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Evaluation of **phage therapy** as an alternative option for the treatment of bacterial wound infection

Presentation of a new European study

Thomas ROSE
Jean-Paul PIRNAY
Gilbert VERBEKEN
Daniel DE VOS

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Conflict of interest slide

- Involved in a EU funded study evaluating a product developed by a commercial company.
- I have no financial interests.

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This talk

Goal: To establish safety and efficacy of **phage therapy** for the treatment of *Pseudomonas aeruginosa* and *E. coli* burn wound infection

Phage therapy: The use of bacteriophages to combat uncontrolled bacteria.

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(Bacterio)phage

Basic structure

Head
(a protein coat encapsulating a DNA or RNA genome)

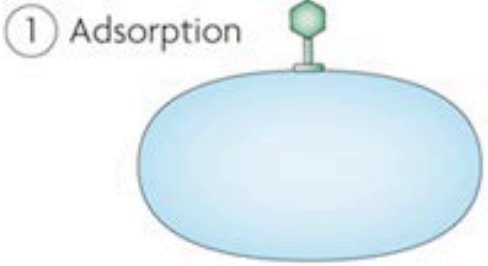
Tail
(a genome injection system)

Attach to bacterial receptors
- **Tail fibers**
- **Tail spikes**

Viruses that infect bacteria

Bacterial cell

TM4 mycobacteriophage. Credit: Lawrence Broxmeyer.



'self-replicating' antimicrobial

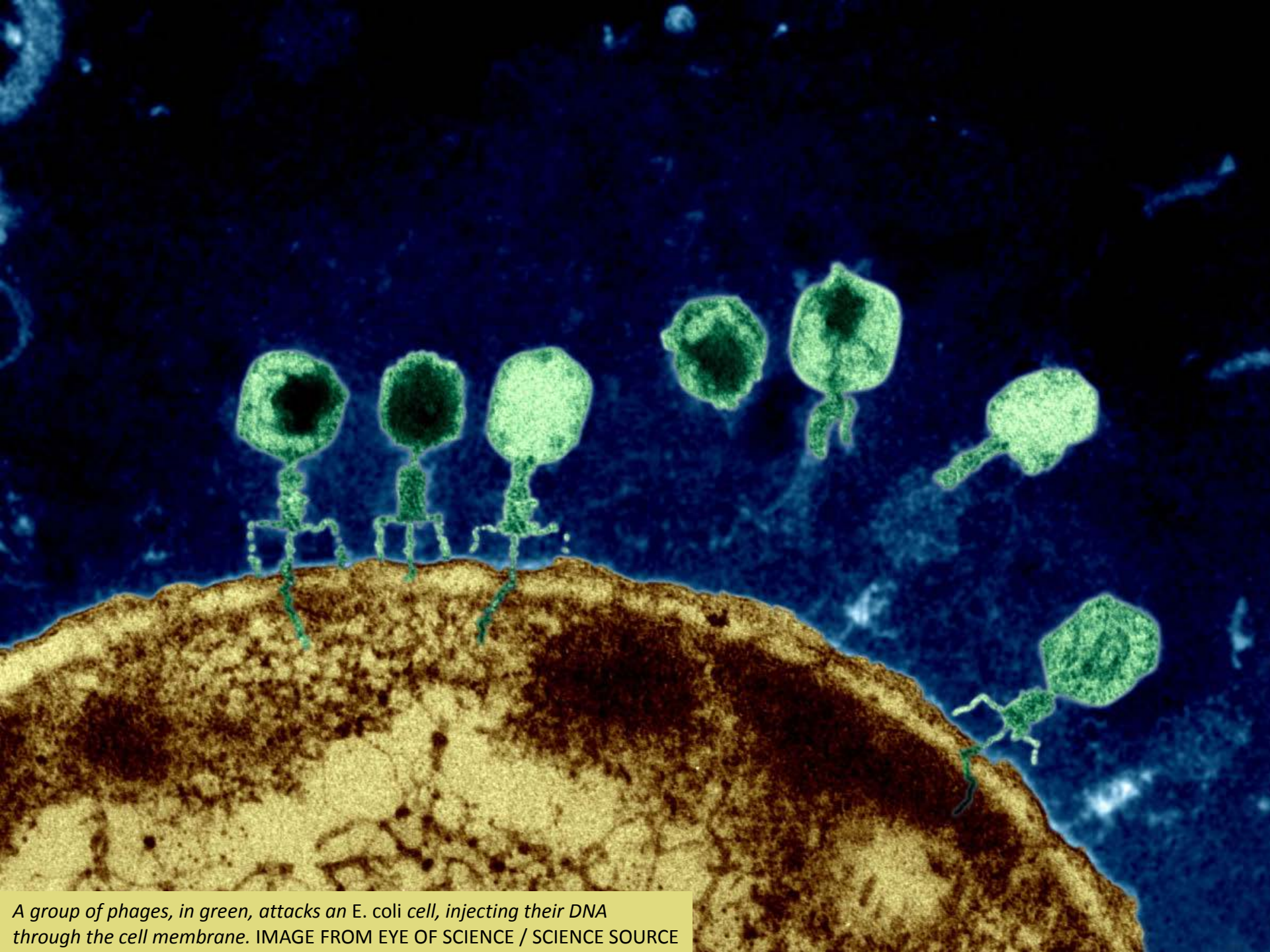
Lytic cycle

The phage hijacks the machinery of the bacterial cell, forcing it to replicate the phage's genetic material and protein coat.

Many copies of the phage are produced and the bacterium bursts.

The phage offspring is set free to infect other bacteria.

Nature Reviews | Microbiology



A group of phages, in green, attacks an E. coli *cell, injecting their DNA through the cell membrane.* IMAGE FROM EYE OF SCIENCE / SCIENCE SOURCE

Phages are everywhere…

The most abundant biological lifelike entities of our biosphere.

*They are present **wherever bacteria are**, outnumbering them 10 to 1.*

Estimated 10^{31} phages on our planet

- Soil
- Plants
- Rivers and lakes
- Ocean water & sediment
- Ocean ice

Human body & live organisms

- Oral cavity
- Intestines
- Vagina
- Skin
- Urine

Everyday life

- Food (cheese, yoghurt, salami,..)
- Drinking water
- Live polio vaccines

We live in a sea of phages

Up to 1 billion of phages/ml of water

Yet, no infection of human cells by phages has been reported.

Photo: Kirk Weddle

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Because

Bacterial cell (prokaryote)

Animal cell (eukaryote)

It is virtually impossible for phages to enter directly into eukaryotic cells since it requires **prokaryotic cell wall receptors** for its attachment.

It is virtually impossible for phages to multiply in eukaryotic cells since it requires a **prokaryotic biochemical machinery** for replication.

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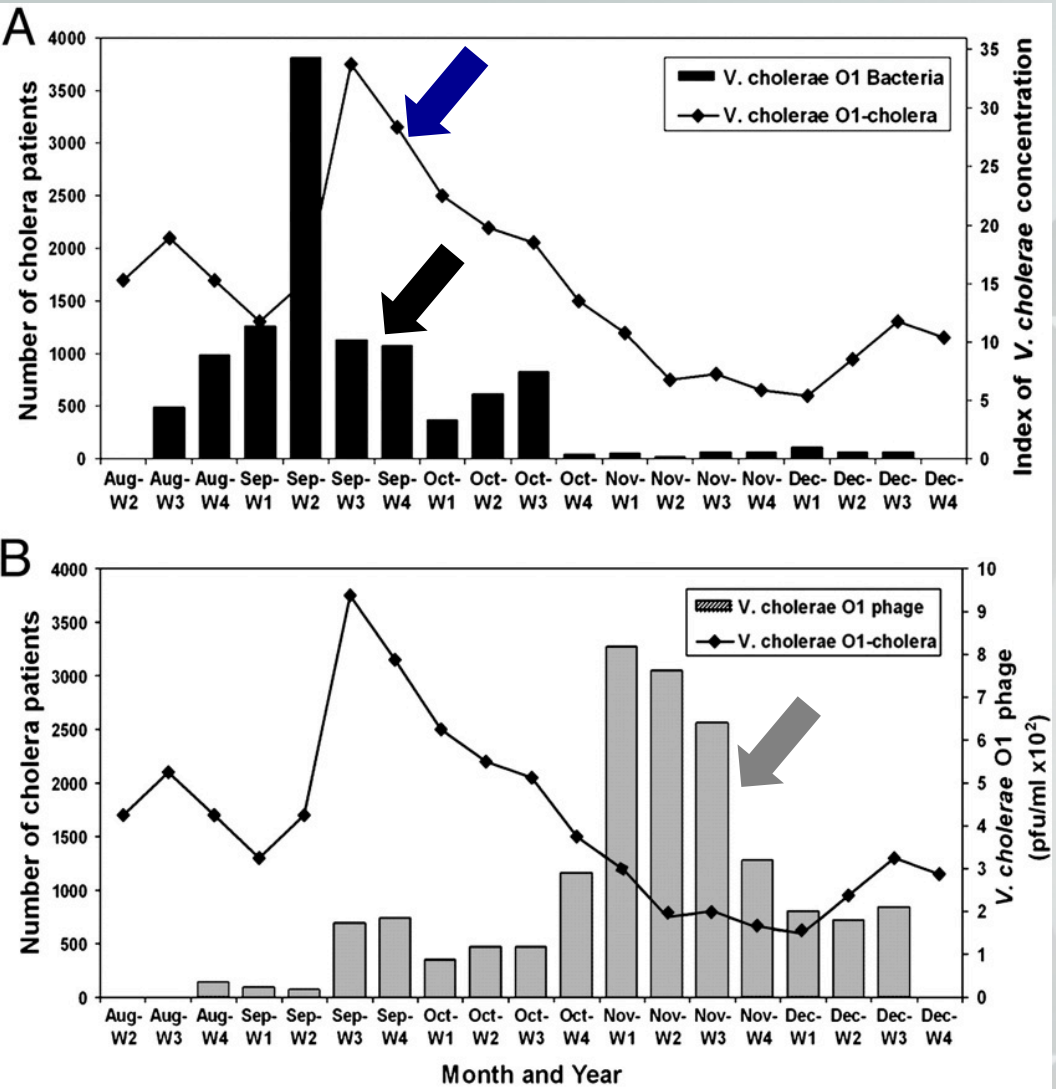
Natural controllers



- Phage will rapidly reduce the population of the most abundant bacteria. They equilibrate/control bacterial populations.
- Example: The self-limiting nature of seasonal cholera epidemics in Dhaka, Bangladesh.

Curve: Number of cholera patients over a 5-month period.

Black bars: *Vibrio cholerae* concentration in river water.

Grey bars : *Vibrio cholerae* phage concentration in river water.

Faruque *et al.* PNAS 2005

Phage therapy timeline

Phage discovery

1896: Hankin (UK): river water can kill cholera pathogen

1915: Twort (UK): a mysterious agent that kills bacteria

1916: d'Herelle (FRA): a microbe destroys shigella pathogen

1917: d'Herelle calls the microbe "bacteriophage"

1923: the Eliava **phage institute** is established in Tbilisi (GEO)

1928: Fleming (UK) discovers **penicillin**

WWII:

Red Army (USSR)

German Army (North Africa campaign)

Japanese Army

Phage therapy

1919: d'Herelle treats dysentery in a boy using phages.

1921: Bruynoghe and Maisin (BEL) publish on the first use of phages in a therapy context

1930s: phage products are **marketed** by:

- Laboratoire du Bactériophage (FRA)
- Robert et Carrière (FRA)
- L'Oréal (FRA)
- Eli Lilly (USA)
- Squibb & Sons (today Bristol-Myers Squibb) (USA)
- Swan-Myers division of Abbott (USA)

1940s: Antibiotics overshadow phage therapy

Since then: decline of phage therapy in the West, while it is further developed in the USSR

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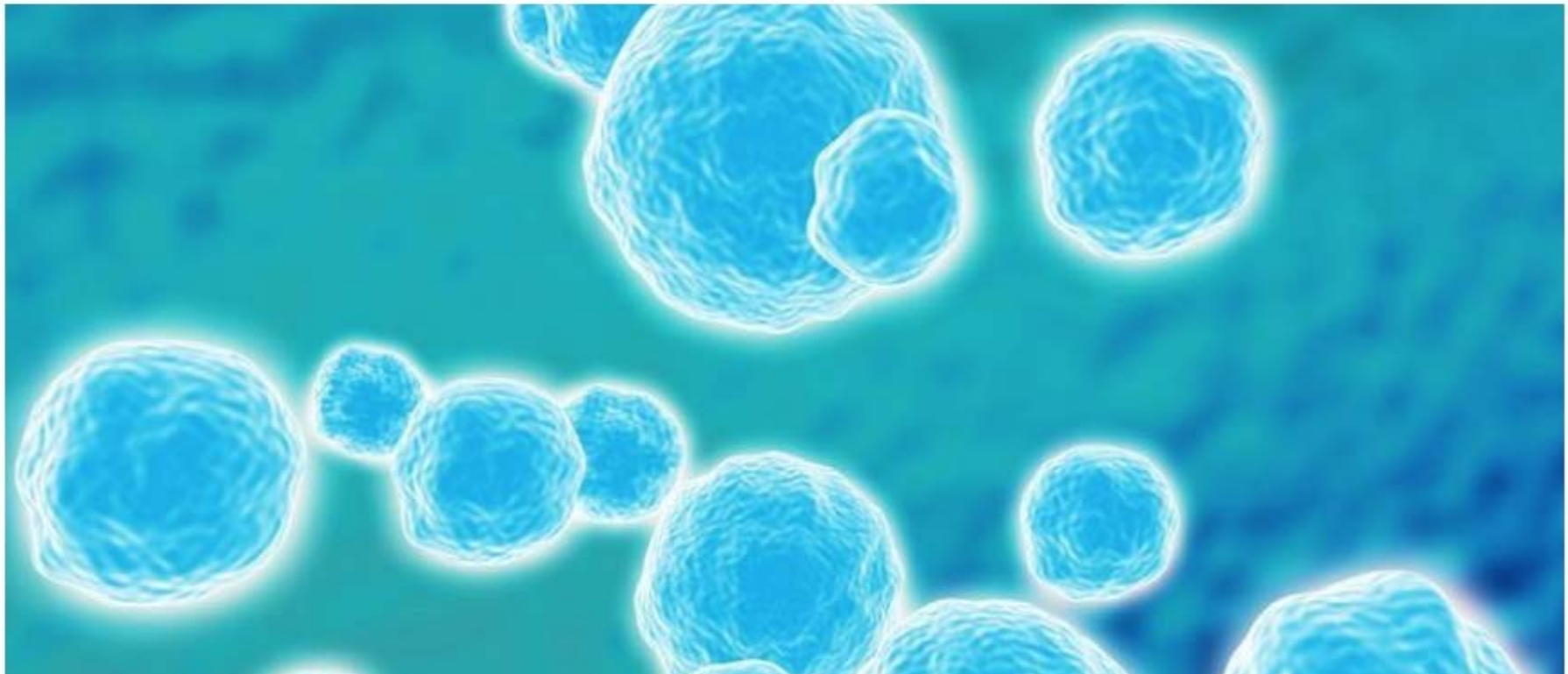
Renewed interest

'The world is headed for a post-antibiotic era,' WHO official warns



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Reasons for the decline

We must understand the reasons for the initial decline of phage therapy in the West, to successfully re-introduce phage therapy in Western medicine.

- **Microbiological issues**
- **Prejudices**
- **Pharmaco-economical issues**

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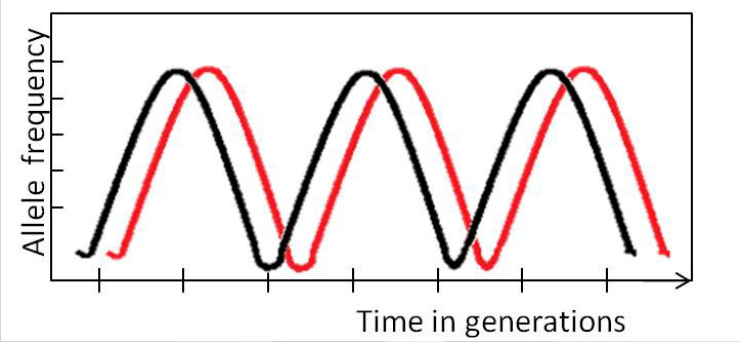
Microbiological issues

Phages are species or even strain specificity.

- Do not disturb the commensal flora.
- Infecting bacteria need to be known (**cocktails** could partially solve this).
- Problematic, particularly in empiric antimicrobial therapy.

The bacterium and it's phage are a co-evolving host/parasite couple.

- Phages **will not eradicate** their hosts. They reduce bioburden, but the patient's immune system and/or other antimicrobials need to finish.
- They are involved in arms race, consisting of the repeated emergence **bacterial resistance (even to cocktails)** and **new phage infectivity**.



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Solution

✓Select phages, from the environment or from collections, matched (personalised) to the infecting bacteria.

✓Apply different phages sequentially (not in a cocktail) to stay ahead of bacterial resistance.

✓Combine phages with other antimicrobials.

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Sustainable approach

- In line with evolutionary **“Darwinian” medicine** concepts.
- Phages are the **natural controllers** of bacterial populations on Earth (and also in the human body).

Sustainable approach is not compatible with current trends

Get me Phages… Now!

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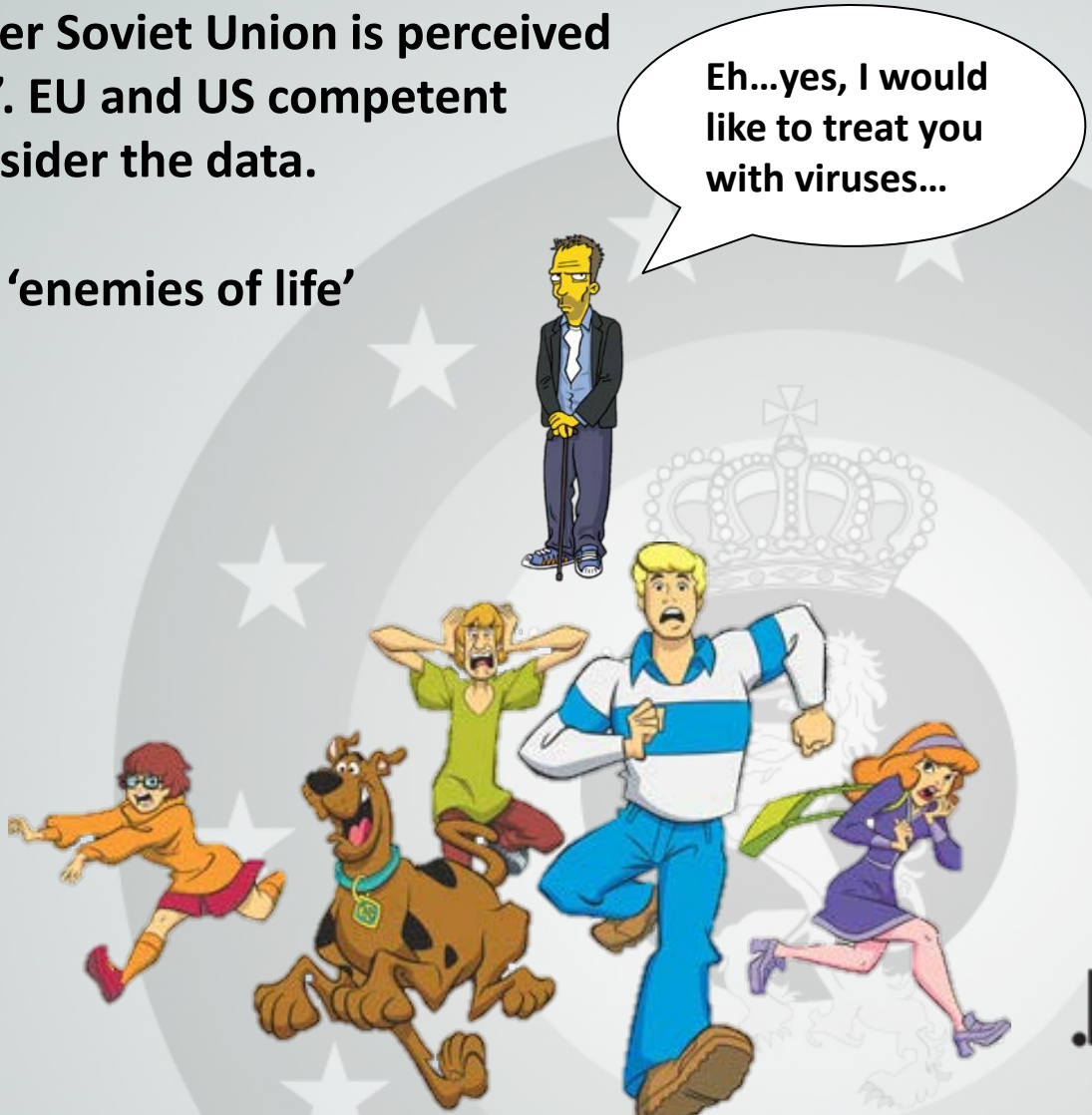
Prejudices

- **Work performed in former Soviet Union is perceived as ‘academically inferior’. EU and US competent authorities refuse to consider the data.**

- **Viruses are perceived as ‘enemies of life’**



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Pharmaco-economical issues

- Phage products were classified as **medicinal products** (drugs).
  - Need to follow conventional medicinal product licensing pathways.
    - ✓ **Manufactured according to Good Manufacturing Procedures (GMP).**
    - ✓ **Preclinical studies.**
    - ✓ **Phase I, II and III clinical trials.**
    - ✓ **Marketing.**
  - Takes **many years** and costs **millions of EUR**.
  - **Developed for conventional 'static' drugs** such as antibiotics.
  - **Not suitable for sustainable (personalised) phage therapy** approaches.
- Investments require strong intellectual property protection.
  - Phage therapy is in the public domain since 1920s.
  - Discussions about patenting natural organisms such as phages.

In the past

Phages

- Often, not matched to the infecting bacteria.
- Not adequately purified.

Advantages of antibiotics

- No need to match.
- Industrially produced in stable and pure preparations.
- Were marketed and used in large quantities.

These advantages tipped the balance in favour of antibiotics, but ultimately resulted in the current antibiotic resistance crisis!

Have both

Industrial phage therapy medicinal products.

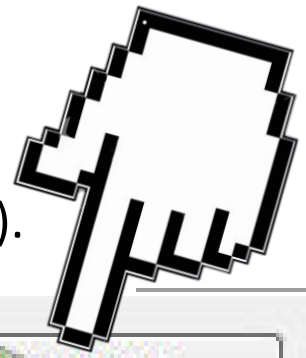
- **Phage products, manufactured, tested and marketed as if they were antibiotics.**
- **Global** supply of products for first line (empiric) treatment.

Windows Security Alert

Sustainable phage therapy approaches.

Local supply of phage therapy products for:

- 'Personalized therapy' (e.g. chronic wound infections).
- Public health or medical emergencies (e.g. EHEC outbreak).



Keep blocking

Unblock

Today's players

- **Hospitals (phage therapy centres) and universities** are not able/willing to bring phage medicinal products to the market.
- **Big Pharma** is sitting on the fence.
- A handful of **small and medium-sized companies** are trying to market phage cocktails.
  - Venture capital (high risk, high return).
  - Public sources (e.g. EC funding).

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Phagoburn

SEVENTH FRAMEWORK
PROGRAMME

PhagoBurn

- 3.85 milj. EUR funding by EC, within the FP7 framework.
- Started: June 1, 2013

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Two main parts

I) **GMP Manufacturing** of a **phage therapy medicinal product**.

II) **Multicentric clinical trial.**

SEVENTH FRAMEWORK PROGRAMME

PhagoBurn

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Phagoburn partners

Pherecydes Pharma (FRA): Developers of the phage therapy medicinal product and co-coordinator of the project.

Clean Cells (FRA): Manufacturing of the phage therapy medicinal product under GMP conditions.

Service de Santé des Armées (FRA): Coordinator of the project.

Centre hospitalier universitaire vaudois (SWI): Coordinator of the Swiss section of the clinical trial.

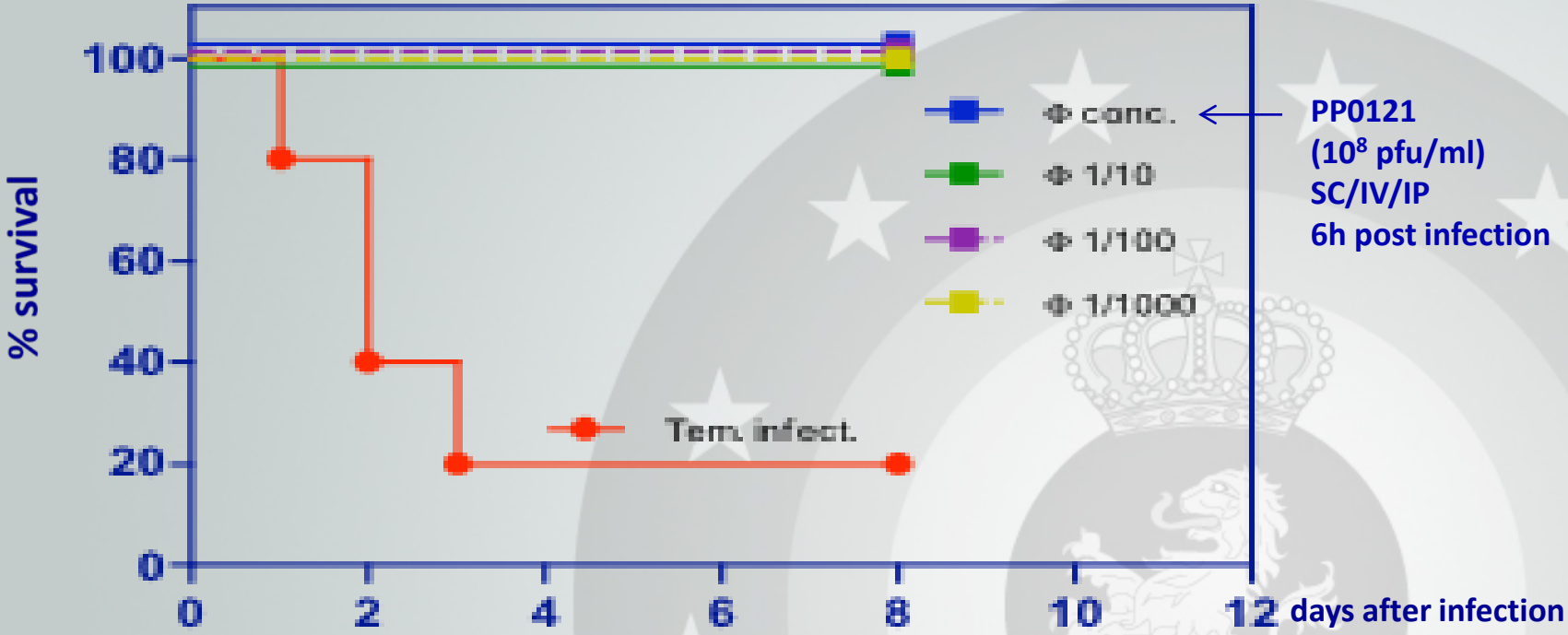
Royal military academy (BEL): Coordinator of the Belgian section of the clinical trial.

The product

- Two Pherecydes phage cocktails:
  - PP0121: 13 natural *E. coli* lytic phages.
  - PP1131: 12 natural *P. aeruginosa* lytic phages.
- Manufactured according to GMP.
- Carrier for burn wound application: Algosteril™ dressing (Les Laboratoires Brothier).

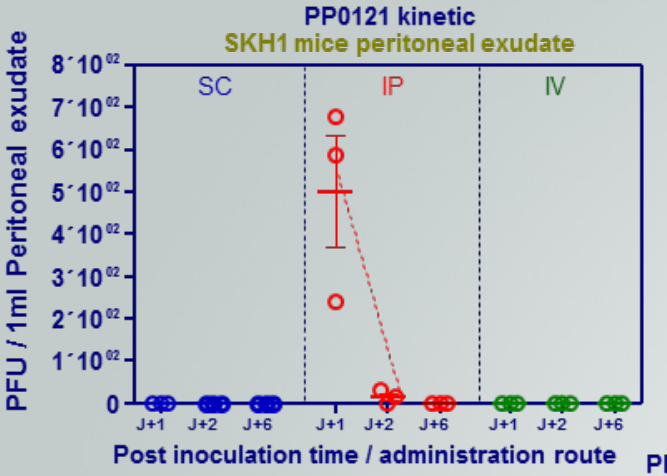
Role of dose (preclinical)

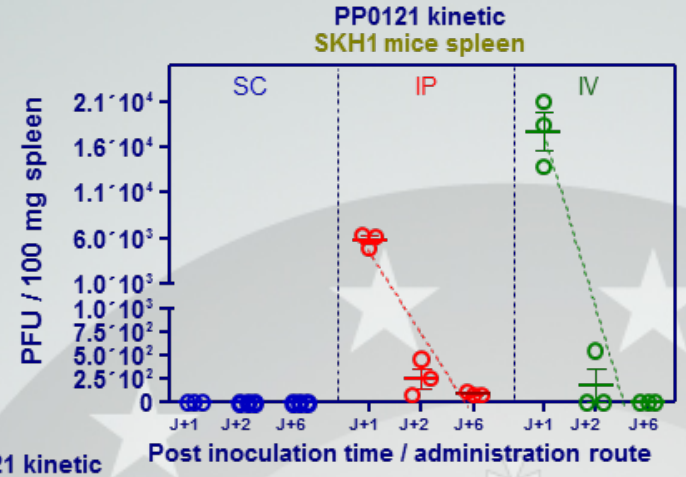
Immuno-depressed mice + mustard gas burn + SC MDR *E. Coli* (10^7 cfu/ml)

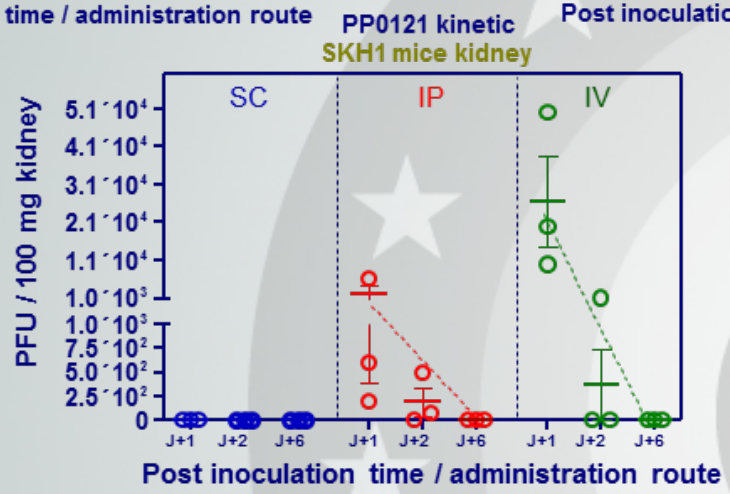


100% efficacy whatever the dilution (1 to 1/1000)

Pharmacokinetics (preclinical)







PP0121
Dose at D0: 10^8 PFU/ml

IV or IP: phages eliminated from spleen and kidneys after 2 days.
SC: no phages detected in mice.

Competent authorities

July 7, 2015: Approval of GMP products issued by the French, Belgian and Swiss agencies for medicines.

Completion of Part I: Manufacturing!

Approximately 1 year delay.

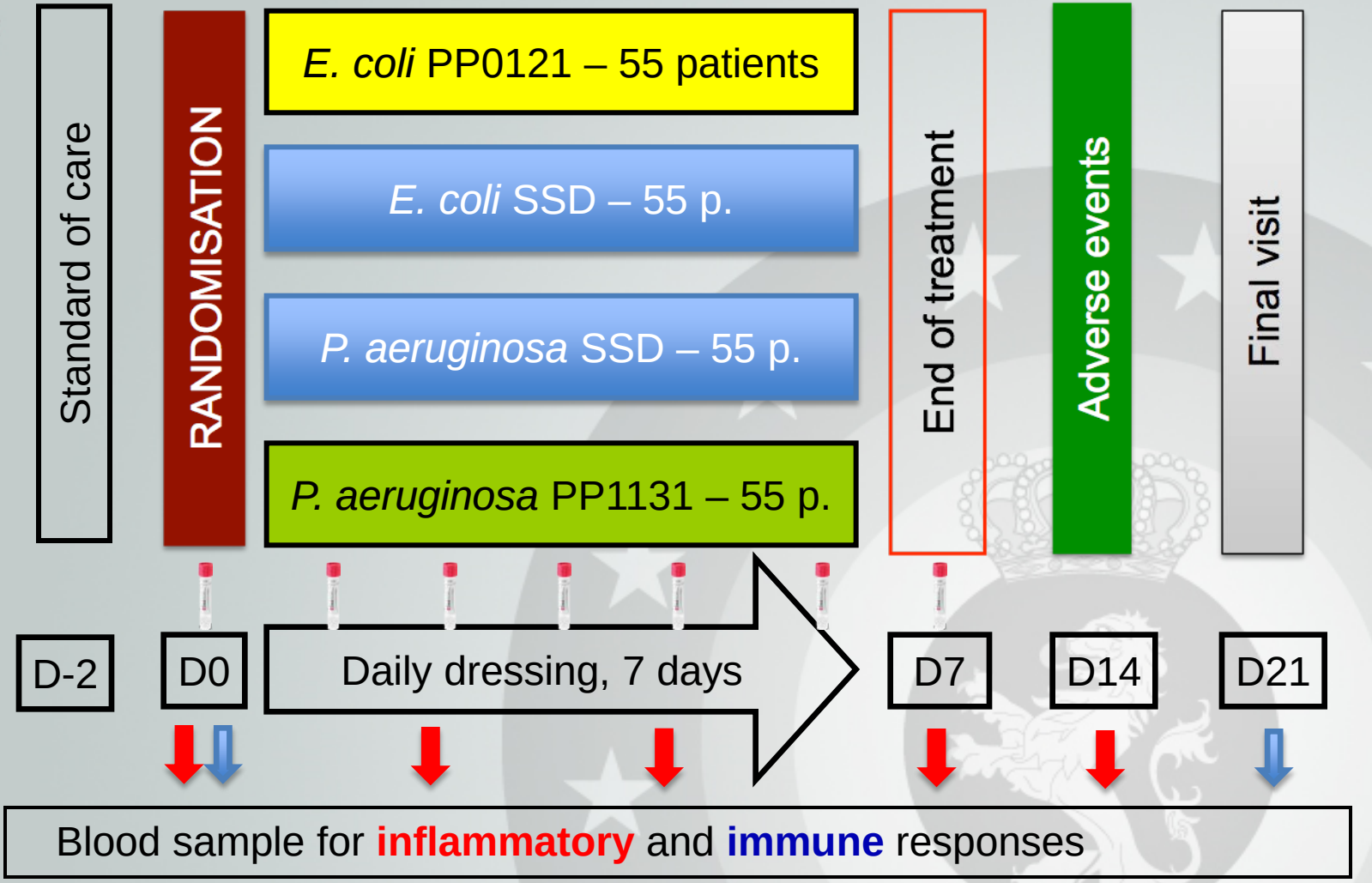
Trial set up

- **Phase I/II** clinical trial.
- 220 patients with 3rd degree burn wounds infected exclusively by *E. coli* or *P. aeruginosa*.
- **Controlled** (1% silver sulfadiazine (SSD) cream)
- **Randomised**.
- **Blind** (to patients and assessors (microbiologists)).
- eCRF (electronic case report form) accessible to the competent authorities.

11 burn wound centres

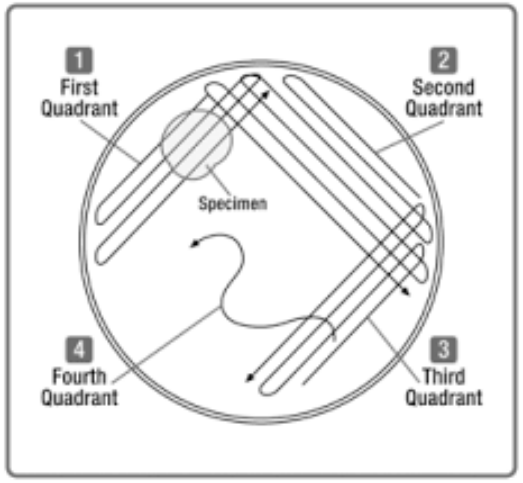
FRANCE	**Dr. Patrick JAULT (coordinator) & Prof. Thomas LECLERC**	Instruction Military hospital Percy – Paris (Clamart)
SWITZELAND	**Dr. Yok Aie QUE**	CHUV - Lausanne
BELGIUM	**Dr. Serge JENNES**	Queen Astrid military hospital Bruxelles
FRANCE	**Dr. François RAVAT**	Centre hospitalier Saint Joseph Saint Luc - Lyon
FRANCE	**Dr. Ronan LEFLOCH**	CHU - Nantes
BELGIUM	**Dr. Anne-Françoise ROUSSEAU**	CHU - Liège
BELGIUM	**Dr. Jean-Philippe FAUVILLE & Dr. Ghüder SAIDANE**	Hôpital de Charleroi - Loverval
FRANCE	**Dr. Hervé CARSIN**	Centre Hospitalier Hôpital de Mercy Metz-Thionville
FRANCE	**Dr. Sandrine Wiramus**	Hôpital de la Conception – APHM Marseille
FRANCE	**Dr. Nathalie Bénillan**	Centre FX Michelet CHU Bordeaux
FRANCE	**Dr. Eric Meaudre**	Hôpital d'instruction des armées Sainte-Anne - Toulon

Global design



Primary endpoint

- ESwabs from D0 to D8 to collect bacteria.
- **Endpoint: Time for 2 quadrants bacterial reduction relative to D0.**

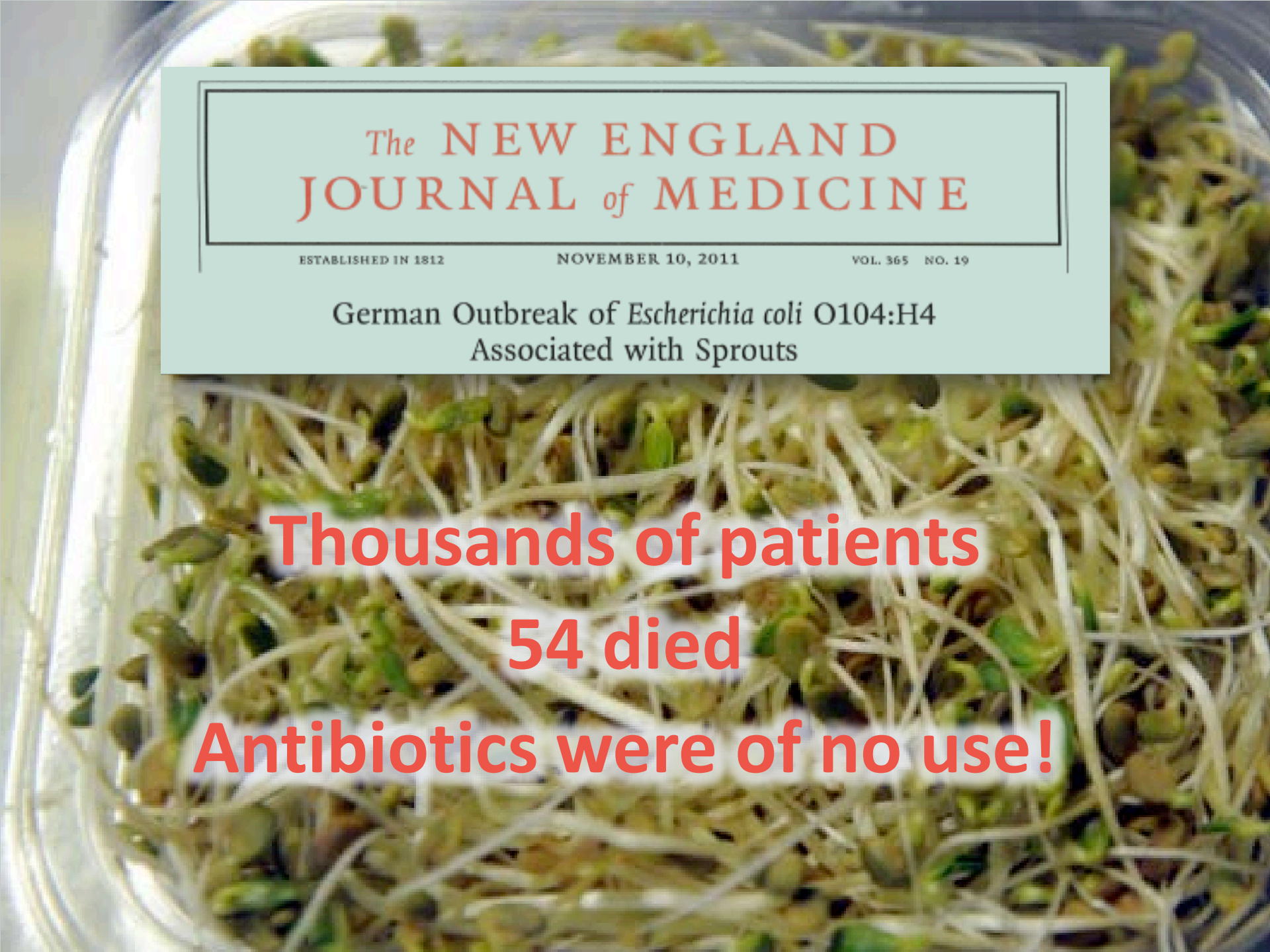


- A semi-quantitative parameter assessed blindly by microbiologists.
- + Bacterial species identification.
- + Antibiogram.
- + Evaluation of the wound bacteria's response to the phages (resistance).

Results

Ongoing clinical trial (first patients inclued in July 2015)

Whatever the result, it is a <u>mandatory</u> step in the re-introduction of phage therapy in Western medicine (if the case should arise).


The NEW ENGLAND JOURNAL of MEDICINE
ESTABLISHED IN 1812 NOVEMBER 10, 2011 VOL. 365 NO. 19
German Outbreak of Escherichia coli O104:H4 Associated with Sprouts
Thousands of patients
54 died
Antibiotics were of no use!

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Consider phage therapy?

AAC
Journals.ASM.org

Virulent Bacteriophages Can Target O104:H4 Enteroaggregative *Escherichia coli* in the Mouse Intestine

Damien Maura,[a,b]* Matthieu Galtier,[a,b] Chantal Le Bouguénec,[c] and Laurent Debarbieux[a]

Institut Pasteur, Molecular Biology of the Gene in Extremophiles Unit, Department of Microbiology, Paris, France[a]; Université Paris Diderot, Sorbonne Paris Cité, Cellule Pasteur, Paris, France[b]; and Institut Pasteur, Biology of Gram-Positive Pathogens Unit, Department of Microbiology, Paris, France[c]

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439 VIEWS

RESEARCH ARTICLE

Selection and Characterization of a Candidate Therapeutic Bacteriophage That Lyses the *Escherichia coli* O104:H4 Strain from the 2011 Outbreak in Germany

Maia Merabishvili, Daniel De Vos, Gilbert Verbeken, Andrew M. Kropinski, Dieter Vandenheuvel, Rob Lavigne, Pierre Wattiau, Jan Mast, Catherine Ragimbeau, Joel Mossong, Jacques Scheres, Nina Chanishvili, Mario Vaneechoutte, Jean-Paul Pirnay

AEM
Journals.ASM.org

MINIREVIEW

Shiga Toxin-Producing *Escherichia coli* O104:H4: a New Challenge for Microbiology

Maite Muniesa,[a] Jens A. Hammerl,[b] Stefan Hertwig,[b] Bernd Appel,[b] and Harald Brüssow[c]

Department of Microbiology, University of Barcelona, Barcelona, Spain[a]; Bundesinstitut für Risikobewertung, Berlin, Germany[b]; and Nestlé Research Center, Lausanne, Switzerland[c]

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Authorities didn't

« In fact, Nestlé Research Center offered a lytic phage to the German public health sector during the epidemic »

H. Brüssow, Virology 2012

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Second chance

Just as in the last century, a possible broad acceptance of phage therapy will depend on:

The credibility of the scientists.

The **socio-economic and political context** in which they work!

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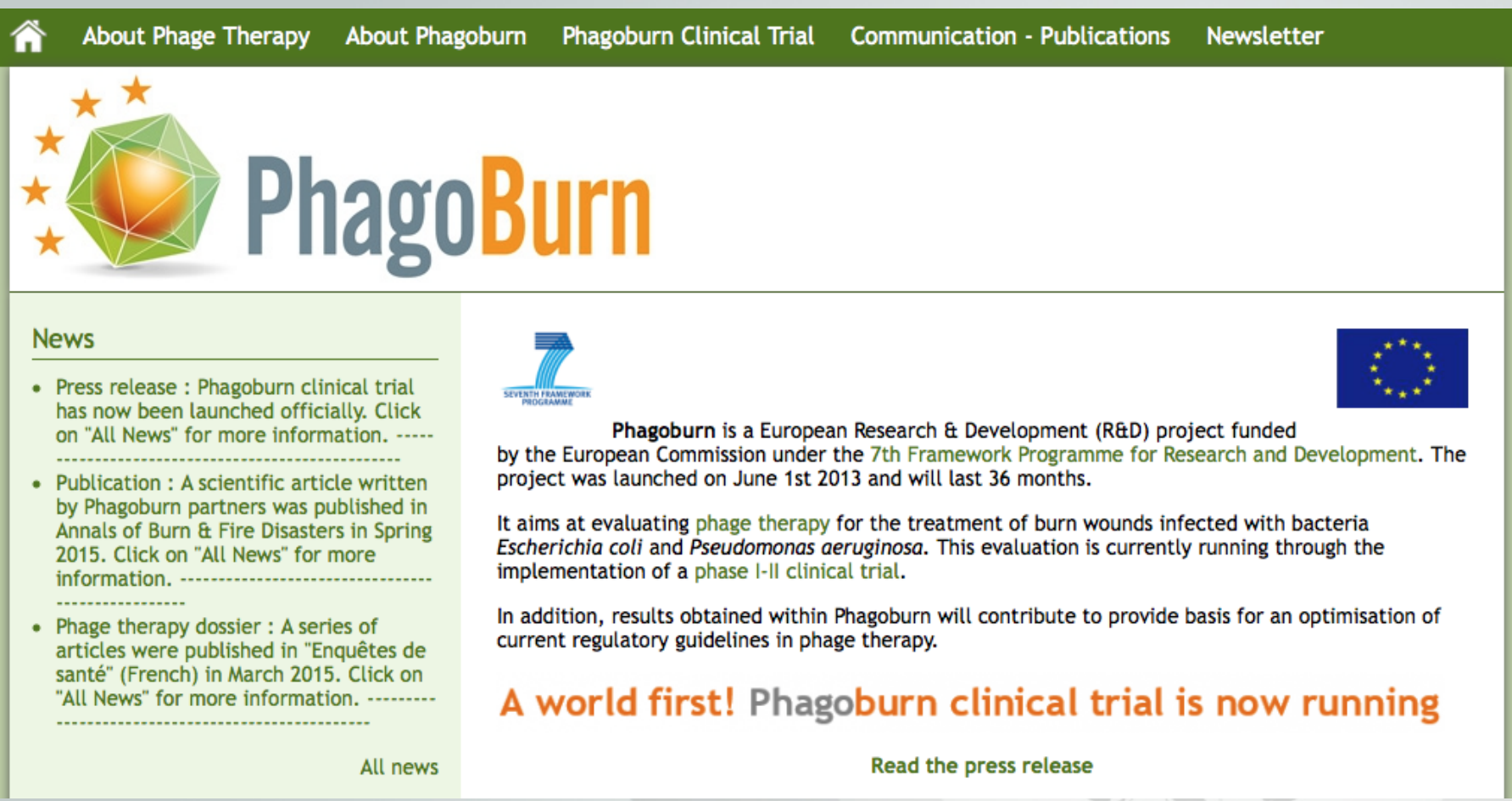
Thank you!

http://www.phagoburn.eu



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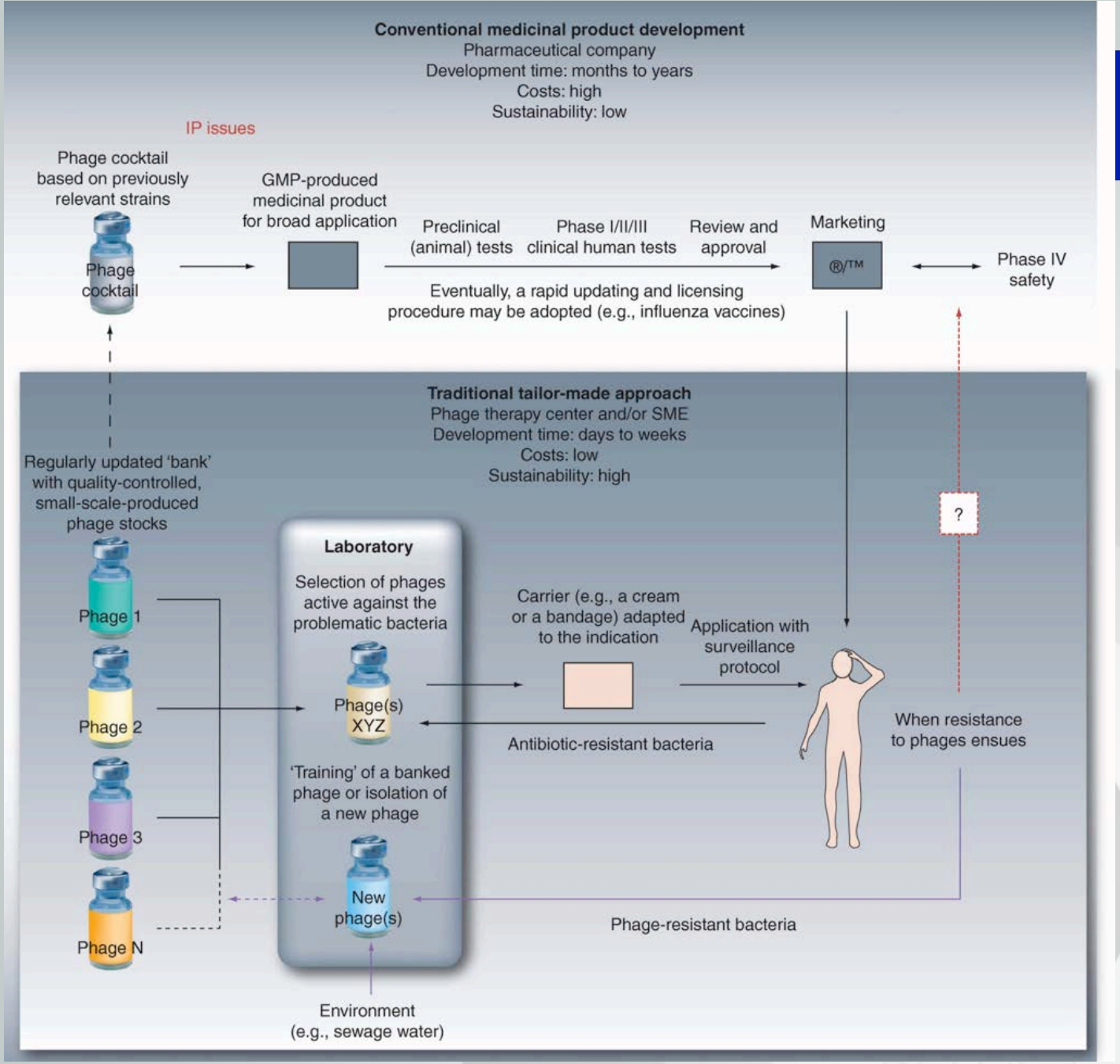
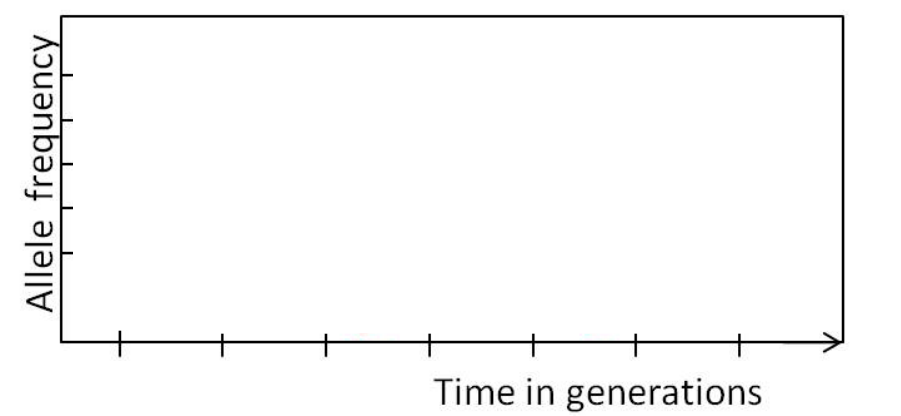


Pirnay *et al.*, Future Virology 2012

Arms race



Typical antagonistic host-parasite co-evolution:

- (1) Successful **bacteria (black curve)** thrive.
  (2) **Phages (red curve)** emerge to lyse these bacteria.
- (3) Bacterial density decreases.
  (4) Phage density decreases (due to the decline of their host).
- (5) Lytic phages impose a strong **selection for bacterial resistance**, and bacteria resistant to these phages emerge.
  (6) There is strong **selection to overcome this bacterial resistance**. New – evolved – infective phages emerge.
- This leads to an arms race, consisting of the repeated emergence of new phage infectivity and bacterial resistance mutations.

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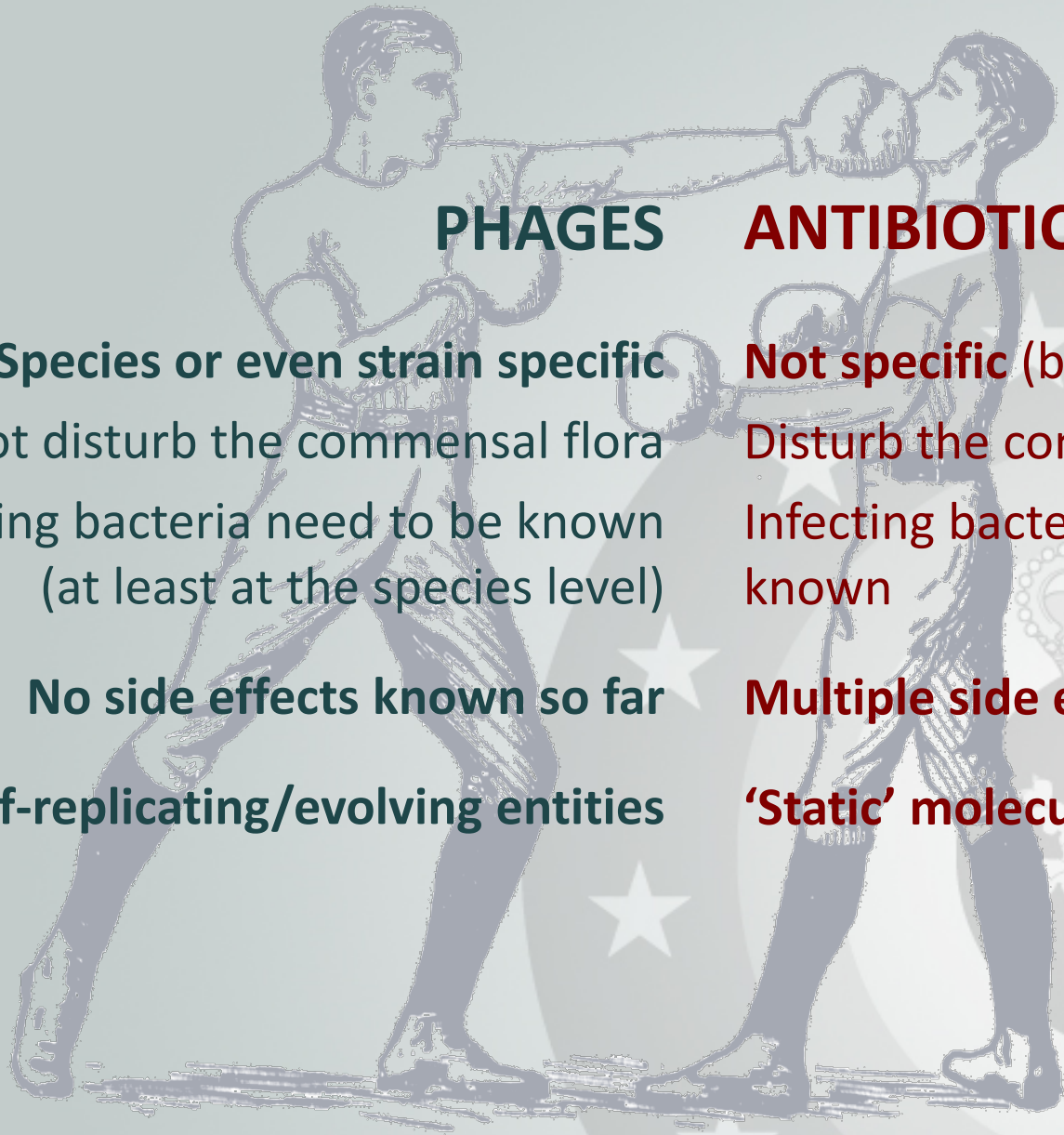
Phages vs Antibiotics



PHAGES	ANTIBIOTICS (ABs)
Species or even strain specific	**Not specific** (broad spectrum ABs)
Do not disturb the commensal flora	Disturb the commensal flora
Infecting bacteria need to be known (at least at the species level)	Infecting bacteria don't need to be known
No side effects known so far	**Multiple side effects**
Self-replicating/evolving entities	**'Static' molecules**

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- Thomas.Rose@mil.be

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