months[52].

Moreover, virologists agreed to suspend further Gain of Function research, but then a group of virologists revoked this in 2013 already and argued that their laboratories were safe enough[53]. However, the research was stopped again in October 2014 due to safety concerns including an incident where the CDC

[48] EMEA 2002, Cetaruk 2002a
[49] Imai et al. 2012, Guterl 2012
[50] Fouchier et al. 2012, Herfst et al 2012
[51] Taubenberger/Morens 2013, p.1-2 provide the full official wording of these rules.
[52] Fouchier et al. 2012, Herfst et al 2012, Imai et al. 2012
[53] Fouchier et al. 2013

erroneously sent aggressive influenza viruses to a research laboratory instead of the requested harmless version[54].
But on 19 Dec 2017, the US lifted the ban on funding of this research, but accompanied this by a new regulatory framework on potentially contagious organisms. In particular, an expert panel should do oversight of the activities[55].
Nevertheless, during 2014-2017, ten GoF studies got waivers and were continued, 5 on influenza virus and 5 on the Coronavirus MERS (Middle East Respiratory Syndrome).

The idea to have more aggressive viruses for better understanding of infections was however not new. In 2005, the 1918 influenza virus (Spanish Flu) which caused millions of death was recreated by reverse genetics[56].
Gain of Function can also occur as ‚**Bio-Error**'[57]. In late 2000, in Australia the insertion of the IL-4 gene into a mousepox vaccinia virus was intended to increase antibody response of mice, but instead it led to increased letality of infected mice[58].

3.2 Debate

For a detailed overview on the debate, please refer to the research workshop documents of the National Academy of Sciences from 2015 on GoF research[59].
In short, there are major safety concerns about GoF research, in particular the risk of inadvertent release from a laboratory which could cause an influenza pandemic. Also, there are concerns that the information from this research opens the way to weaponize influenza viruses. Both human beings and animals could be affected by this.

The supporters of the GoF research argue that the nature is driving mutations away and that this research is the only chance to be ahead of nature with preventive research. Until now, after an outbreak of a new agent, there is no time left to develop countermeasures, GoF research could give more time for new treatments.

Taubenberger/Morens argue that the nature is breaking all the rules of the DURC anyway even without any human activities, as Influenza viruses mutate regularly and some of them were more aggressive, were able to disrupt immunity (e.g. by affecting the interferon-based antivirus reactions), developing resistance to the antiviral oseltamivir, by breaking the species barriers by adaption or reassortment

[54] Grunert 2015, McNeil Jr 2017
[55] McNeil Jr 2017
[56] Tumpey et al 2005
[57] EC2007
[58] Frankfurter Allgemeine Zeitung No. 10/2001
[59] Sharples et al. 2015

(Swine Flu 2009), they enhance secondary infections like the Spanish Flu virus, and it seems that extinct virus variants may return after decades sometimes, as sometimes elderly populations have immunity against 'new' viruses[60].
In conclusion, their point is GoF research helps to understand viruses better and to have more time to be prepared for future challenges.

The defense against potential GoF viruses was already discussed in Section 2.4 above

4. Other Biothreats

4.1 Synthetic biology

As shown in the analysis of Variola, the synthetic biology has made substantial progress with the production of entire genomes, even of extinct viruses, just from paper knowledge[61].

Since 2010, Craig Venter and his team worked to develop a **minimal genome** cell, this is the smallest possible genome that allows autonomous life and replication[62]. Mycoplasma was the smallest known autonomous cell type and thus used as model organism since 1984. In 2016, a new cell, called *Syn 3.0*, was created by replacing the genome of *Mycoplasma capricolum* with the genome of *Mycoplasma mycoides*, with removal of unessential DNA. It has only 473 genes, but still the function of 149 genes is unknown.
If the function of these 149 genes could be clarified, it may theoretically be possible that some of other 'essential' genes are only needed to keep the *mycoplasma* design and may be replaceable by other genes. This however would be the breakthrough to **freely designable artificial cells**. From that point on, these extremely 'lean' and fast replicating cells may represent a significant bio-threat, so this research area needs to be under tight supervision by security authorities.

Artificial cells may also open the chance to develop **autonomous biohybrids**, which in the long run may be much more promising than cyborg projects. Biohybrids are free combinations of biological and synthetic materials.

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[60] Taubenberger/Morens 2013, p.1-6
[61] Thus, Hollywood and others imagined the recreation of dinosaurs, archaeopteryx and other extinct life forms from old DNA. However, the atmospheric oxygen concentration was much higher in past hundred millions of years than today which allowed the existence of much larger organisms than today. Nowadays, very large animals would not get enough oxygen so they would die immediately. Recreation of such animals is impossible and *not* a biothreat.
[62] Kastilan 2010

In 2016, a swimming robot that mimics a ray fish was constructed with a microfabricated gold skeleton and a rubber body powered by 200,000 rat heart muscle cells[63]. The cells were genetically modified so that speed and direction of the ray was controlled by modulating light. However, the biohybrid was still dependent from the presence of a physiologic salt solution.
Note that **cyborg development** is going much slower than expected[64]. Among other problems, the interfaces between living and computer sections are challenging. Another issue is the energy supply for the machine parts. Maintenance and repair requirements are already used as backdoors for cyber-attacks. Finally, the amount of machine parts that an organism may be able to carry seems to be quite limited.

In the first decade of the century, there were ambitious projects on **artificial life,** also known as **Programmable Artificial Cell Evolution (PACE)**[65]. However, nobody has any idea how things are brought to life, currently still any design requires an already living environment. Thus, artificial life remains hypothetical. Originally, an even more ambitious approach was the **system biology** where researches created repositories of biological elements (**biobricks**) which should allow planned engineering of cells[66].

4.2 CRISPR/Cas9

CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats) are DNA sequences in bacteria which help to detect virus DNA from attacking viruses. There are nearby located genes called **Cas (CRISPR-associated system)**, of which the Cas9 type is of practical relevance, as CRISPR/Cas9 can be used in many cells, including human cells. By linking the CRISPR/Cas9 to a synthetic guide RNA, this system can be used for targeted insertion of new and/or deletion of old genes.
This technique is much more precise and much easier to handle than previous technologies and applicable for many organisms and thus a highly significant technology advance.
In particular, it allows **genome editing** (like a draft movie is cut and stuck together) and **genome surgery**, which means the targeted repair of genetic defects in human beings[67].

[63] Park et al. 2016
[64] For background information on cyborg projects and state of technology in 2017, you may refer to the free Paper "Cyberwar –Methods and Practice" http://www.dirk-koentopp.com/downloads/saalbach-cyberwar-methods-and-practice.pdf, and the literature cited therein.
[65] Epping 2008, Gibbs 2004, p.70f.; ECLT 2006, Smith 2003, PACE 2006; Ailab 2006, Kuhrt 2006, p.30
[66] Boeing 2006, p.35
[67] For a detailed review on methodology and research history, please refer to Porteus 2015

The biothreat is primarily a long-term one: If embryos are cleaned from genetic defects or altered in other ways, this opens the pathway to a **targeted evolution of human beings**.[68]
One particular risk is the radical elimination of genetic defects which could result in a kind of **genetic monoculture** of human beings. However, sometimes genetic defects which seem to be useless or even harmful may provide immunity for future pandemics. The most prominent example is the defect of the CCR5 (C-C chemokine receptor type 5) gene, which affects the first line of defense in the immune system. However, CCR5 is needed by *Human Immunodeficiency Virus* for entry, so a CCR5 defect provides a high infection hurdle for HIV infections (i.e. immunity against AIDS infection). It is disputed whether this defect also provided immunity against Plague pandemics in the medieval age, too.
Another well-known example is sickle cell anemia which provides some protection against Malaria infections as the wrongly structured red blood cells inhibit the replication of the Malaria agent *Plasmodium*.
Irrespective of this, Chinese scientists started experiments with embryos in 2015, but the study showed that the technique is not yet precise enough (not effective in every embryo and also occurrence of **off-target changes**, i.e. inadvertent damage of other genes), all 85 embryos failed the treatment target[69]. Further studies are in progress.

Currently, *cloning of human beings* is globally forbidden[70] and would also due its complexity face many obstacles which make this unrealistic at the moment, but if cloning may be possible and allowed in the later future (e.g. due to the pressures of overaged societies in the Northern Hemisphere), the genetic monoculture problem would also be applicable here and a key biothreat to cloned populations.

While a lot of successful research is done to make CRISPR/Cas9 even more precise and reliable[71], the development is slowed down by *patent disputes* between various actors, which may still go on for some years.

Another biosecurity issue is the *potential enhancement of biohacking* by this new technology. Security authorities are already aware of this. In later 2017, a commercially available CRISPR kit *Odin* has raised concerns[72] as this could help to spread antibiotics resistance and meanwhile the German Federal State of Bavaria has notified the Odin manufacturer that further imports to Germany are unlawful[73].

[68] For a discussion of societal effects, please refer to Carroll/Charo 2015
[69] Johnson/Williams 2005
[70] Kolata 2015
[71] Fischer 2017
[72] Sneed 2017
[73] Lubbadeh 2017

4.3 Antibiotics resistance

4.3.1 Problem statement

Antibiotic resistance is one of the biggest threats to global health, food security, and development today as it can affect everybody[74]. It occurs naturally, but misuse of antibiotics in humans (by non-targeted or inappropriate intake) and in farming and agriculture is accelerating the process. A growing number of infections including pneumonia, tuberculosis, gonorrhea, and salmonellosis are becoming harder to treat. More importantly, there are some new antibiotics in development, none of them are expected to be effective against the most dangerous forms of antibiotic-resistant bacteria[75].

The World Health Organization initiated the Global Antibiotic Research and Development Partnership (GARDP) as a joint initiative of WHO and Drugs for Neglected Diseases initiative (DNDi), GARDP encourages research and development through public-private partnerships. By 2023, the partnership aims to develop and deliver up to four new treatments, through improvement of existing antibiotics and acceleration of the entry of new antibiotic drugs.

Also, many governments, medical societies and authorities have released guidance and education to use the existing antibiotics more carefully and properly. Furthermore, hygienic measures to prevent infections were globally enhanced.

However, the antibiotics pipeline of industry is emptier than in previous decades and there is an urgent need to push antibiotics research. Also, there is an urgent need to look for alternative treatments. The primary option so far is a method that was in particular used by the Soviet Union as substitute for antibiotic, the so-called **bacteriophages**.

Of all potential biothreats, the antibiotics resistance maybe the most relevant. It costs an increasing number of lives and money and may need to be considered as a security problem[76].

4.3.2 Bacteriophages

Bacteriophages are viruses against bacteria which use bacteria for their replication.

Bacteriophages were already used as anti-bacteria viruses in the Soviet Union and today Russia, Poland and Georgia for severe infections[77]. Despite concerns of a

[74] WHO 2017
[75] WHO 2017
[76] WHO 2017
[77] Mandal 2014

coming post-antibiotic era, the research activity is still limited and a specific regulatory framework is still missing in the Western states[78]. In 2009, a small study in 24 patients with ear infection showed positive results[79].

There were a few papers from the Soviet era about bacteriophage use and preparations are available from the *Eliava Institut*e in Georgia, however, there is need for larger and more systemic clinical trials. Another important bacteriophage research unit is located at the *Institute of Immunology and Experimental Therapy* in Wroclaw in Poland.
There were lots of discussions whether bacteriophages could be part of the regulatory framework of the drug regulatory authorities. A main concern is that these are living organisms with the ability of self-replication. However, this argument is not convincing in a time where manipulated living cells and stem cell preparations are injected into patients as part of clinical trials[80]. Also, there are no hints so far for persistence of bacteriophages after they killed all bacteria.

To push the bacteriophage development forward, the *French Ministry of* Defense initiated the Phagoburn study and coordinated the work of *Pherecydes* (French SME) and *Clean Cells* (French SME) for the Good Manufacturing Practice (GMP)-compliant bioproduction of drug products, CHU Vaudois (CH) burn wards, as well as the Royal Military Academy of Belgium (Queen Astrid). Three regulatory authorities of France (ANSM), Belgium (AFMPS) and Switzerland (Swissmedic) agreed to cooperate. The project was supported by the research fund FP7 of the European Union.

A first significant success was the ability to produce standardized bacteriophage preparations in line with GMP, which was seen as a major obstacle in previous phage research and which was indeed very difficult[81]. However, as many burn wounds were infected by many bacteria simultaneously, while bacteriophages are directed against one bacterium only, patient recruitment was lower than expected. However, preliminary results from 25 patients indicated in late 2017 that therapy was effective and safe, so that Pherecydes will continue to work on bacteriophages[82].

Bacteriophage enzymes may have also military relevance, as one bacteriophage product was effective against the standard bioweapon *Bacillus anthracis*, more commonly known as Anthrax[83].

[78] WHO 2014, Verbeken et al. 2014
[79] Wright et al. 2009
[80] Posey/June/ Levine 2017
[81] Pherecydes 2015
[82] Pherecydes 2015, Phagoburn 2017
[83] Zucca/Savoia 2010, p.83

An unconventional matter is viruses against viruses, so called **virophages**. Already nine virophages were found until 2012, all of them directed against a special subclass of viruses, the giant double-stranded DNA viruses[84]. The *Sputnik* virophage is directed against the Mimivirus that can cause human pneumonia[85], meanwhile the related *Zamilon* virophage was discovered[86]. The number of virophages is permanently growing, so several virophage genome sequences have been partially or fully assembled from metagenomic datasets, e.g. from two Antarctic lakes and the Yellowstone Lake[87].
As bacteriophages, this could be an alternative to current anti-infective medications.

4.4 Anti-Material weapons

In the past, there were some discussions whether there is a risk that genetically modified bacteria could infect machines with degradation and depolymerization. However, no such infection was ever reported in practice, so this remained theoretical.

But in 2016, a novel bacterium, *Ideonella sakaiensis 201-F6*, was discovered that is able to utilize Polyethylene terephthalate (PET) that is extensively used worldwide in plastic products as its major energy and carbon source, Yoshida et al. 2016. Two fungal species were already identified in 2011[88]: Two *Pestalotiopsis microspora* isolates were able to grow on Polyurethane PUR as sole carbon source both under aerobic and anaerobic conditions. Young moths (*Galleria melonella*) also consume Polyurethan at much higher rates than Ideonella[89].

The current findings were based on analyses in specific laboratory environments.
There is an urgent need to expand PET- and PUR- degradation research as there is a growing pollution of oceans with plastic garbage. Respective organisms could clean the water biologically.

However, in the later future the research may lead to organisms which are less dependent on temperature and water than the current ones. Then, aerosols with these agents could slowly and silently destruct all plastic elements in a target infrastructure. This would be the opposite of a neutron bomb which only destroys life while anti-material weapons would only destroy the materials. However, this

[84] Zhou et al. 2012
[85] Zhanga et al. 2012
[86] Krupovic et al. 2016
[87] Krupovic et al. 2016
[88] Russell et al. 2011, p.6076ff.
[89] Neuroth 2017

will *remain hypothetical* until less temperature-dependent PETases and PURases are developed/discovered (as e.g. computers are hot and dry).

5. Summary and Conclusions

Recent developments in synthetic biology (horse pox recreation), genetic engineering (CRISPR/Cas9) and the lift of the ban of gain of function (GoF) research are new challenges for biosecurity and biodefense.

For classical biological weapons, a regulatory framework is available that was significantly enhanced after the post-9/11 attacks. However, the paper has shown that large-scale bioterrorism is unlikely without support and funding of state actors. Those however should be aware that biological weapons and attacks can be attributed by various methods and molecular properties.

Principal treatment strategies are preventive, therapeutic, and supportive/symptomatic and an overview was given for the classical bioweapons.
New findings showed the important role of inappropriate release of immune hormones (cytokines) as cytokine storm or Cytokine Release Syndrome CRS for infection dynamics.
So the understanding and the treatment of the Cytokine Release Syndrome CRS could be a key element of biodefense, also it is likely to be the most important defense strategy in case of new GoF-based bioweapons and as the CRS is also important for classical biological weapons like Anthrax and Poxvirus (Variola).

Unfortunately, while the growing resistance against antibiotics gets global attention, the existence of major gaps in antiviral treatment is critical. Bacteriophages, i.e. viruses against bacteria maybe a promising alternative since French researchers recently managed to overcome standardization and quality issues which blocked the progress on these agents for decades.

The genome editing method CRISPR/Cas9 has led to a massive expansion of genetic engineering approaches and available organisms, which is difficult to control. A particular problem could be the expansion of biohacking activities, which was already observed in 2017.

Antimaterial weapons (plastic-eating organisms) remain hypothetical biothreats, the same is true for artificial cells, but the latter may change in the next few years. The paper presented various new options and recommendations to enhance biodefense and biosecurity.

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