Phage therapy in human bacterial infection

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Bacteriophage

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Conclusion

INTRODUCTION

Bacterial infectious diseases endanger human health

~ 25% of deaths are caused by infectious diseases, and bacteria account for 38% them

Infections are major factors in determining life expectancy, which in 1900 in the USA was 47 years

Over 70 years in 1970s, due to the discovery of antibiotics and their widespread use in the 1940s

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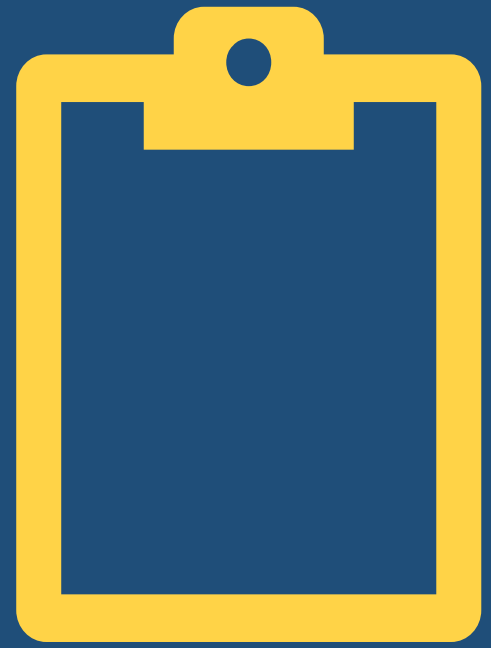
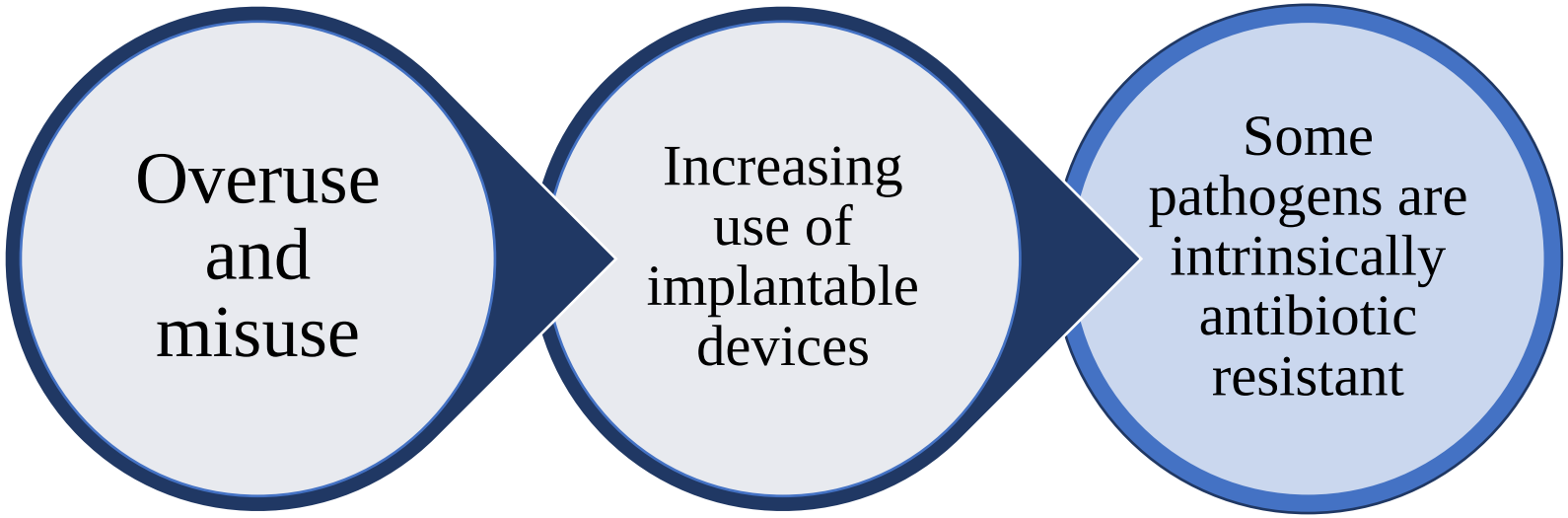


The rapid growth of resistance to antibiotics is driven by



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Antimicrob Agents Chemother.2022

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Lancet Microbe 2022; 3: e956–68

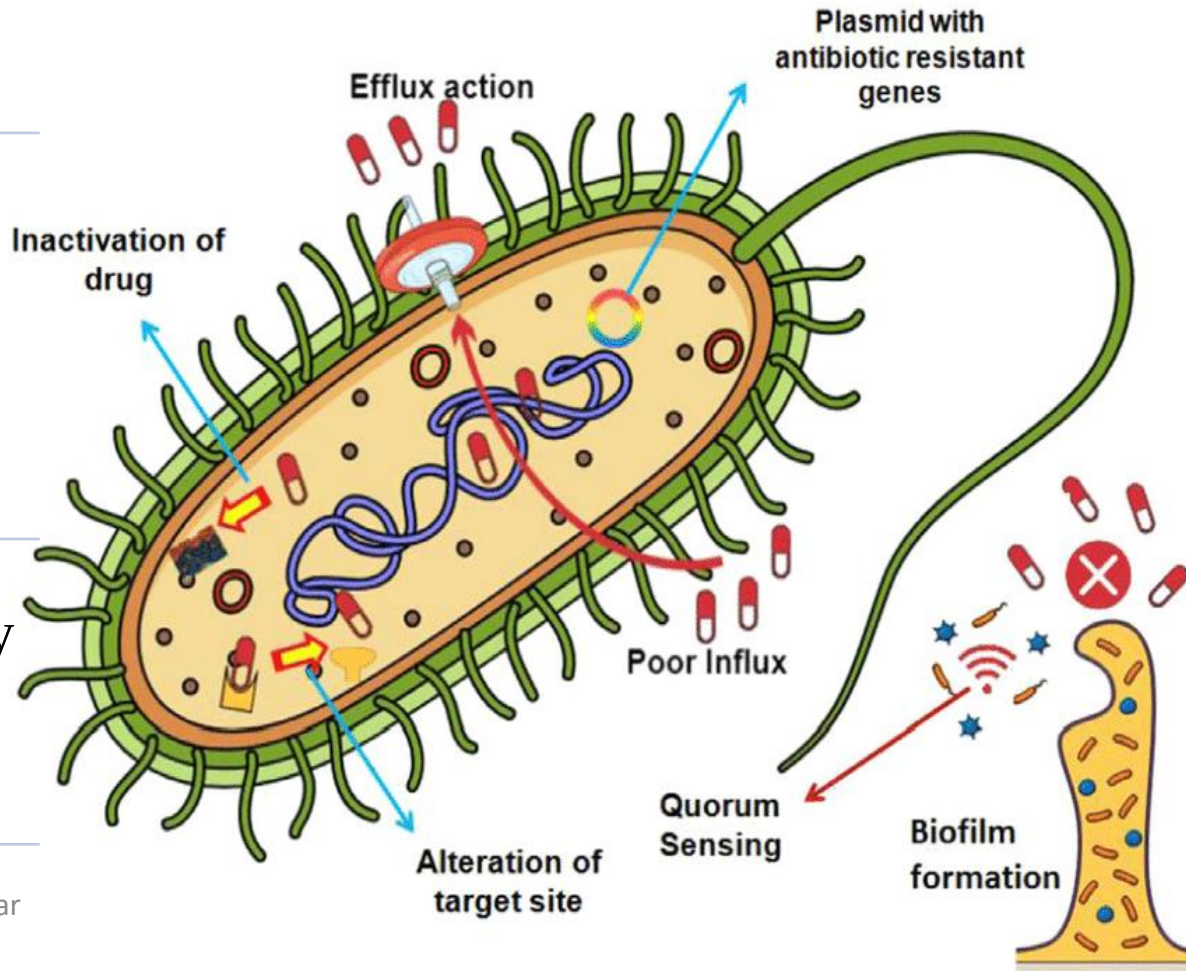
In 2019 ~ 1·3 million yearly deaths to AMR

At least 10 million people will die from AMR by 2050

Cost of one trillion dollars per year

Prolonged hospital stays, inadequate or the intensive use of antibiotics during the pandemic leads to increased prevalence of MDR bacteria

The development of new antibiotics that act differently from extant ones is both expensive and slow



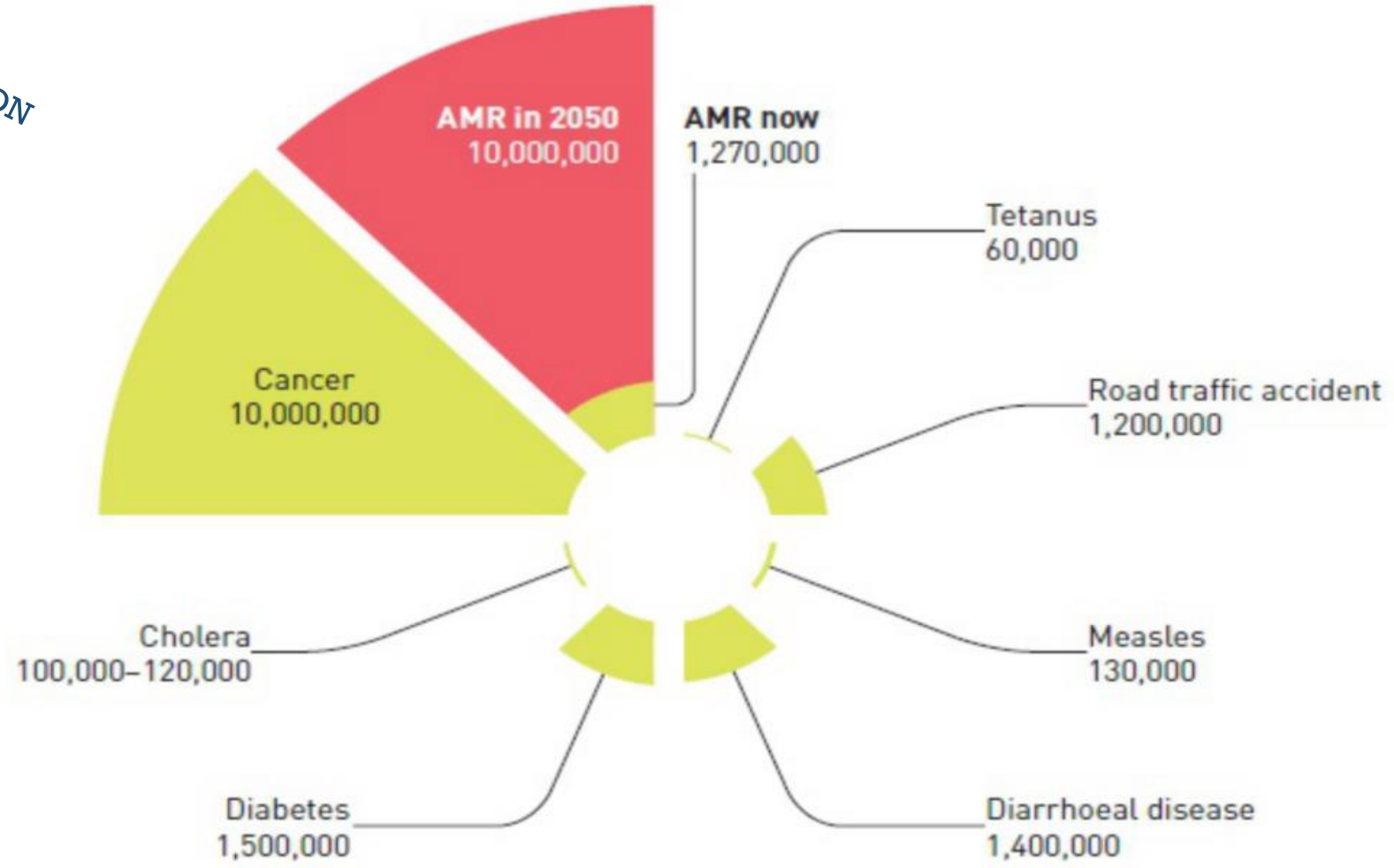


Fig.1 Predicted mortality from AMR compared with common causes of current deaths

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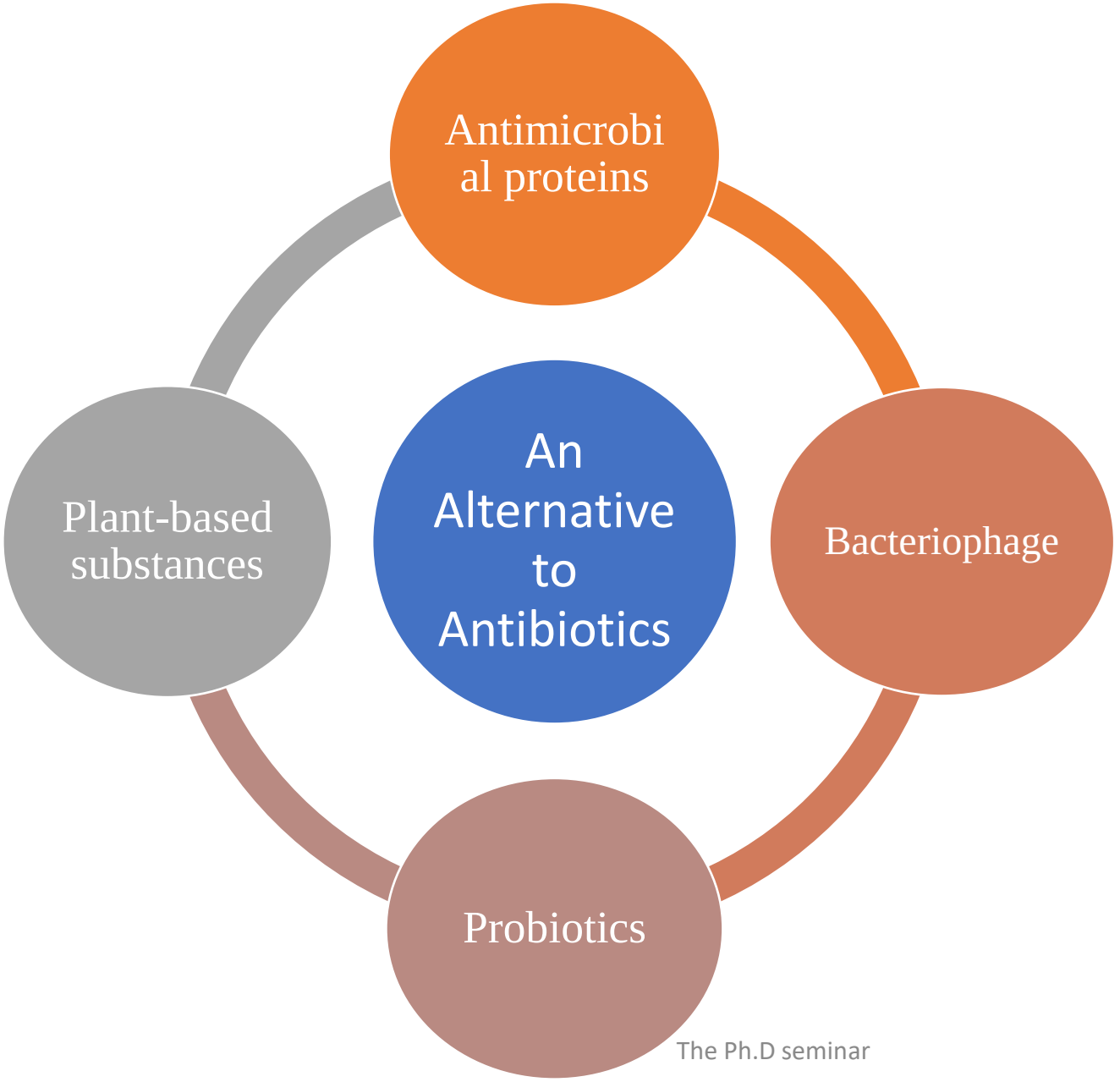

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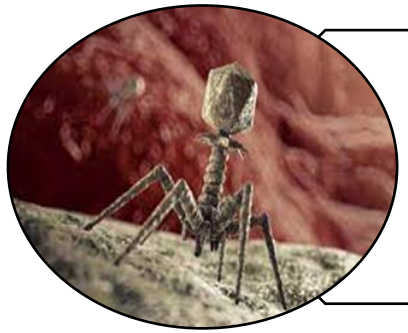
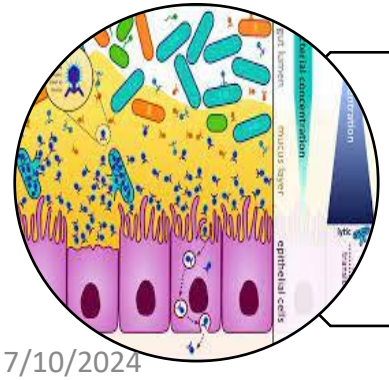
Bacteriophage



Viruses that specifically target bacterial cells

Were first described by Felix d'Herrelle and Frederick Twort



Over 30 billion phage particles are move in and out of human tissues every day, serving as various microbiomes

Annu. Rev. Virol. 2023. 10:503–24

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Antibiotics 2022, 11, 1340.

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Antibiotics 2022, 11, 1340.

- Natural predators of bacteria that have co-evolved with bacteria for ~ 4 billion years.
- ~10^{31} bacteriophage particles in the biosphere, they are believed to be the oldest and most abundant organisms on the planet.

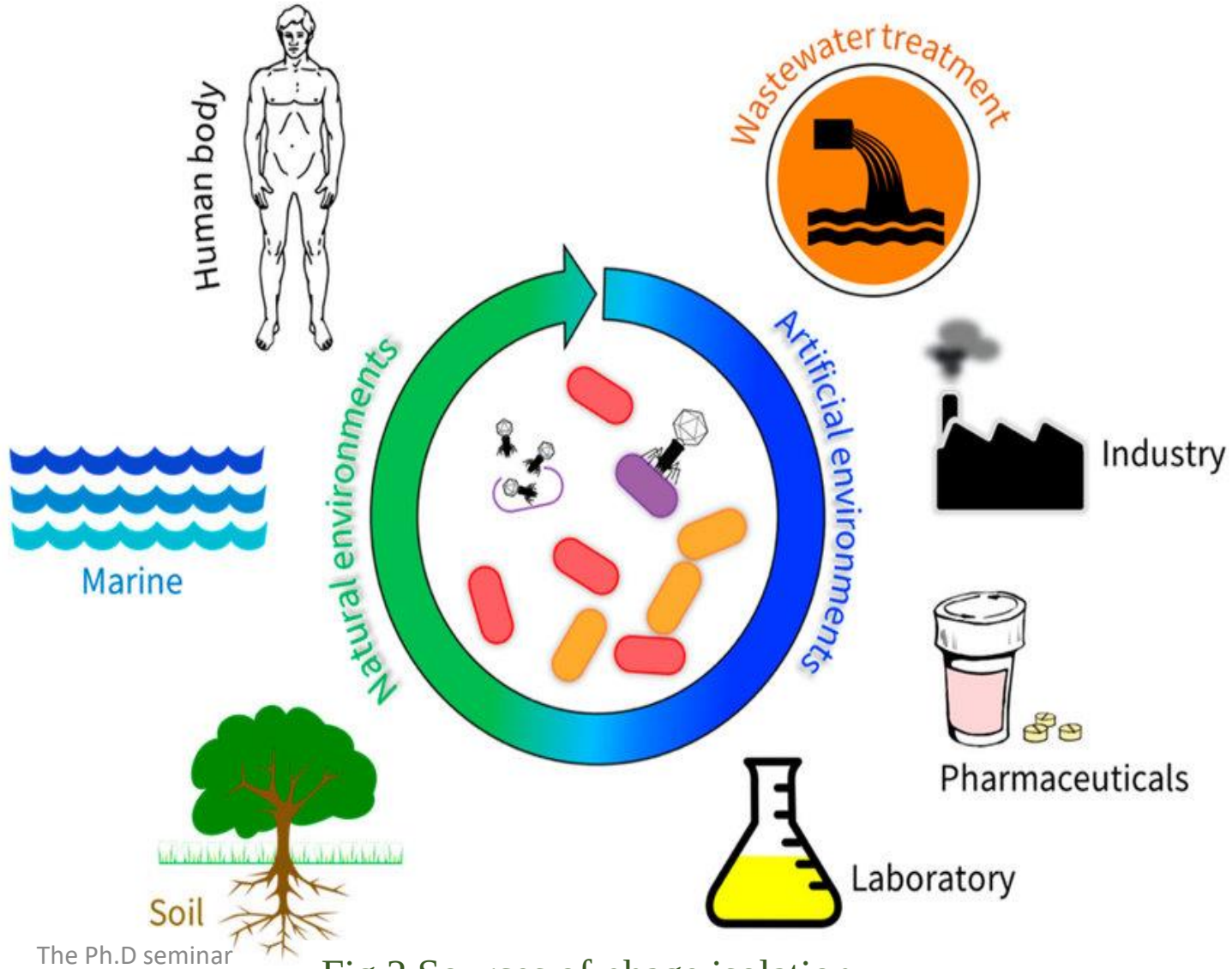

Fig.2 Sources of phage isolation

Bacteriophage

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Cell 186, Jan 5, 2023

Cannot replicate on their own and require a bacterial host

There is genetic diversity among phages

Phages

Small (50–200 nm)

Host range, may be very narrow or broad. A key factor determining the therapeutic potential of phages

Biologics:2022, 173-186

- Phages are made up of proteins or proteolipid capsids containing fragments of deoxynucleic acid (DNA) or ribonucleic acid (RNA).
- Their genome size ranges from a few thousand to 498 kbs.
- Phages have no machinery to generate energy or ribosomes to make proteins and they carry the genetic information needed to replicate in the host cell.

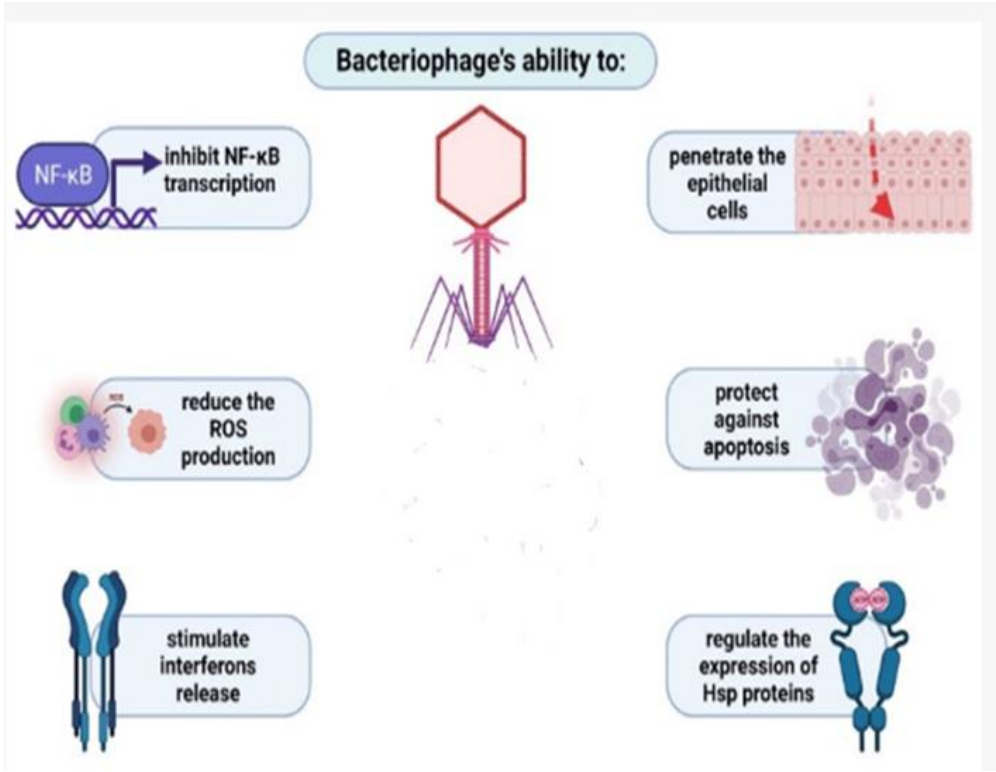

Fig. 3 Bacteriophages features

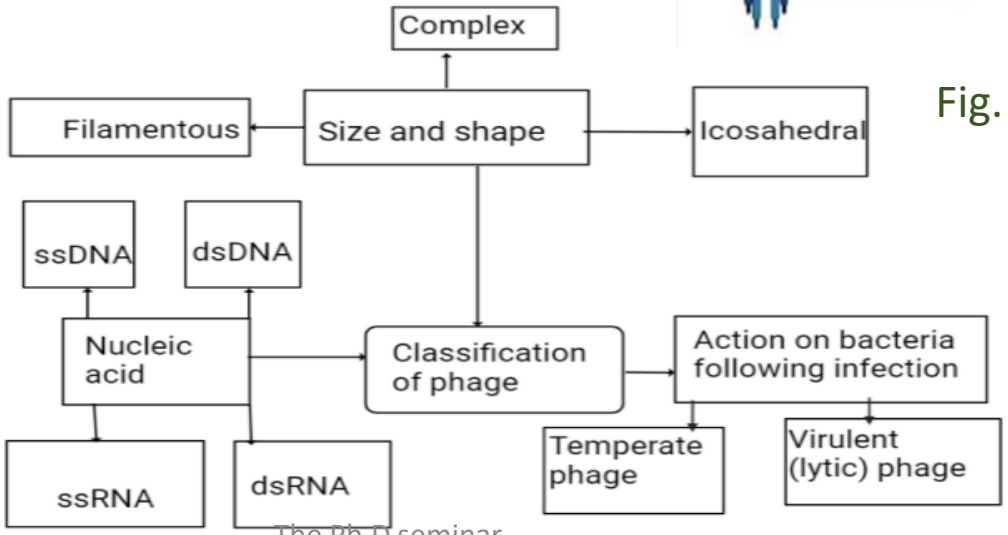

Fig.4 Classification of phages

Phage Therapy

- Phage therapy (PT) was attractive to d’Herrelle in the early 20th century as it offered the first plausible solution for treating bacterial infections.
- After d’Herelle successfully used phage preparations to treat children suffering from bacterial dysentery in 1919, PT was used extensively to treat bacterial infections in humans and animals in the 1930s.
- The first PT program opened in what is now Tbilisi, Georgia, followed by another in Wroclaw, Poland; both programs still exist to this day.

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- The broad-spectrum activity of penicillin and future antibiotics against bacterial infections was considered an advantage over phages, which require that bacteria express specific surface molecules to which the phage can bind and that lack intracellular defenses capable of inactivating the phage following entry.
- Antagonism between the United States and the Soviet Union in the post-war period fueled both distrust of science coming from the former Soviet Bloc and pervasive suspicions about the therapeutic use of phages for decades to come.

Lancet Microbe 2022; 3: e956–68

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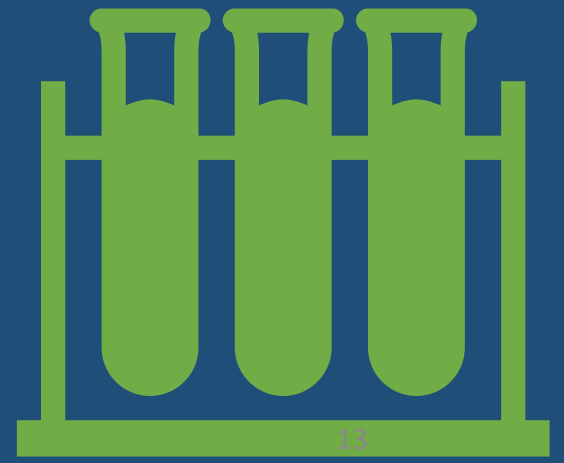
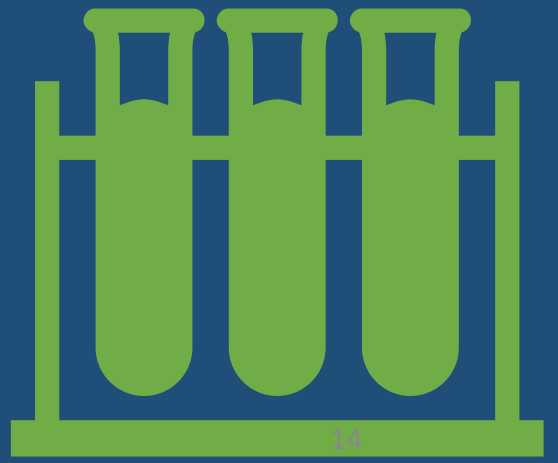


Int. J. Mol. Sci. 2022, 23, 4577

- PT is a way of delivering virulent phages to patient to kill pathogenic bacteria.
- The ability of most lytic phages to encode the enzymes, holins and endolysins, that degrade bacterial structures make them a new weapon against bacterial infections.
- Temperate phages are not used for therapeutic purposes because they integrate their genome into the host chromosome or sometimes maintain it as a plasmid to be transmitted to daughter cells during cell division or horizontally across the bacterial community.



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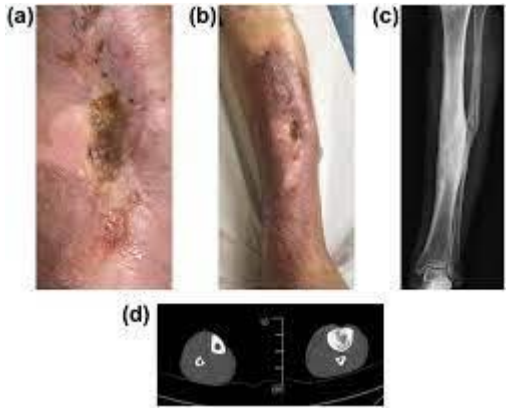
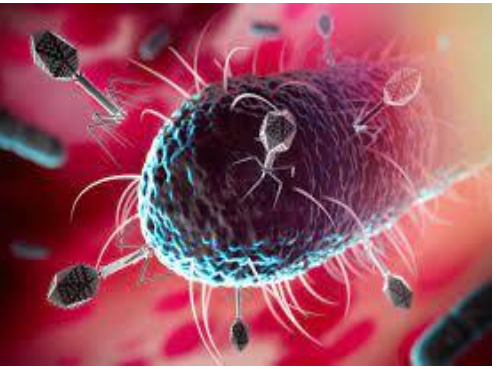
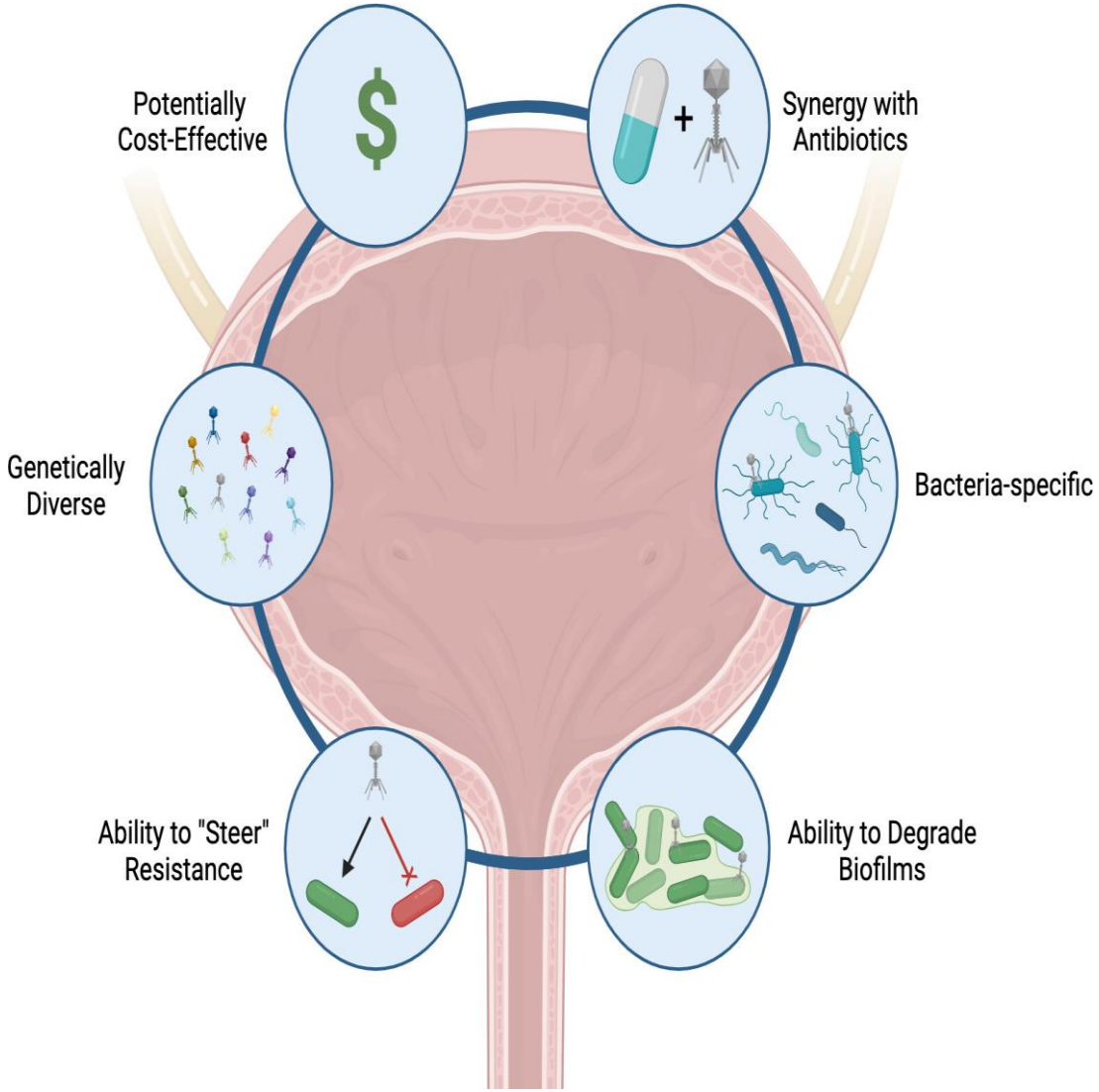




PT has been used for the treatment of infections related to burn injuries or soft tissue and skin trauma, osteomyelitis, sepsis, bacteremia, and otitis media as well as urinary tract, pulmonary, and prosthetic device-associated infections.



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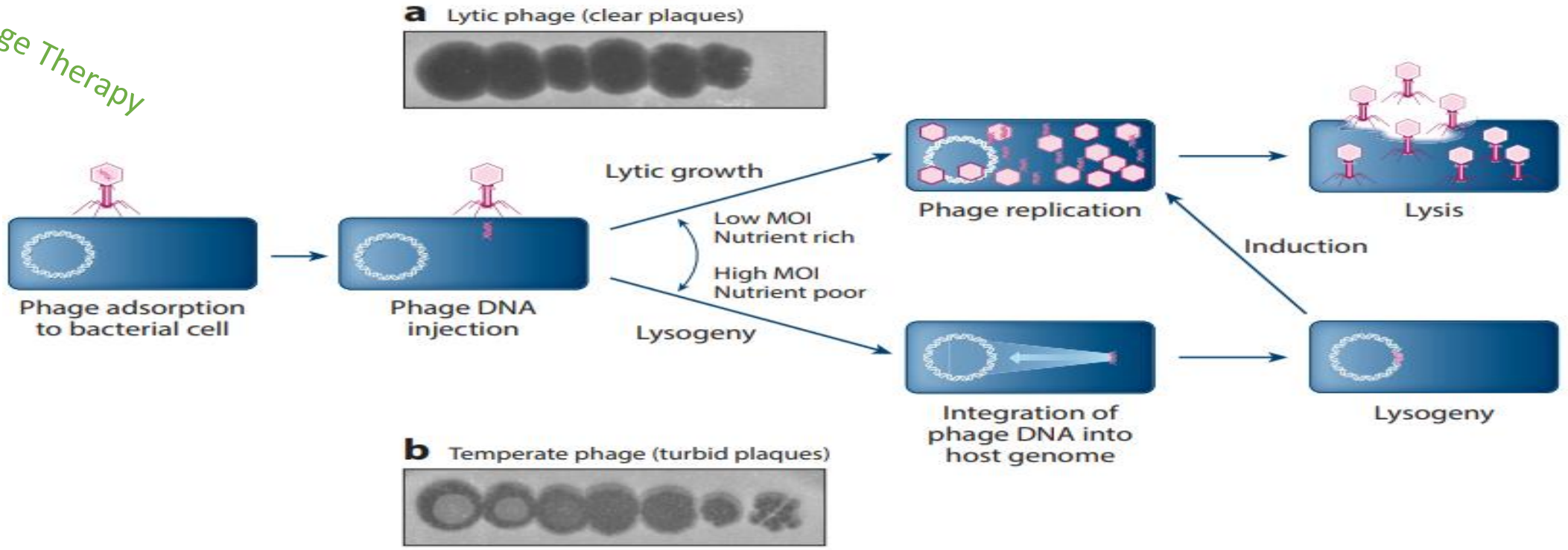

Fig. 9 Bacteriophage life cycles. Lytic phages typically infect the host bacterium through adsorption, DNA injection, DNA replication, assembly of progeny phage particles, and lysis of the bacterial cell. The only outcome is phage growth and cell death (a). The spots appear dark, compared to the surrounding lawn of cells, because all the cells in the spots are dead. Temperate phages similarly adsorb to the host bacterium and inject their DNA, but then "choose" to establish lysogeny in which the phage genome is integrated into the host chromosome (to form a prophage), and the lytic genes are switched off (b); turbid plaques form in which bacterial lysogens are growing. The path followed is influenced by many factors including the multiplicity of infection (MOI) and nutrient availability.

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- Only lytic phages are used for therapeutic purposes.
- They inhibit the emergence of resistant bacteria by disrupt bacterial metabolism and killing the bacteria that they infect and are preferable to antibiotics as they cause less damage to the general microbiome.

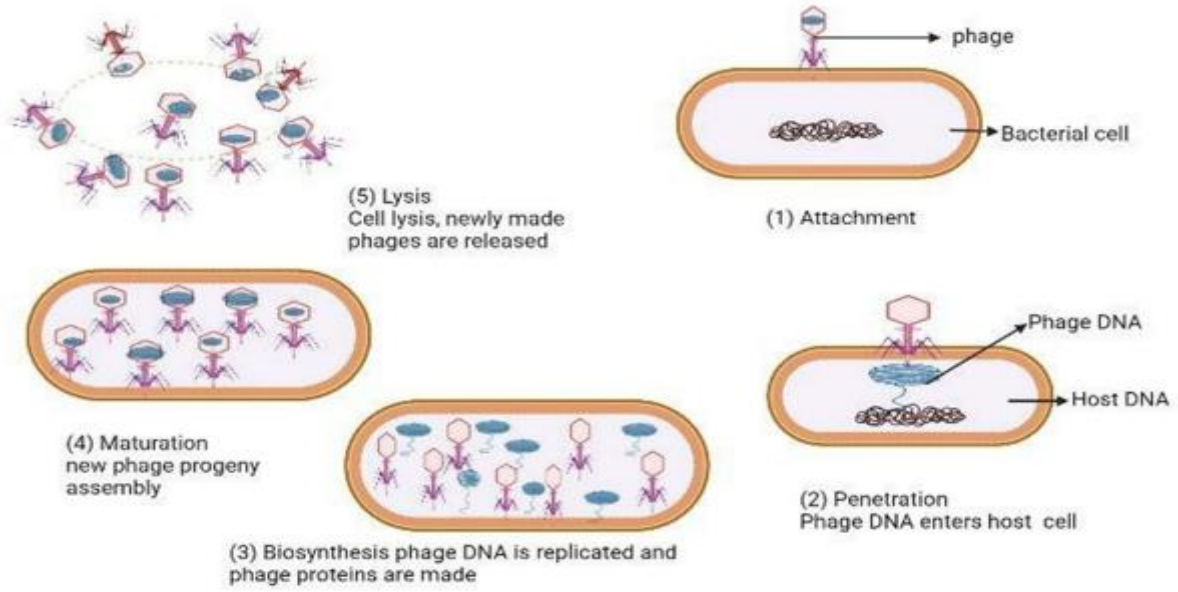

Fig. 10 Phage lytic life cycle

Advantages of phage therapy

- Biofilm control
- Re-sensitization to antibiotics
- Easier recognition by immune system cells
- Competitive ability relative to native microbiome species

Phages advantages over traditional antibiotics

- Highly specific for their hosts
- The most abundant biological entities on Earth, providing an enormous reservoir for PT
- Self-amplify and disseminate in the body to encounter susceptible bacteria
- Safe, based on rare cases of toxicity in animals and patients
- Target different bacterial mechanisms than antibiotics, minimizing evolution of cross-resistance

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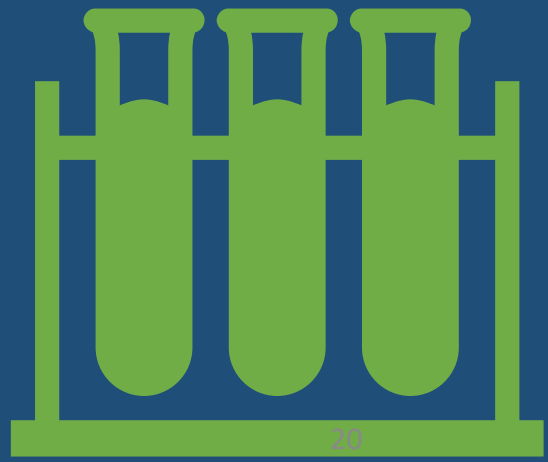
Pulm Ther (2023) 9:91–107

Key consideration in phage therapy

- Kill the specific bacterial pathogen
- Easy to propagate, to produce in high-titer preparations, and to purify
- Stable over a range of concentrations, storage for extended time periods at refrigerator temp. is not associated with loss of infectivity
- The phage genomes should not include any genes known or suspected to be toxic

AND… the phages should not be able to act as generalized transducing phages.



The mechanisms of phage effects on bacteria

Reduced bacterial adherence and colonization

Decreased host invasion

Impaired ability to evade host immune system

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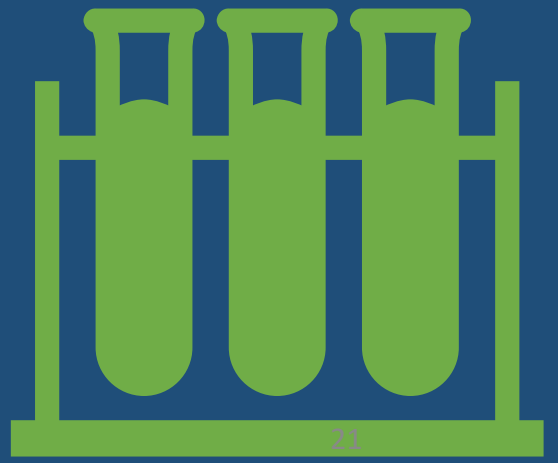

Reduced bacterial adherence and colonization

- *Listeria monocytogenes*, an intracellular pathogen, requires a complex glycosylation pattern acquired through teichoic acids in its cell wall for successful infection.
- Acquiring resistance to a phage specifically recognizing this pattern resulted in loss of these sugars from the bacterial cell surface.
- The phage-resistant mutants of *L. monocytogenes* were unable to target the putative receptors on host mammalian cells and were unable to adhere and infect the host tissue.
- *E. faecalis* mutants are resistant to 19 phages evolved to acquire mutations in the *epa* gene cluster, a known virulence factor in enterococci.
- These *epa* mutants exhibited altered cell-surface properties (made them more susceptible to daptomycin and vancomycin, antibiotics that target the cell wall).

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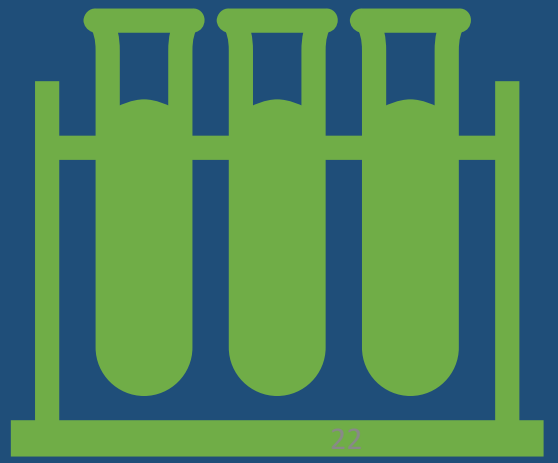

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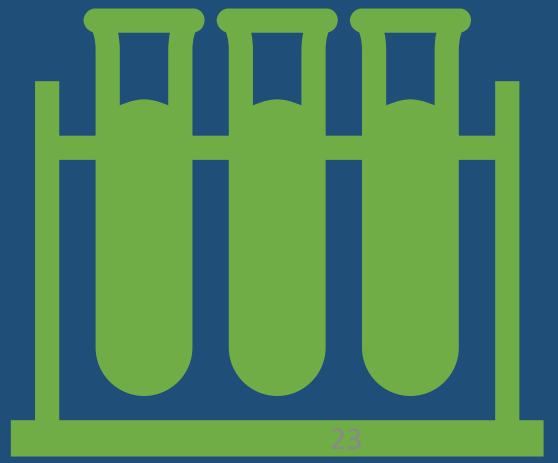
Decreased host invasion

- To spread between cells in the human intestine, *Shigella flexneri* requires outer membrane protein A (OmpA) as well as the O-antigen (a component of LPS).
- *Shigella* phages A1-1 and Sf6 require OmpA for entry into the cell.
- The phage-resistant mutants, unlike the ancestral wild-type bacteria, were unable to spread between eukaryotic host cells in a tissue-culture pathogenicity model.



Impaired ability to evade host immune system

- *K. pneumoniae*, when subjected to selection by phage GH-K3, evolves to downregulate three glucosyltransferase-encoding genes involved in capsule synthesis. The phage-resistant mutants, defective in capsule synthesis, were less virulent.
- Immunofluorescence assays revealed that the phage-resistant mutants had a much higher probability of being endocytosed by macrophages.

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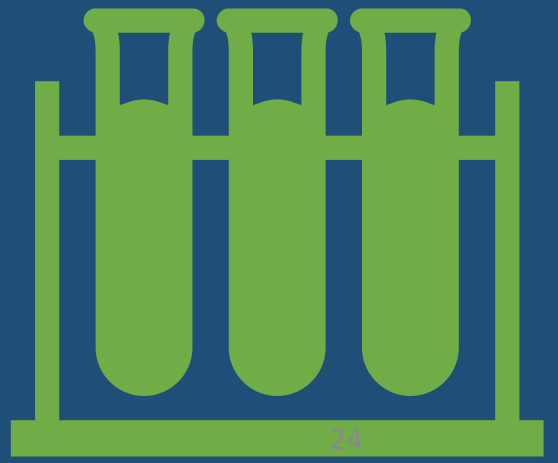


Phage effects in Immunology

Biologics:2022, 173-186

- A phage selected for PT should be resistant to phagosomal degradation.
- Therapeutic phages are naturally immunogenic, so they stimulate interactions between innate and adaptive immune cells that may affect the PT.
- The role of phagocytic cells is to recognize and eliminate foreign antigens and to activate the adaptive immune system response.
- When phages express proteins that mediate bacteria–phage interaction, they bind together and macrophages become activated.
- Leukocytes bind to phages in a time-, concentration-, and temperature- dependent manner, and endocytose them to remove them.

Phage–Phagocyte Interaction

Biologics:2022, 173-186

- PMN and macrophages can degrade phages and endocytose the phages along with them. Phage degradation is the first step in stimulating antigen presentation and the development of an adaptive immune response.
- Phagocytosis is stimulated via bacteria and phage-derived PAMPs and continual phagocytosis of phage-infected bacteria occurs.
- Antibodies produced against the bacteria opsonize the bacteria and facilitate phagocytosis by MQ, which promotes bacterial clearance.

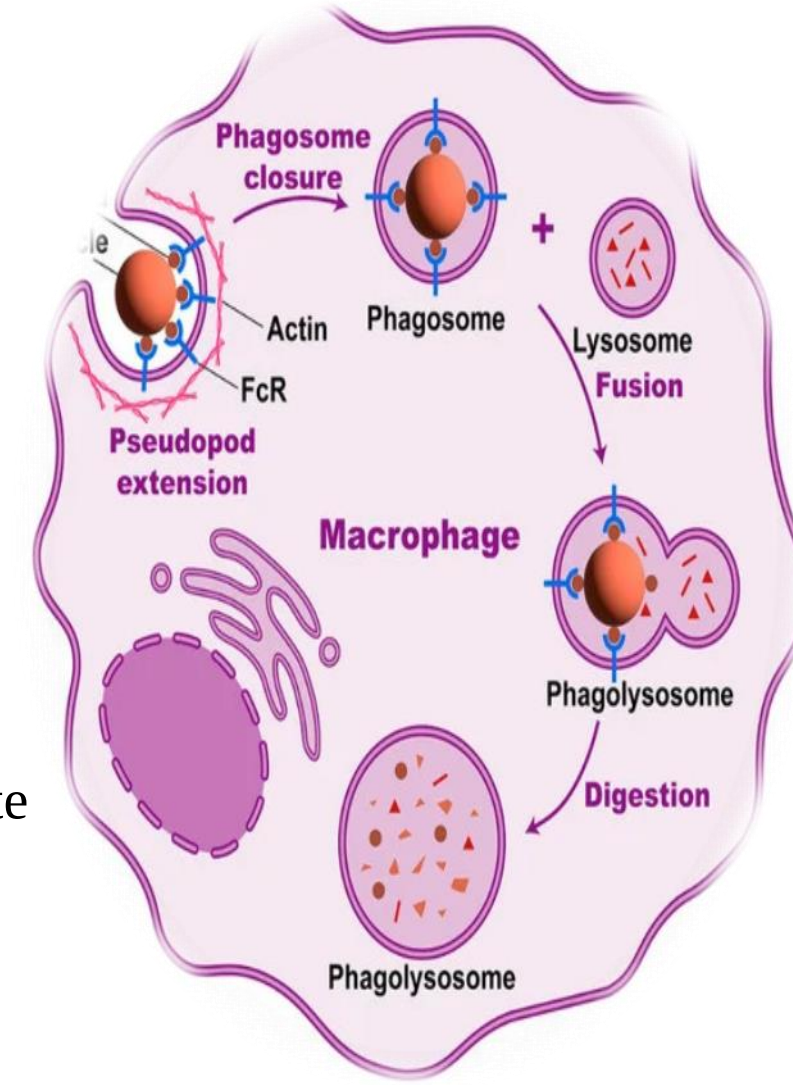


Fig. 6 Mechanisms of phagocytosis

Phage–Phagocyte Interaction

Biologics:2022, 173-186

- Phage–antibody complexes bind to Fc receptors on macrophages, which triggers endocytosis and subsequent phage clearance.
- For PT to be effective there must be a strong interaction between host-derived ligands and host PRR.
- Weak PRR activation in immune-deficient individuals affects the individual innate immune response.

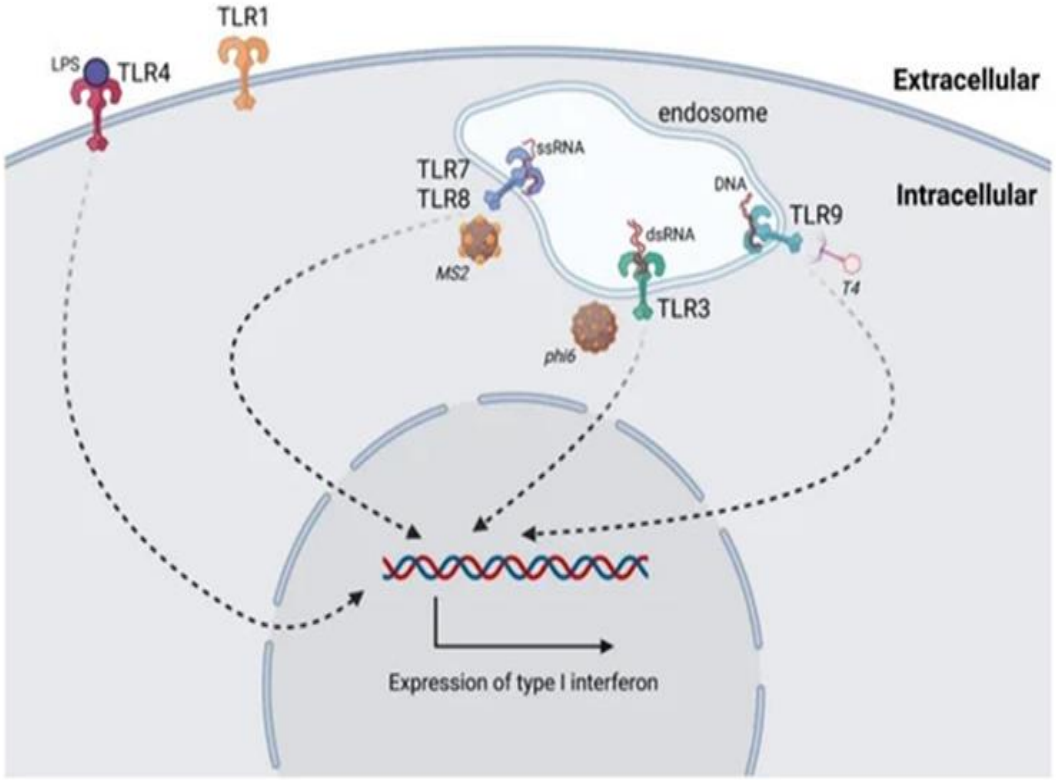
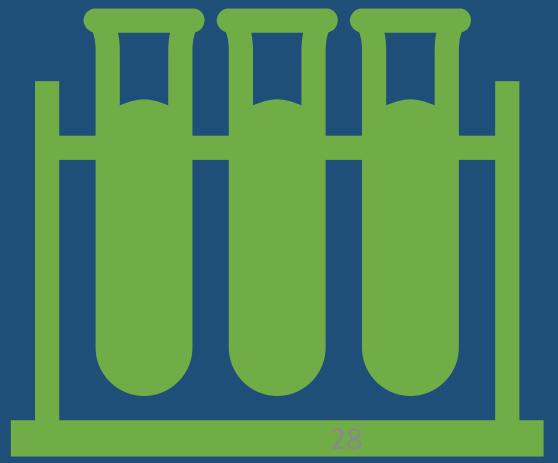


Fig. 7 Phage- PRR interaction

Phage–Adaptive Immune System Interaction

Biologics:2022, 173-186

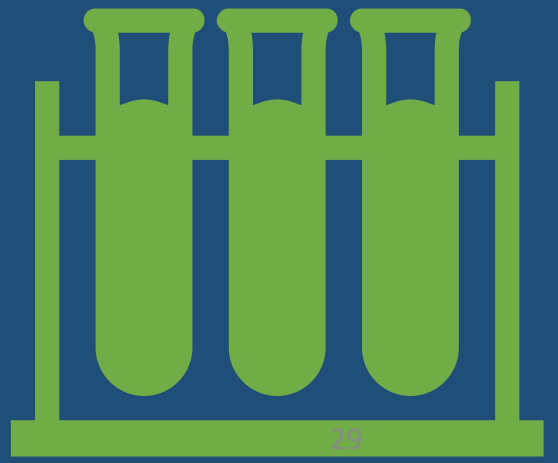
- Phages strongly influence adaptive immunity via their effects on humeral immunity.
- Phages alone are not sufficient to fight bacterial infection. The combined effect of the immune system along with PT is essential to fight bacterial infections.
- Phage-mediated bacterial lysis stimulates the human adaptive immune response, which enhances the efficacy of PT. Adverse phage treatment may cause toxicity owing to the release of endotoxins as a result of bacterial lysis.
- Phage–immune interactions depend on immune recognition through PRR, the immunogenic nature of the phage, and the multiplication rate of the phage.



Phage–Adaptive Immune System Interaction

Biologics:2022, 173-186

- The PRR recruits phagocytes to the site of infection to resolve the infection.
- The commitment of the PRR and the level of immune activation depend on phage type, phage dose, and nucleic acid synthesis activity.
- Anti-T4 phage antibodies are frequent in the human population. The multiplication rate of phages can be reduced by IgG or IgA. One of the drawbacks of PT is that antibodies are neutralize the phages and hinder infection of a bacterium by phages.



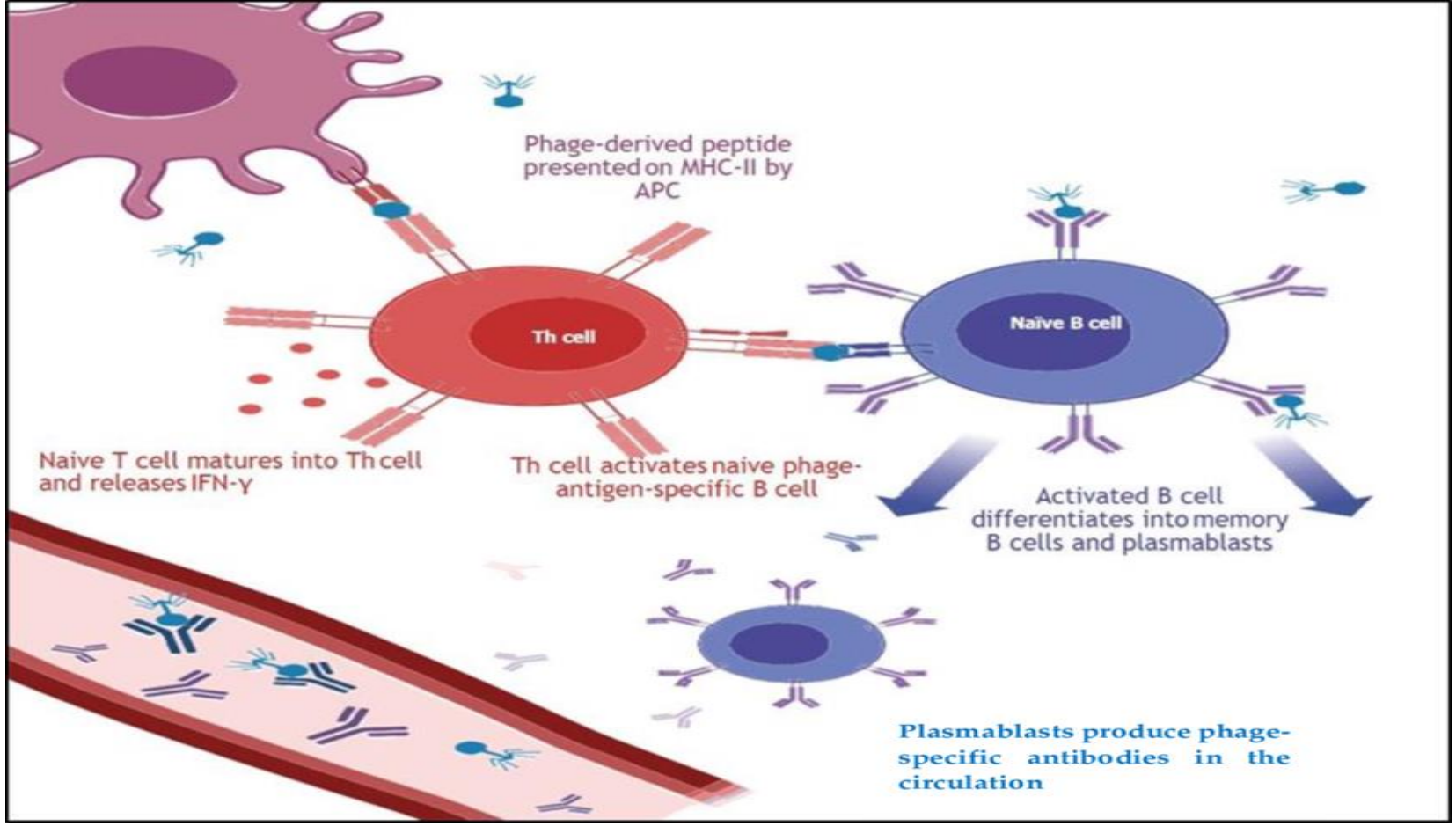


Fig. 8 Bacteriophage interactions with adaptive immune cells. Phage peptides are presented by APCs to naive T cells on MHC-II. Naive CD4 + T cells via the release of IFN-γ proliferate into Th cells. Phage-specific Th cells are then capable of activating naive phage-specific B cells. Activated B cells differentiate into plasmablasts, short-lived cells that produce large quantities of phage-specific antibodies, and memory B cells, which may initiate the production of further antiphage antibodies. Antiphage antibodies bind and inactivate phages in the circulation and within tissues.

Int. J. Mol. Sci. 2023.24. 1536. 10.3390

Phage Preparation, Dosage, and Administration

Annu. Rev. Med. 2022. 73:197–211
Curr Opin Virol. 2022 Feb: 52:9-14
Clin. Infect. Dis. 2023;77 (S5):S407–15

- ✓Most bacteriophages can be amplified to high titer (>10^{10} PFU/ml) either in liquid or on solid media, concentrated, and purified. According to Clin. Infect. Dis. 2023;77 (S5):S407–15, it ranges between 10^6 to 10^{10} PFU/mL
- ✓It is important that the preparations are sterile and have endotoxin levels below FDA-approved levels.
- ✓Phage stability is an important factor, and it varies greatly for different phages and different concentrations.
- ✓Many phage preparations retain viability when stored cold (4–10°C) and concentrated.
- Diluted phage samples (e.g., 10^3 PFU/ml) may lose viability rapidly.

Phage Preparation, Dosage, and Administration

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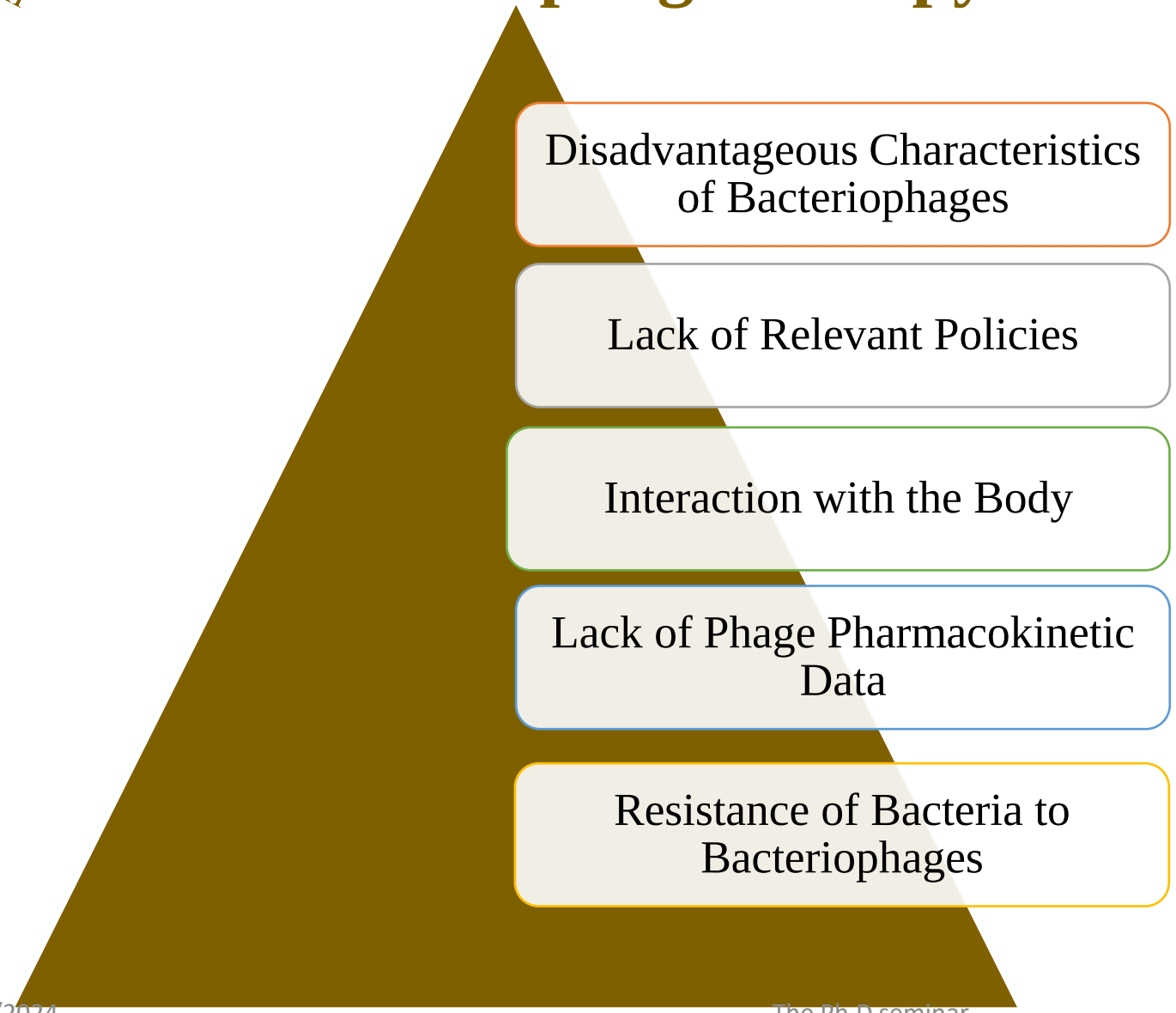
- ✓Phage levels depending on when the phage infection occurs and where the bacterial infection is located.
- ✓Intravenous administration has the advantage of delivering a defined dosage, and twice-daily administration at 10^{1o} PFU/dose has been used in several compassionate use cases.
- ✓Similar dosages for pulmonary infections, especially for cystic fibrosis patients.
- ✓Various dosages have been used for skin infections and direct injection into infected joints.
- ✓Because the safety profile of phages is generally very good, complications from relatively high dosages are less concerning than they are for powerful small-molecule antibiotics.

Limitations of phage therapy



Molecules 2022, 27, 1857

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Phage limitation

Disadvantageous Characteristics of Bacteriophages

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1

- The phages spectrum is narrow due to its high specificity. (Phage mixture)

2

- Useful in diseases caused by a single bacterium, but the clinical cases are often caused by a variety of pathogenic bacteria

3

- Some lysogenic phages inhibit the lytic effect of other phages on their host bacteria after integration with the host bacteria.

The lysogenic state can also transmit toxins and antibiotic resistance genes to bacteria

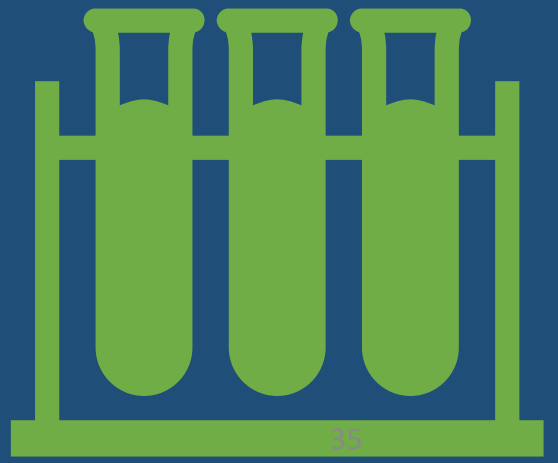
Lack of Relevant Policies

Molecules 2022, 27, 1857

- Lacking policies and regulations on the clinical application of PT.
- No clear standard for phage isolation and purification, which makes the efficacy of isolated phage preparations variable.
- No standardized procedure in clinical treatments with bacteriophages.

Solution

- Establishment of Relevant Policies and Standards should be issued in time.
- Standardized phage purification and operating procedure has been repeatedly considered.
- A national standard scheme should be established for personalized PT.



Interaction with the Body

- Bacteriophages release bacterial toxins, such as endotoxins, when lysing bacteria, which worsens bacterial infections. This even resulted in septic infections.
- Foreign proteins carried by bacteriophages may induce immune reactions. This reaction is an exception, as phages have been shown to be safe; the reactions were allergic.

Solution

- Clinical Experience is necessary to observe the treatment objects in PT, record the dosage of phage and the symptoms due to adverse reactions, accumulate experience through a large number of clinical treatments, and thoroughly investigate the immune response phages may cause.

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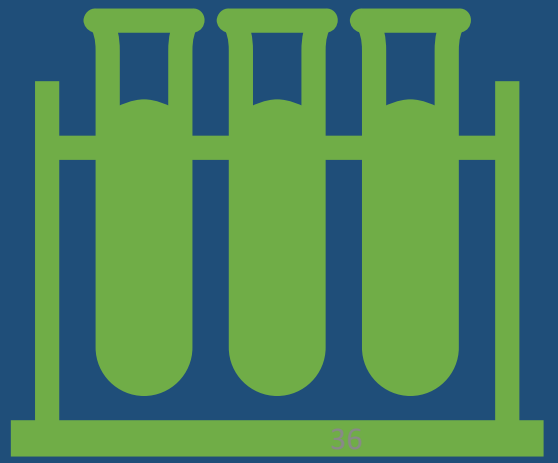

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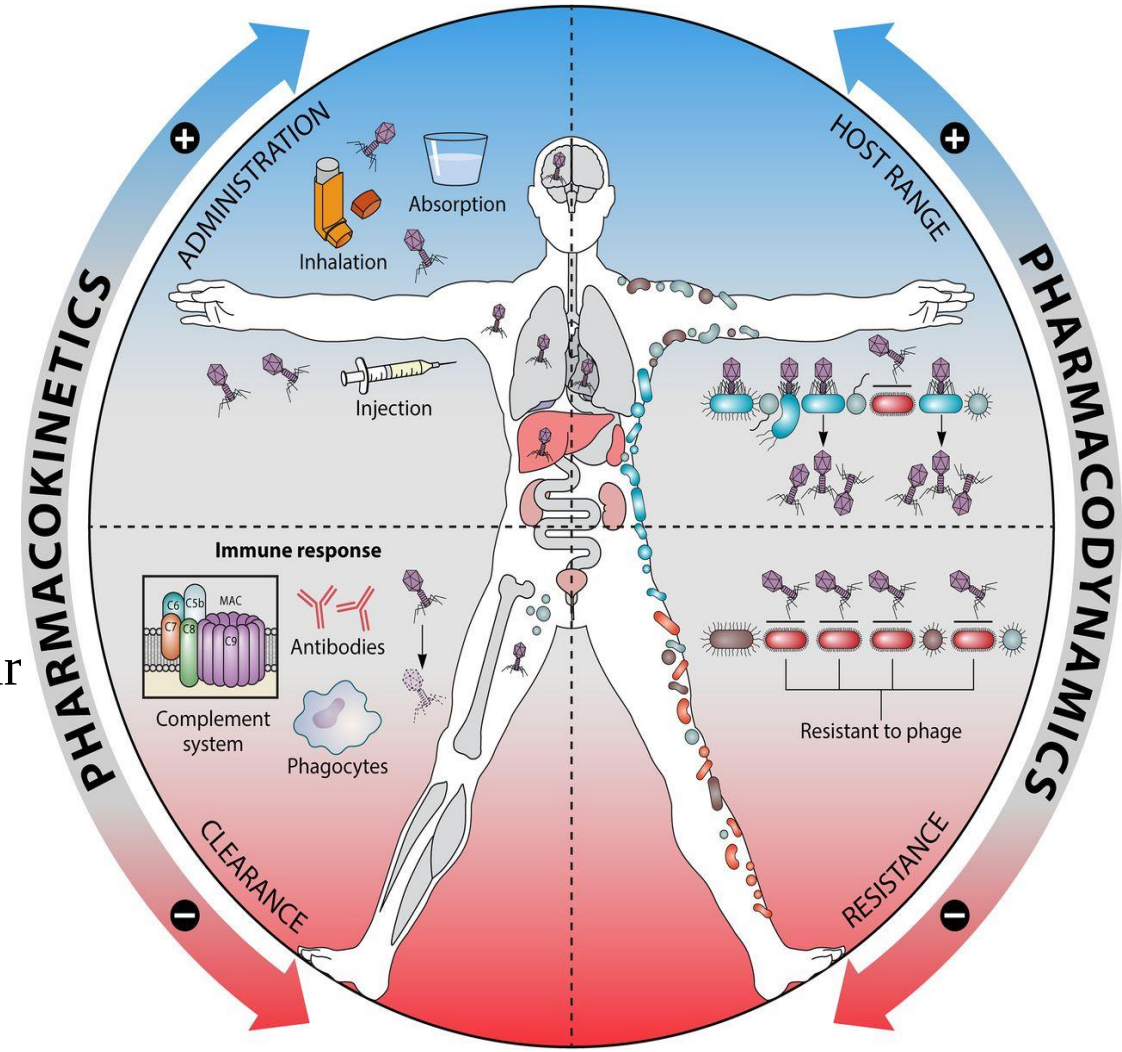
Lack of Phage Pharmacokinetic Data

- PT preparations are difficult to standardize. The definition of dosage and the mode of administration remains unclear.
- Phages are composed of proteins and DNA or RNA, so they can easily be degraded when they interact with human metabolism, such as in the stomach and liver.
- Related pharmacokinetic studies showed that a quarter of bacteriophage infusions lasted for 36 h after treatment, but their effective concentration was diluted by body fluids.



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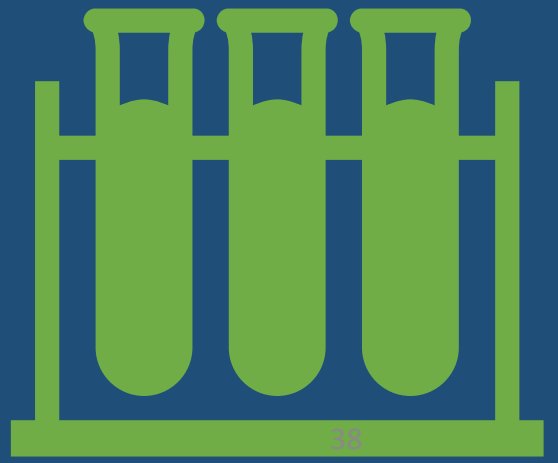
- Oral administration was the most suitable mode for humans.
- Induced low immunogenicity compared to other drug administration methods.
- **Solution**

✓Optimization of the Administration needs to consider whether the phage can survive in the body and be transported to all parts of the body.

✓Many phages are sensitive to the low pH of the stomach. microencapsulation of phages in a natural biopolymer matrix is used as a protective barrier against the gastric environment to reduce the inactivation of phages after ingestion; this ensures the efficacy of phages.



Molecules 2022, 27, 1857

Resistance of Bacteria to Bacteriophages

- If a phage is used repeatedly for a long time, bacteria evolve phage-resistant strains in the process of natural selection.

1. Adsorption inhibition → reduced interactions between phages and bacteria
2. Restriction modification systems (RM)
3. Injection blocking
4. Abortion infection → both phages and bacteria die
5. Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR–Cas) system: provides adaptive immunity to bacteria against foreign invaders such as plasmids and phages.

Solution

✓ The combined use of phages with antibiotics

Mechanisms of phage resistance in bacteria

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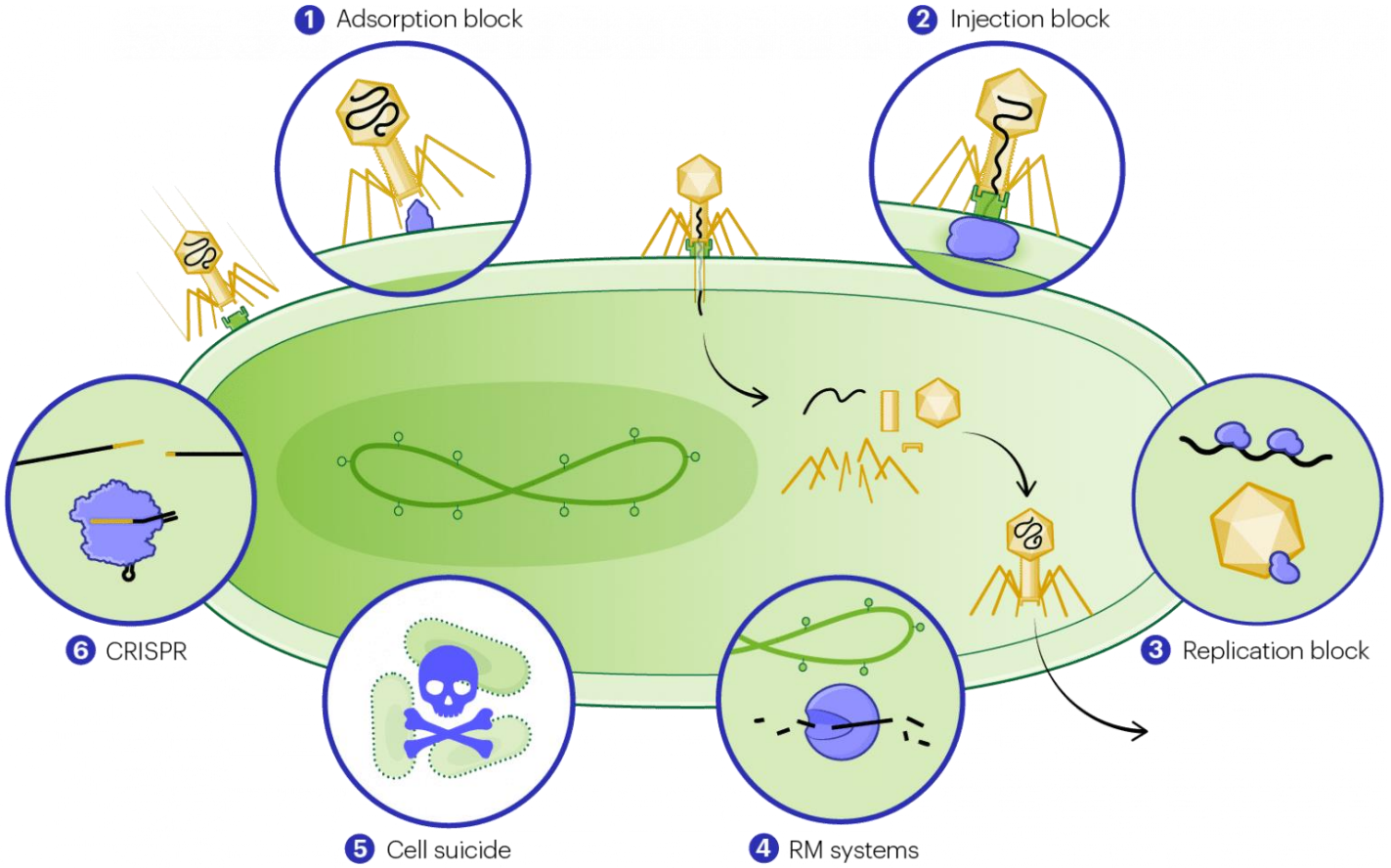
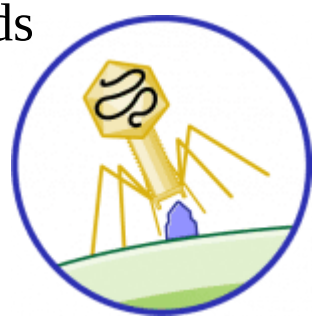


Fig.11 Bacteria have many ways to fend off phage infection, such as blocking adsorption, injection, or assembly; or through cell suicide or RM systems. These are all innate mechanisms that defend bacteria against phages in general rather than targeting a particular type of phage. CRISPR is an adaptive immune system. It defends bacteria against specific phages and adapts to recognize new threats.

1. Bacteria can prevent phage entry to the cell by

A. Altering surface phage receptors such as LPS, OMPs, Teichoic acids

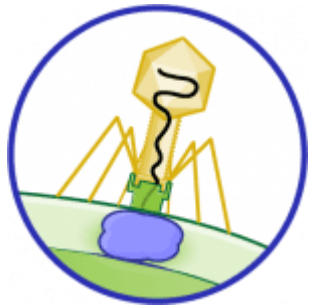


B. Altering appendages such as flagella and pili

C. Can be shielded from phage binding by capsules that cover the cell surface

D. Can avoid lytic phage infection by biofilm

2. Some bacteria block phage DNA from entering the *cytoplasm*



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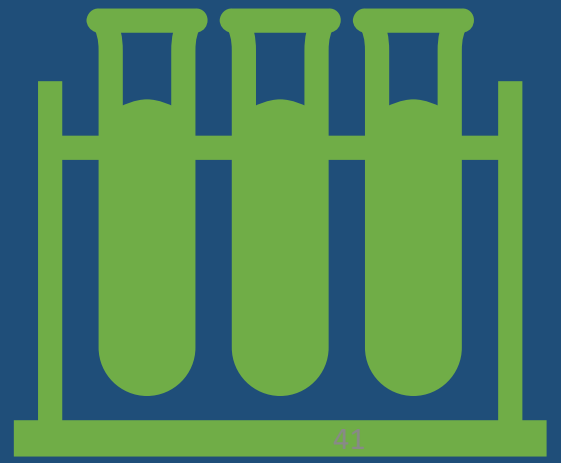

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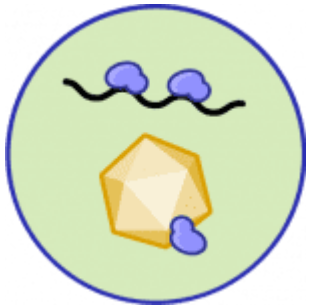
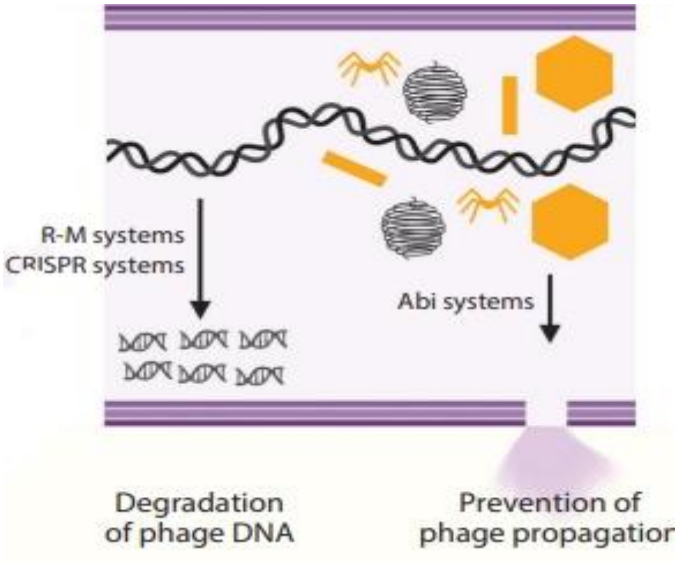
3. If a phage adsorb to the cell surface and successfully injects its genetic material into the cell, bacteria can deploy defense mechanisms that prevent phage replication by targeting and degrading the phage nucleic acid.



- These defenses are analogous to both innate and adaptive immune systems in eukaryotes.
- Innate immunity comprises restriction-modification systems.
- These defenses rely on recognition of DNA modifications to discriminate between bacterial DNA and foreign DNA.



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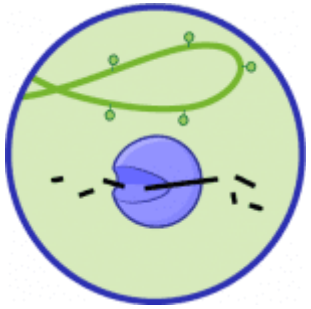
4. RESTRICTION MODIFICATION SYSTEMS

✓ Many bacteria deploy RM systems to destroy phage DNA that is injected into the cell. These defense systems are composed of scissor-like proteins called restriction enzymes. These enzymes cut phage DNA apart, thereby destroying the instructions for making more phages.



✓ To prevent their own DNA from being damaged by restriction enzymes, bacteria add protective chemicals called methyl groups to their genomes. Restriction enzymes have evolved to ignore methylated DNA and don't cut it up. Thus, methylation keeps the bacterial genome safe.

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5. Adaptive immunity in bacteria can occur via CRISPR-Cas systems which defends against specific types of phages. This system can adapt, rapidly generating immunity against new phage challengers.

- These systems are found in about half of all bacterial species. CRISPR systems work by capturing small pieces of invading phage DNA, stashing them in the host cell's genome, and using these molecular memories to find and destroy matching phages.

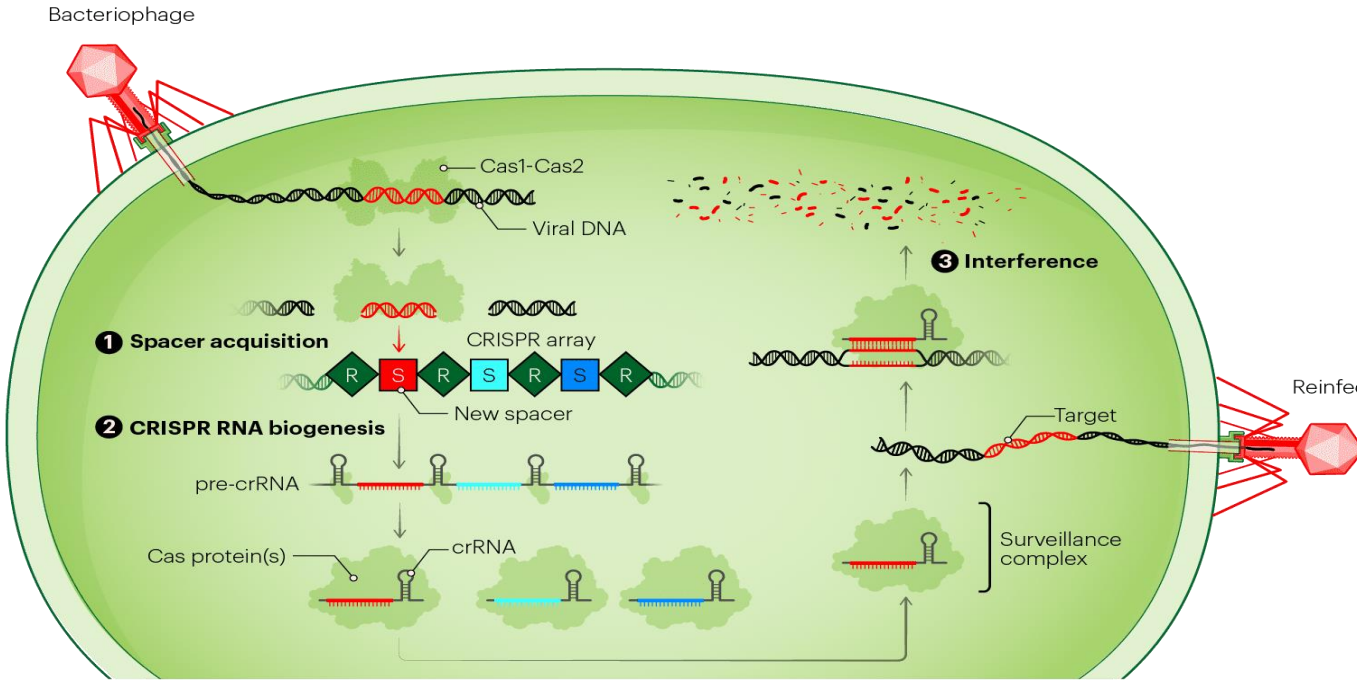

Fig. 12 CRISPR is an adaptive immune system found in bacteria and archaea. Cas proteins store pieces of phage DNA as a memory of infection. Other Cas complexes use these memories as guides to find and destroy matching phage genomes to stop subsequent infection.

Innovative Genomics Institute | CRISP

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- Two Cas proteins, Cas1 and Cas2, work together to capture pieces of injected phage DNA. The Cas1–Cas2 complex works with accessory proteins to measure and cut out a precisely-sized piece of DNA and holds onto the phage DNA and inserts it into one end of the CRISPR array as a new spacer. The system incorporates a new repeat in the process, so all spacers are always flanked by repeats on each side. The bacterium thereby stores a memory of the phage.

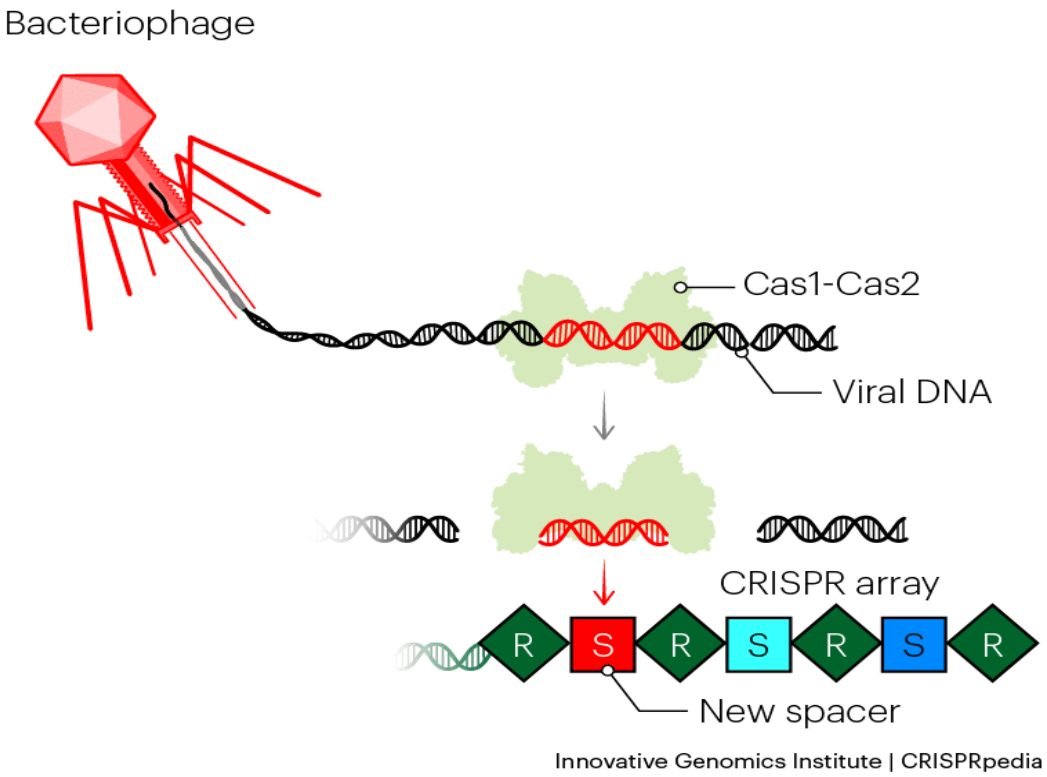

Fig. 13 The first step in establishing CRISPR immunity is to capture a short snippet of an invading phage's DNA.

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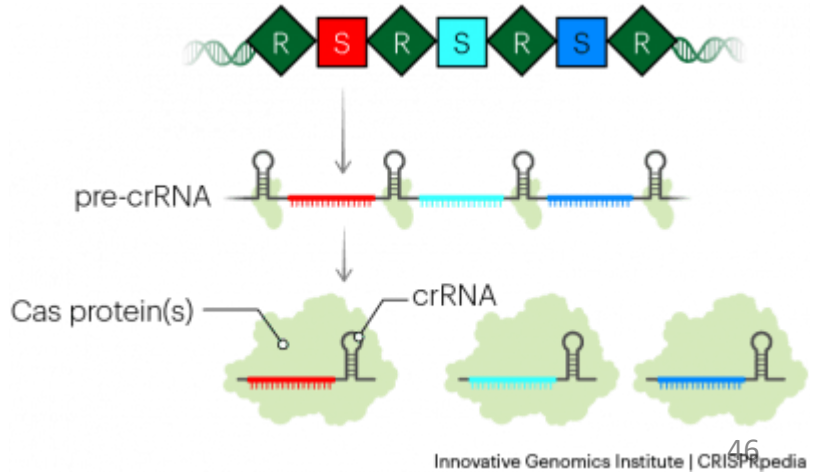
- CRISPR systems store these memories of phage infection in their DNA, but don't use the DNA to recognize subsequent phage infections directly. Instead, they convert the DNA memories into a similar molecule called RNA. RNA is chemically different from DNA and often exists as a single strand. Bacteria make many RNA copies of the phage memories and use them to find newly invading phages. It is useful to make these RNA "working copies" because they can be broken down and recycled without destroying the original, permanent memory.
- Cas proteins then cut or 'cleave' the long RNA into short, individual segments. These contain one spacer and parts of the repeats. The final, fully trimmed RNA copies of the phage memories are called *CRISPR RNAs (crRNAs)*. Next, Cas proteins will use each crRNA to hunt for and positively identify matching phage DNA.

Fig. 14 The CRISPR array is transcribed into one long RNA molecule and then sliced into shorter pieces that each contain a single spacer.



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- The final step in CRISPR immunity leads to the destruction of invading phage DNA. This process is called *interference*, because it involves the CRISPR system "interfering with" or stopping the phage's life cycle. Together, the Cas protein(s) plus crRNA form the *surveillance complex*.
- When a phage injects its DNA into a bacterial cell, the surveillance complex scouts the phage's genome for a sequence that matches the spacer in its crRNA. This target sequence is called the *protospacer*. Because the crRNA is a copy of the original phage DNA

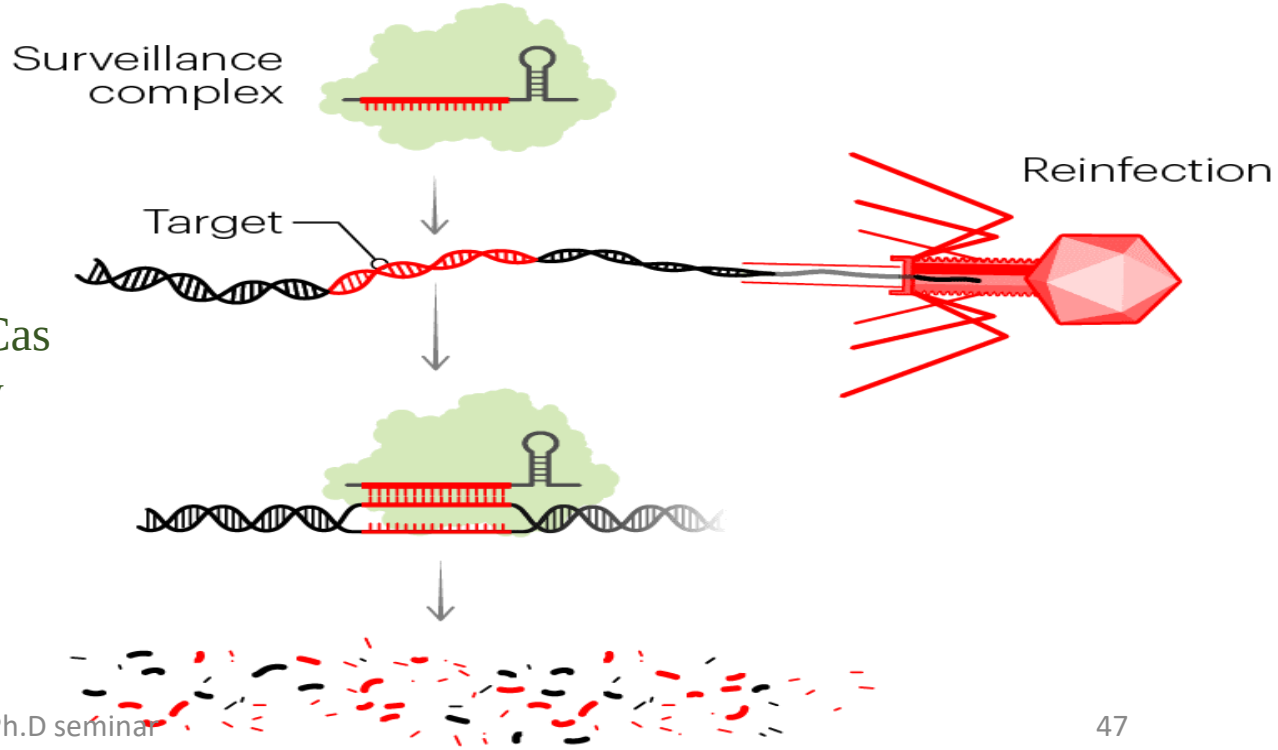

Fig. 15 Surveillance complexes composed of one or more Cas proteins carry crRNA guides and search for complementary targets to destroy. When a phage's genome is cut, it cannot replicate and infection is stopped.

6. If a phage manages to bypass all these safeguards, the bacterium's last line of defense is cell suicide. This "altruistic" act kills the individual bacterium, but prevents the production of more phage copies that could go on to infect neighboring cells.

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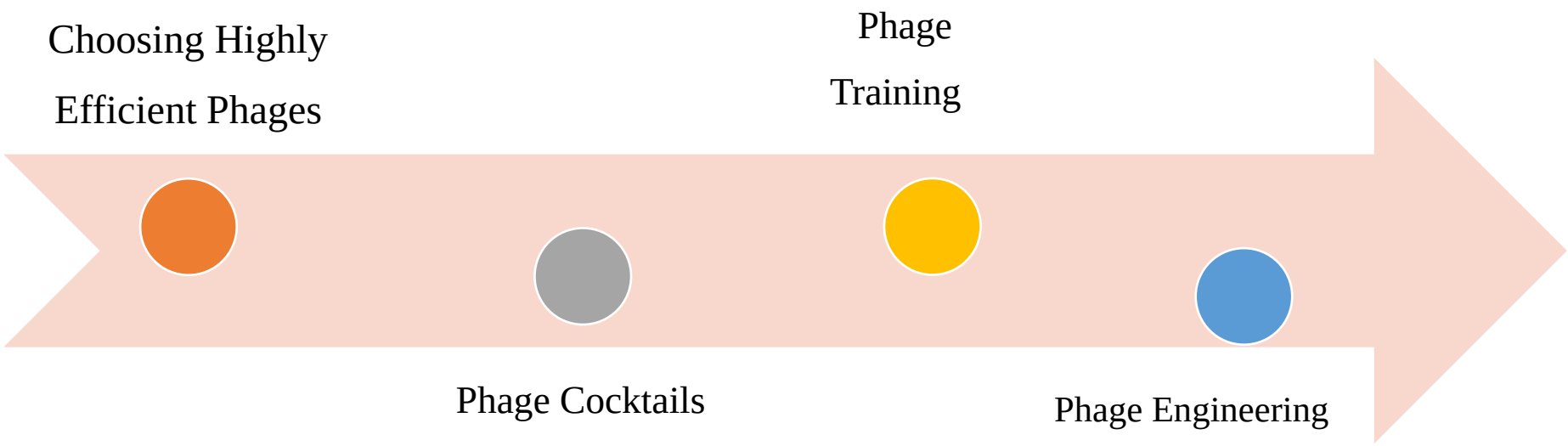
Minimizing bacterial resistance



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Choosing Highly Efficient Phages

✓Using efficiently phages can reduce bacterial numbers.

✓Some traits of the lytic phage replication cycle that may be desirable for maximizing therapeutic phage killing include:

➢Maximizes rapid and irreversible adsorption to susceptible cells.

✓Latent period should be minimized to achieve more rounds of infections per unit time.

✓Large burst sizes result in generation of up to thousands of new offspring phage particles per cellular infection.

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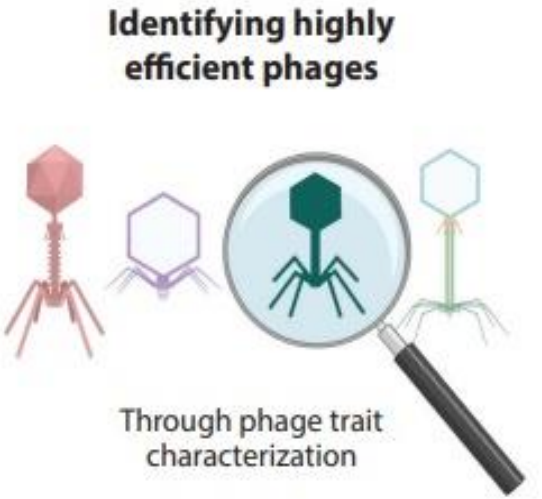
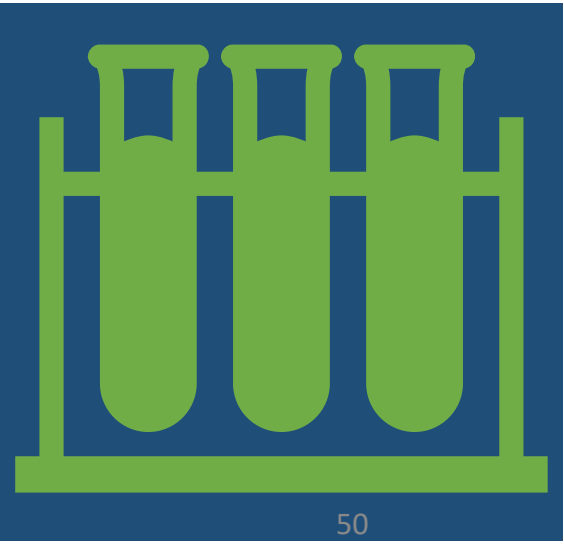
Int. J. Mol. Sci. 2022, 23, 4577





Phage Cocktails

- ✓Narrow host ranges, limit their usefulness in single therapy due to frequent mismatches between a phage and bacterium.
- ✓To limited host range, multiple phages with complementary (non-overlapping) host ranges can be combined into a cocktail preparation.
- ✓Phage cocktails can be designed to target different genotypes of the same bacterial species or to attack multiple species, therefore, cocktails may be more effective at maximizing killing in a bacterial infection, especially when treating uncharacterized polymicrobial infections.
- ✓Phage cocktails have the most popular and widely used strategies for PT.

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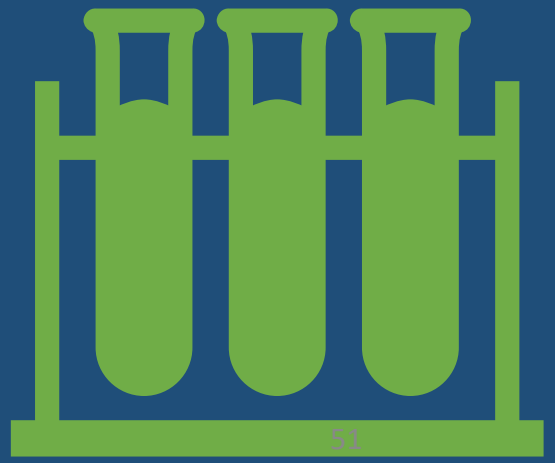
Int. J. Mol. Sci. 2022, 23, 4577



Phage Training

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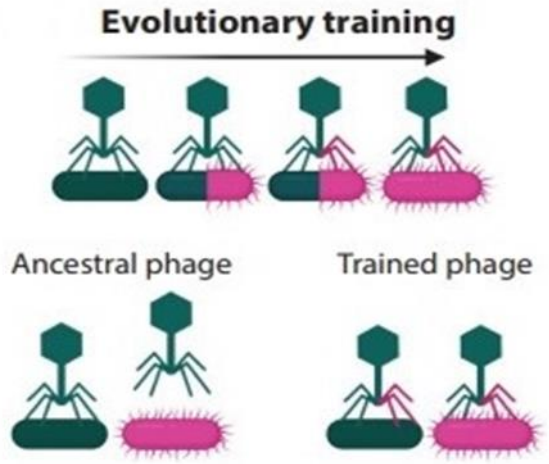
- ✓Known as phage adaptation, exploits the natural potency for phage populations to quickly evolve to overcome bacterial defense mechanisms.
- ✓The Phage is trained to anticipate how the target bacteria will evolve phage resistance.
- ✓This approach allows the phages to accumulate random point mutations or gene insertions/deletions to counter bacterial resistance. So, phages can be trained to acquire expanded host ranges and to evolve traits that minimize the phage-resistant bacteria during therapy.
- ✓One popular approach in phage training is to propagate viruses in vitro for serial rounds of infection on a non evolving host strain.



Phage Training

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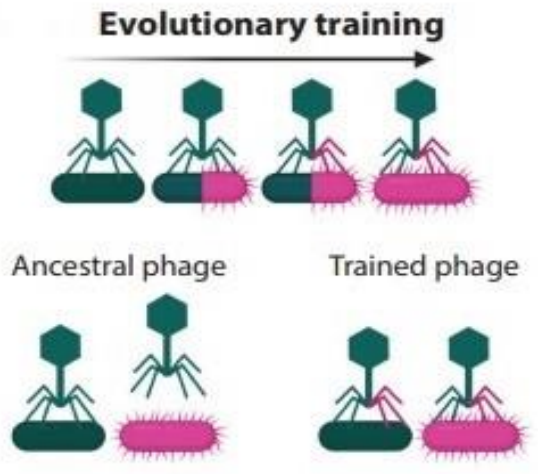
✓ Phage training is based on allowing the host to coevolve with the phages during serial passage in the lab.

✓ After five consecutive passages in liquid culture with *Pseudomonas aeruginosa* host strain, a trained phage population evolved to kill the host bacteria with a tenfold greater efficiency than the ancestral naturally phage.

✓ Or coevolved a lytic phage with its *E. coli* host for 28 daily serial transfers resulted in:



1. The host evolved to reduce the expression of the phage's receptor LamB.
2. Phages coevolved to gain the ability to infect through a secondary phage receptor, OmpF.

This trained phage was found to suppress bacterial growth and minimize evolved phage resistance more efficiently than the ancestral phage strain.

Phage Engineering

- Phage engineering has already been used to enhance properties of naturally phages, like expanding their host ranges, reducing phage resistance in bacteria, increasing phage safety, and improving stability of phages and phage products.
- In 2019, Dedrick et al. reported the first successful case of human PT using engineered phages. Following a lung transplant, a 15-year-old cystic fibrosis patient acquired a disseminated *Mycobacterium abscessus* infection that was resistant to multiple antibiotics. The researchers screened hundreds of naturally isolated mycobacterial phages and identified a single lytic phage that efficiently killed the clinical strain.

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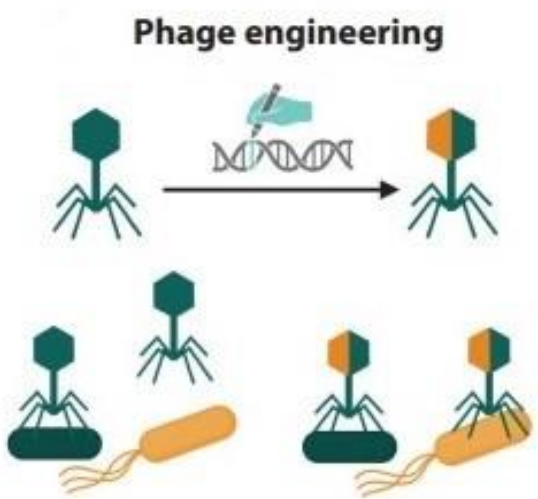

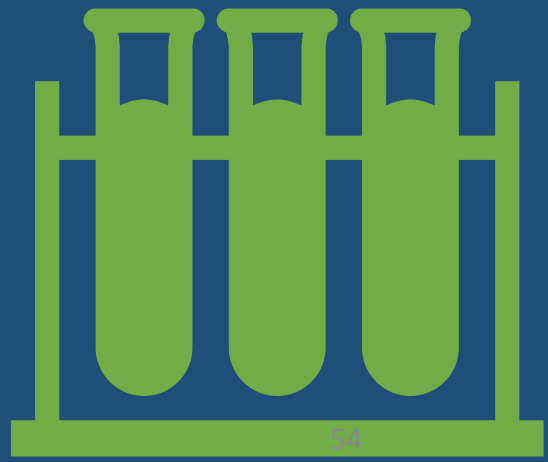

Phage Engineering

- They genetically engineered the temperate phages by precisely removing their immunity repressor gene. The resulting lytic derivatives of the temperate phages showed enhanced killing activity against the target host strain. A cocktail composed of these two (one natural and one engineered) lytic phages was administered intravenously to the patient, which resulted in clinical improvement and alleviation of the infection.

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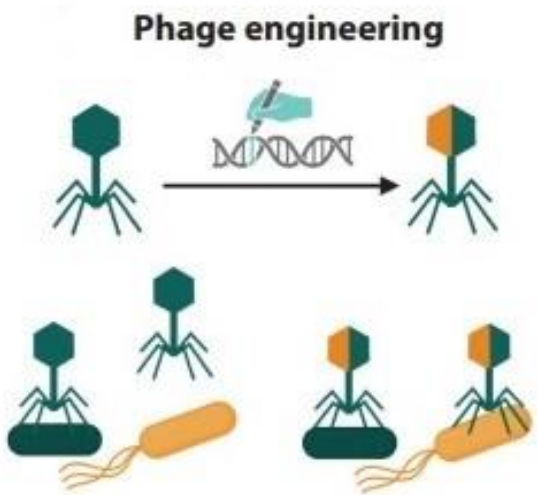

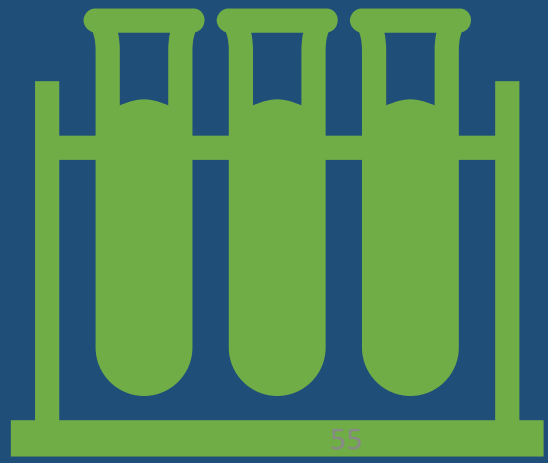

Conclusion

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1. PT is a alternative to antibiotics for the treatment of bacterial infections.

2. Specificity of any particular phage may be restricted to only a small subset of clinical pathogen.

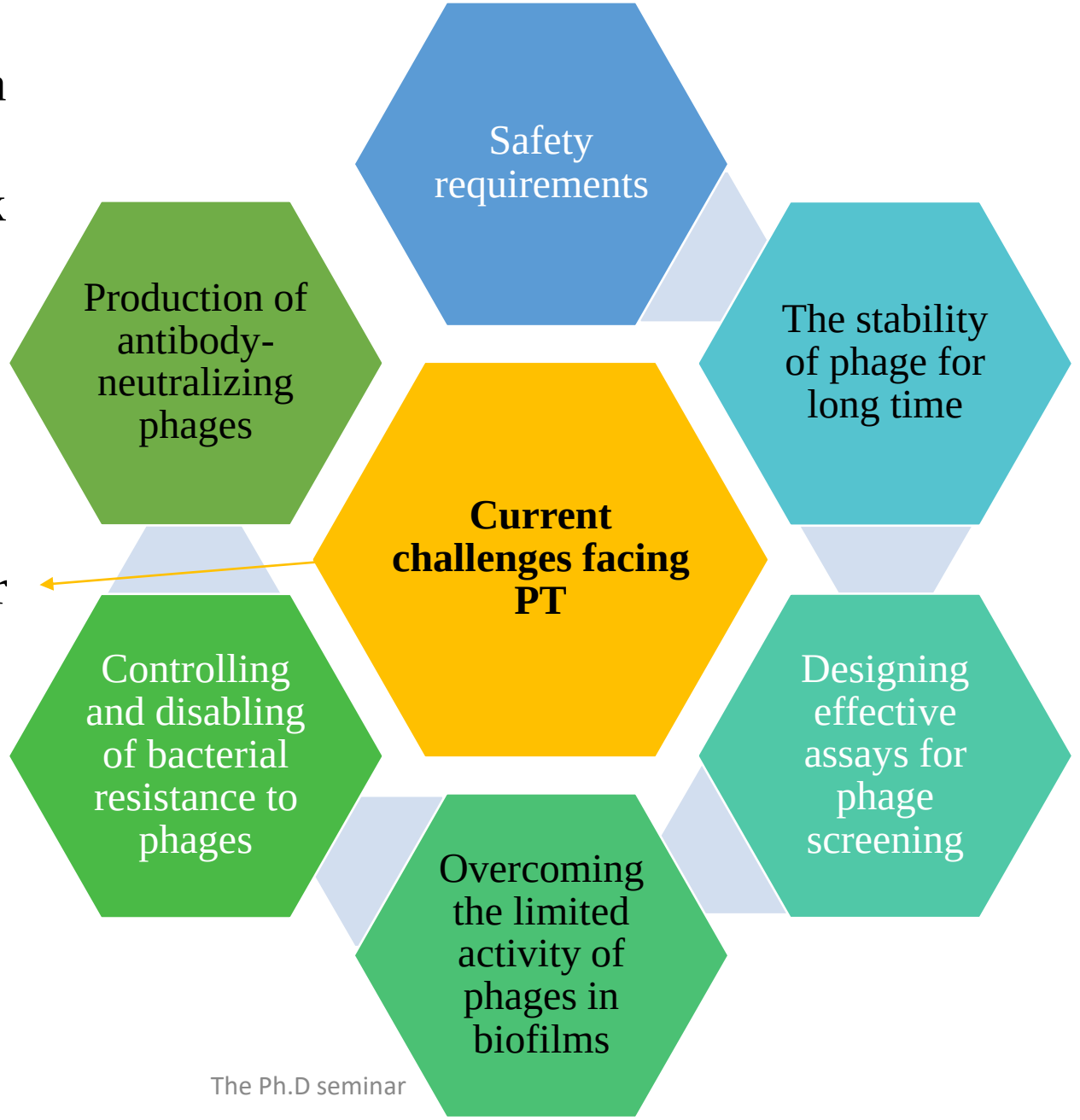
3. Phages generally have good safety features, and escalating dosage is not required.

4. Phage preparations for therapy must be sterile and endotoxin free.

5. Cocktails of more than one phage can help to minimize phage resistance.

6. PT has some limitations, such as a narrow host range, lysogenicity, lack of relevant policies, lack of pharmacokinetic data, and so on.

Sequestration by the spleen and liver



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Thanks for your attention