41e
RÉUNION INTERDISCIPLINAIRE DE CHIMIOTHÉRAPIE ANTI-INFECTIEUSE

LUNDI 13 & MARDI 14 DÉCEMBRE 2021

Palais des Congrès
Paris

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RICAI

Photo: Karoly Effenberger

Phagothérapie en compassionnel: l'expérience Belge

Jean-Paul Pirnay, hôpital militaire, Bruxelles

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Avertissement!

Je ne suis pas francophone

18 years of phage therapy research

Isolation → Characterization → Production → QA/QC → Selection → Therapy

Research laboratory → Cleanroom facility → Pharmacy → Clinic

Fundamental research
(Inter)national collaborations

Experience/phages
Eliava Institute

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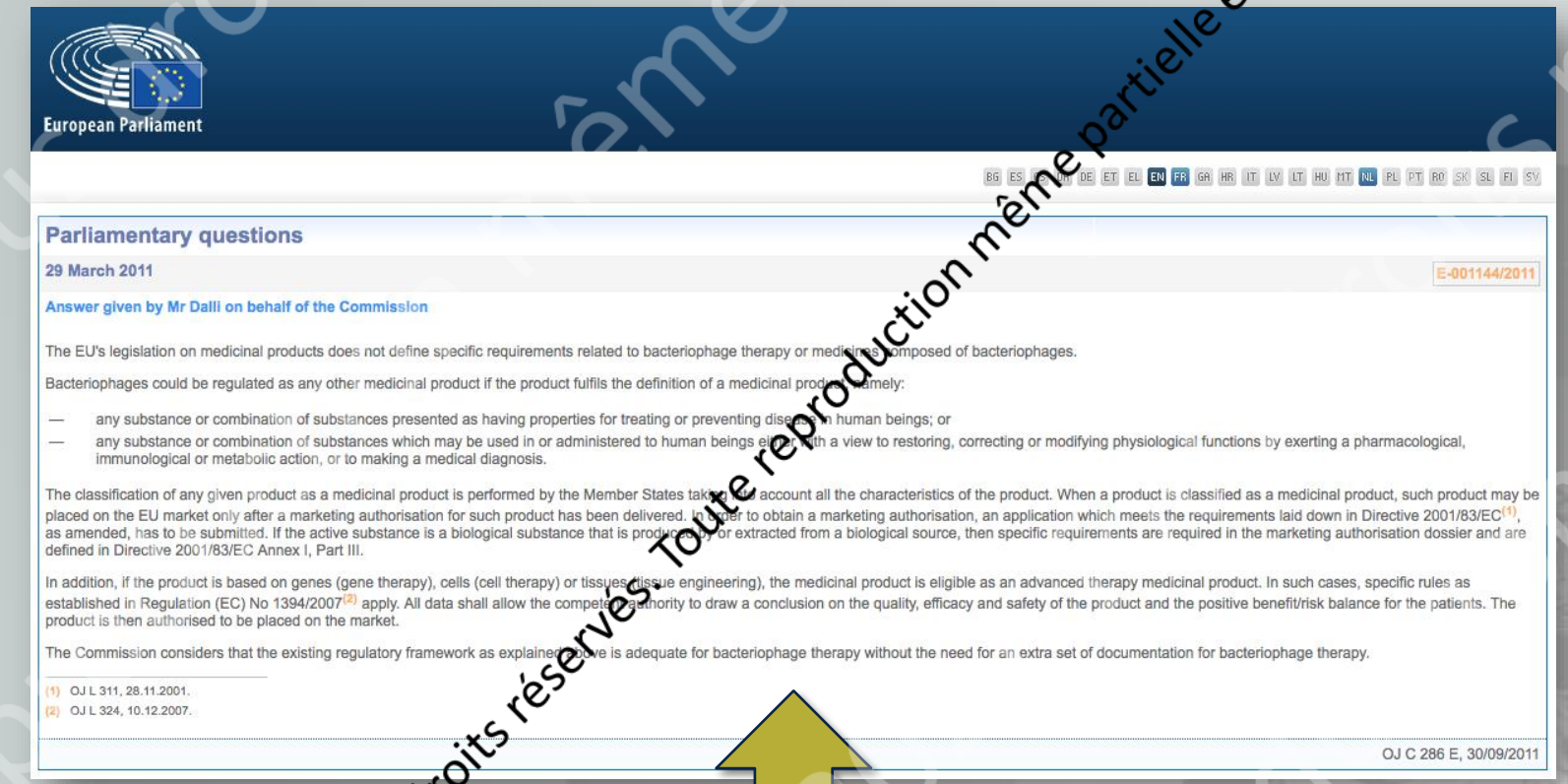
The Eliava Institute

Dedicated to phage therapy since 1923

It's a Medicine (EU and US)



European Parliament

BG ES CS DA DE ET EL EN FR GA HR IT LV LT HU MT NL PL PT RO SK SL FI SV

Parliamentary questions

29 March 2011 E-001144/2011

Answer given by Mr Dalli on behalf of the Commission

The EU's legislation on medicinal products does not define specific requirements related to bacteriophage therapy or medicines composed of bacteriophages.

Bacteriophages could be regulated as any other medicinal product if the product fulfils the definition of a medicinal product, namely:

— any substance or combination of substances presented as having properties for treating or preventing disease in human beings; or

— any substance or combination of substances which may be used in or administered to human beings either with a view to restoring, correcting or modifying physiological functions by exerting a pharmacological, immunological or metabolic action, or to making a medical diagnosis.

The classification of any given product as a medicinal product is performed by the Member States taking into account all the characteristics of the product. When a product is classified as a medicinal product, such product may be placed on the EU market only after a marketing authorisation for such product has been delivered. In order to obtain a marketing authorisation, an application which meets the requirements laid down in Directive 2001/83/EC[1], as amended, has to be submitted. If the active substance is a biological substance that is produced or extracted from a biological source, then specific requirements are required in the marketing authorisation dossier and are defined in Directive 2001/83/EC Annex I, Part III.

In addition, if the product is based on genes (gene therapy), cells (cell therapy) or tissues (tissue engineering), the medicinal product is eligible as an advanced therapy medicinal product. In such cases, specific rules as established in Regulation (EC) No 1394/2007[2] apply. All data shall allow the competent authority to draw a conclusion on the quality, efficacy and safety of the product and the positive benefit/risk balance for the patients. The product is then authorised to be placed on the market.

The Commission considers that the existing regulatory framework as explained above is adequate for bacteriophage therapy without the need for an extra set of documentation for bacteriophage therapy.

(1) OJ L 311, 28.11.2001.
(2) OJ L 324, 10.12.2007.

OJ C 286 E, 30/09/2011

"The Commission considers that the existing Medicinal Product framework is adequate for bacteriophage therapy"

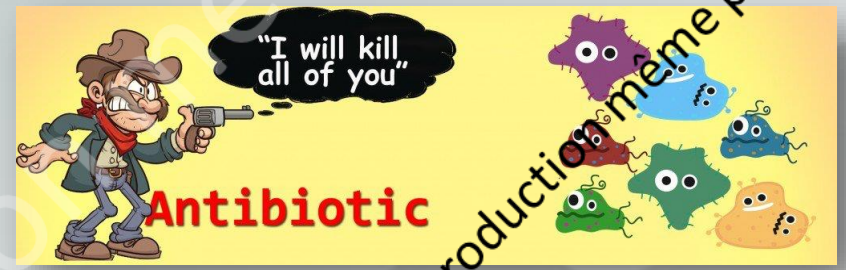
Is it a medicine?

Pirnay JP, Kutter E. Lancet Infect Dis 2020;S1473-3099(20)30464-3.

It's a medicine, Jim, but not as we know it. **Phages have a number of peculiarities.**

Peculiarity 1

Phages are species or even strain specific



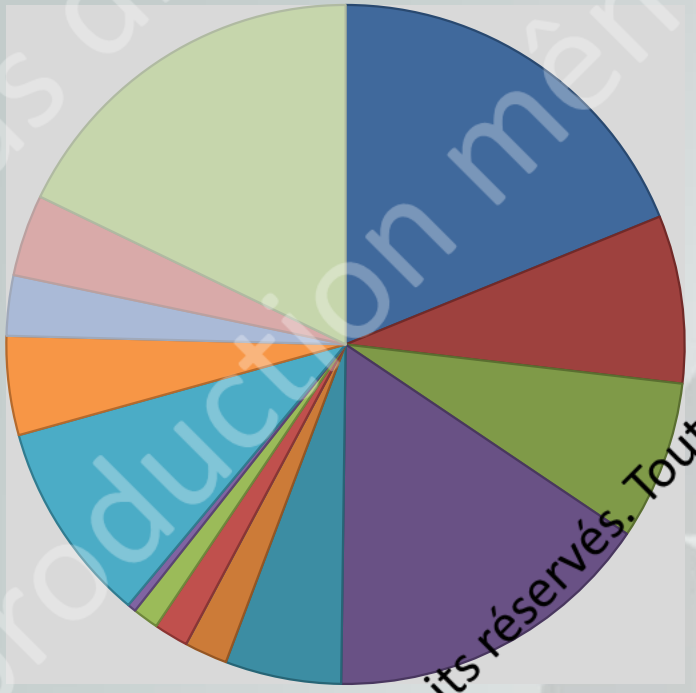
Do not disturb the patient's commensal flora

Infecting bacteria need to be known

Problematic, particularly in empiric antimicrobial therapy

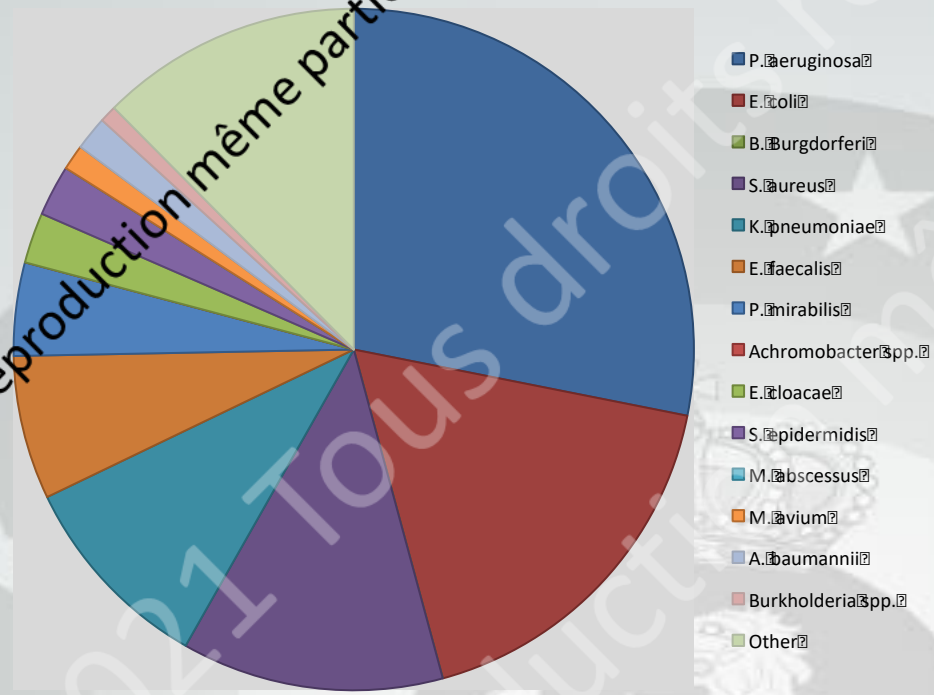
Analysis of PT requests

San Diego: 35 sp./488 req.



Aslam S. et al. Open Forum Infect Dis. 2020; 7(9):ofaa389.

Brussels: 31 sp./249 req.

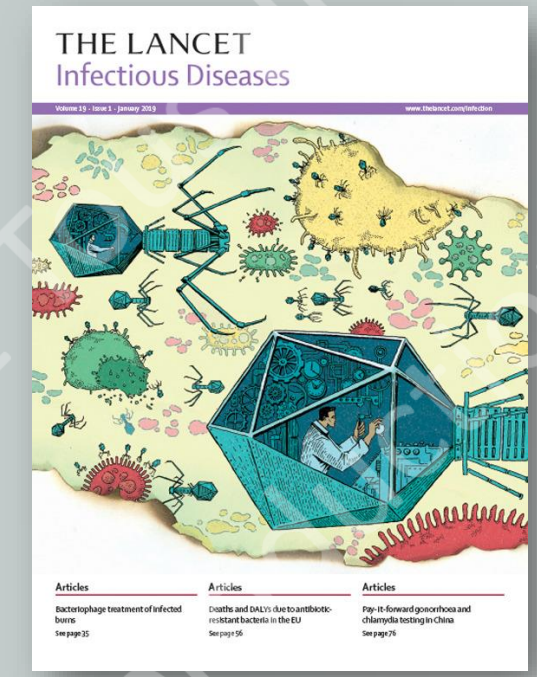


Djebara S. et al. Viruses. 2019;11(3):265.

- >30 sp.
- Geographical variations
- Variations in time

Hundreds of phages, requiring regular updates

PhagoBurn



Efficacy and tolerability of a cocktail of bacteriophages to treat burn wounds infected by *Pseudomonas aeruginosa* (PhagoBurn): a randomised, controlled, double-blind phase 1/2 trial

Patrick Jault, Thomas Leclerc, Serge Jennes, Jean Paul Pirnay, Yok-Ai Que, Gregory Resch, Anne Françoise Rousseau, François Ravat, Hervé Carsin, Ronan Le Floch, Jean Vivien Schaal, Charles Soler, Cindy Fevre, Isabelle Arnaud, Laurent Bretaudeau, Jérôme Gabard

- RCT
- Defined cocktail of 12 lytic anti-*P. aeruginosa* phages
- The susceptibility of the bacterial strains, isolated on **day 0**, was tested in 10 patients in the PT group
- **3/10 patients harboured phage resistant strains at day 0**

Peculiarity 2

Phage co-evolve with bacteria!

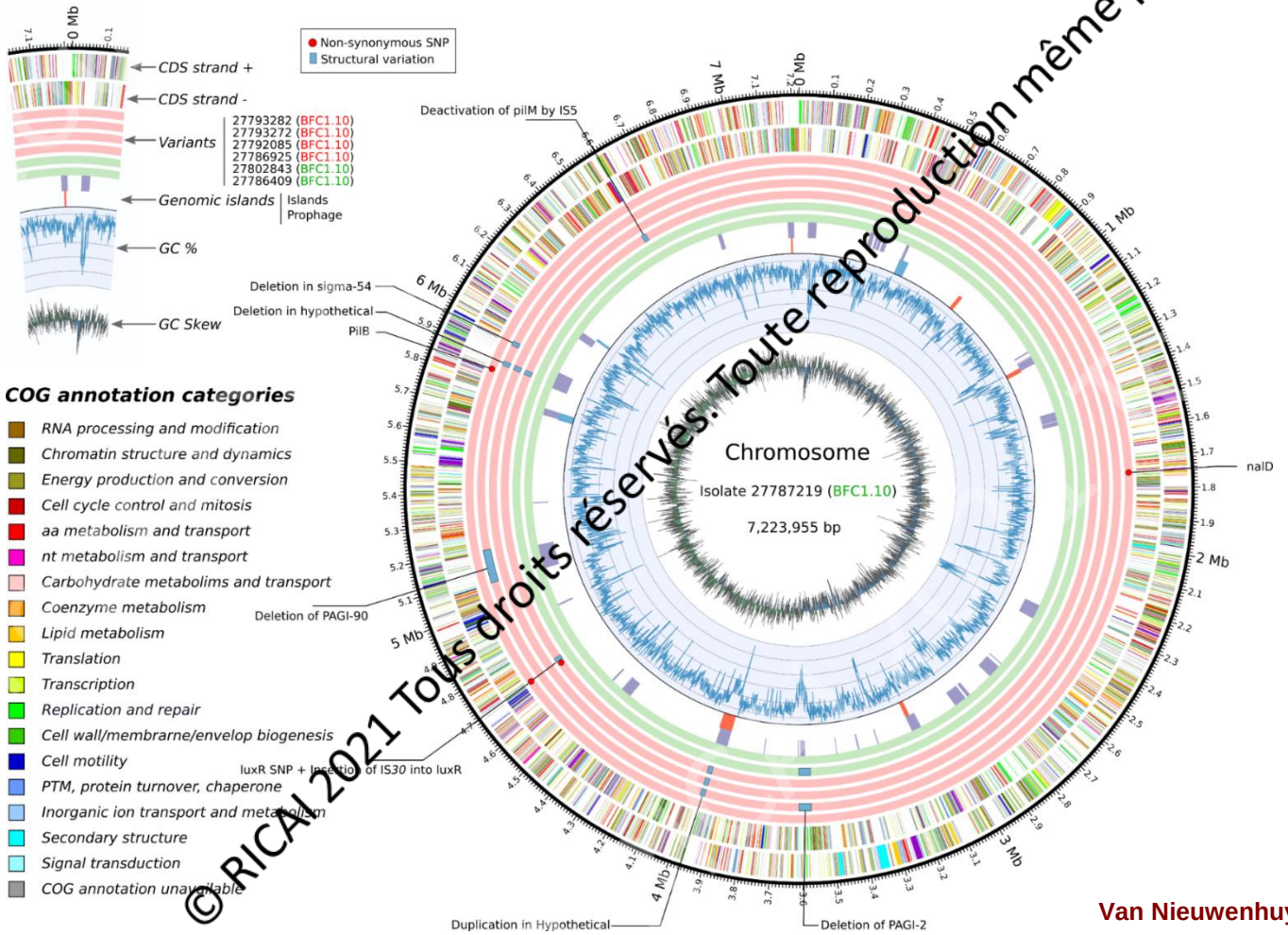
Bacteria/phage involved in host/parasite relationship

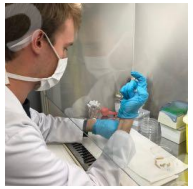
Phages will not eradicate their hosts

- **Bacterial phage resistance (BPR)** will readily emerge
- The patient's immune system and/or antimicrobials need to eradicate the bacteria -> **Phage antibiotic synergy (PAS)**

Example of *In vivo* emergence of BPR

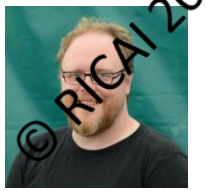
- 1-yr-old patient with *P. aeruginosa* septicemia, post-liver transplant -> 86 days MT (IV)
- WGS showed that four isolates (red circles) emerged that expressed BPR. They possessed genetic alterations, which affect the development of the Type IV pili complex
- Phage insensitive mutants: **No reduced virulence** in *Galleria mellonella*




Brieuc Van Nieuwenhuyse


Cédric Lood


Jeroen Wagemans


Rob Lavigne

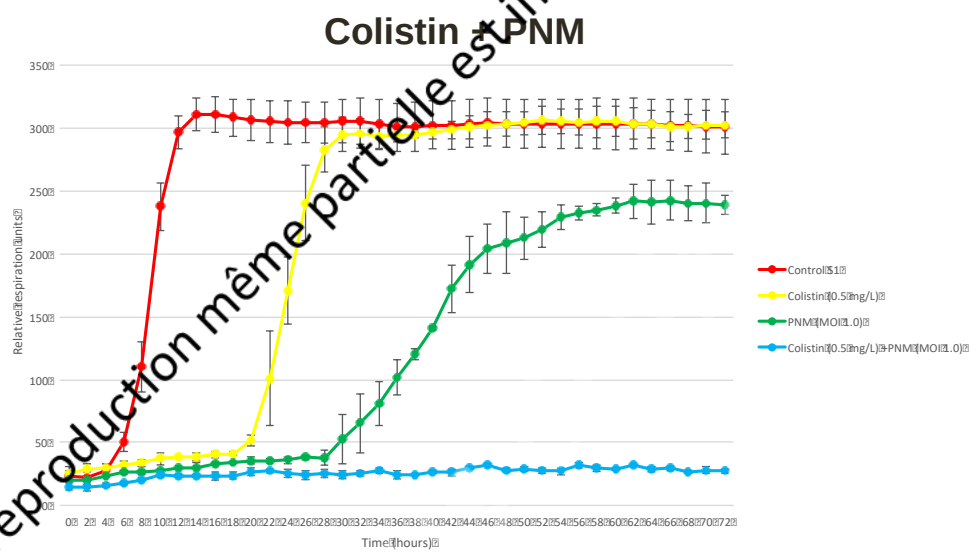
Van Nieuwenhuyse B. *et al.* (Manuscript submitted).

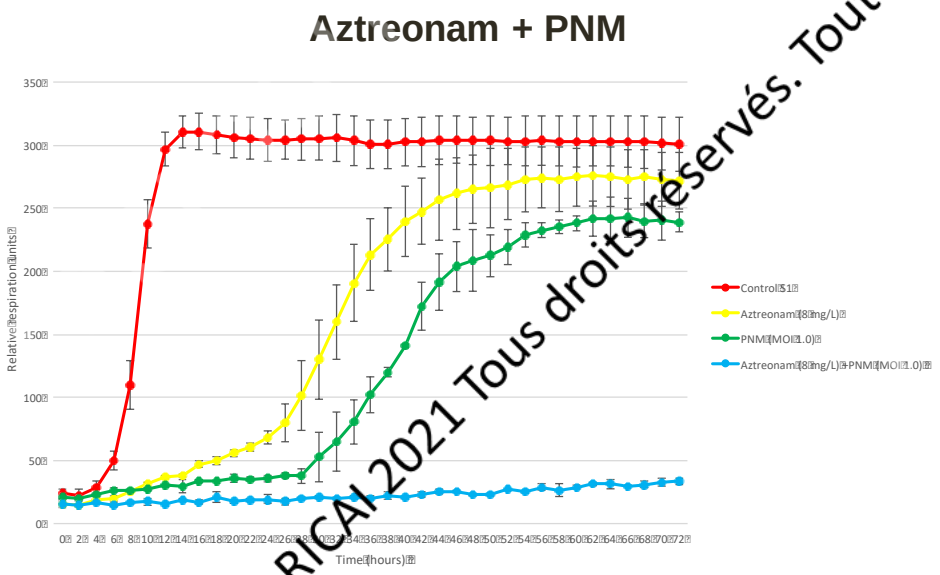
Example of *in vitro* PAS

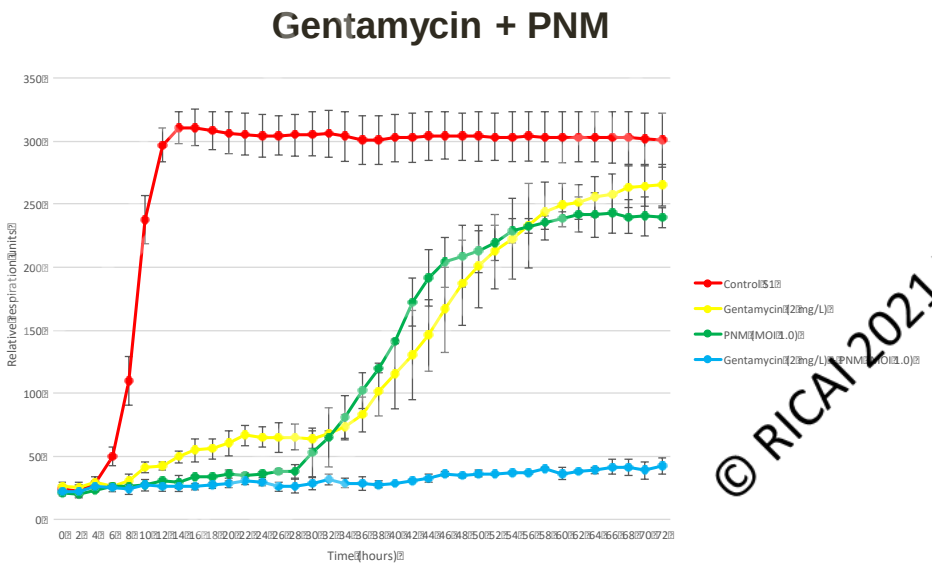
Liver transplant baby saved by "trained" virus at Saint-Luc hospital

Wednesday, 22 May 2019

© Pxhere







Van Nieuwenhuyse B. *et al.* (Manuscript submitted).

Phage “Training”

- Phages selected from collections, matched to the infecting bacteria
- Pre-adapted (trained) to the patient’s bacteria

JOURNAL OF Evolutionary Biology

eseb

doi: 10.1111/jeb.12774

Pre-adapting parasitic phages to a pathogen leads to increased pathogen clearance and lowered resistance evolution with *Pseudomonas aeruginosa* cystic fibrosis bacterial isolates

V-P. FRIMAN*†, D. SOARES-BROWN*, P. SIEROCINSKI*‡, S. MOLIN§, H. K. JOHANSEN§¶, D. MERABISHVILI**†††‡, J-P. PIRNAY**, D. DE VOS** & A. BUCKLING*

Friman *et al*, Evol Biol. 2016; 29(1):188-98.

Example of “phage training”

Host range

CLINICAL ISOLATE	ISP	ISPepi
STEP_UZL/TRH_1		
STEP_UZL/TRH_2A		
STEP_UZL/TRH_2B		
STEP_UZL/TRH_3		
STEP_UZL/TRH_4		
STEP_UZL/TRH_5		
STEP_UZL/TRH_6		
STEP_UZL/TRH_7		
STEP_UZL/TRH_8		
STEP_UZL/TRH_9		
STEP_UZL/TRH_10		
STEP_UZL/TRH_11A		
STEP_UZL/TRH_11B		
STEP_UZL/TRH_11C		

- Patient with *S. epidermidis* infection
- *S. aureus* phage **ISP**
- Training (8 rounds of co-evolution using Appelmans’ method) on each of 3 clinical *S. epidermidis* strains

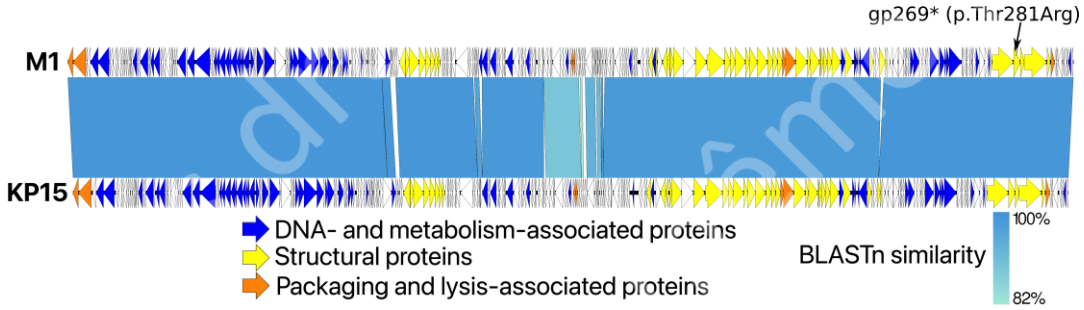
➔ **ISP$_{epi}$**

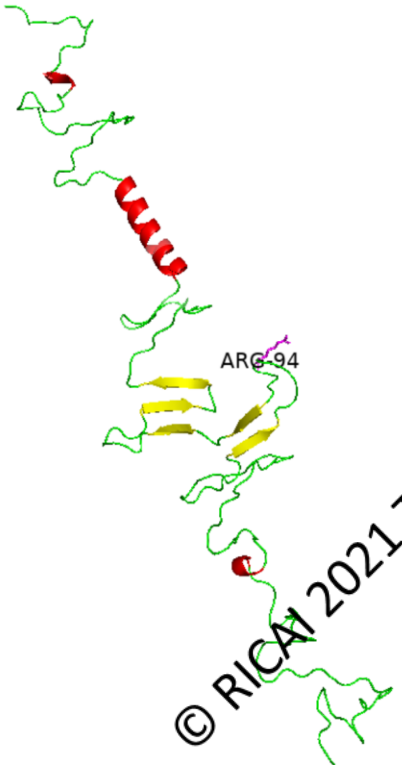
- 48 h lysis without emergence of resistance, and this at low MOI
- Training resulted in 4 missense mutations
- Reduction of host range (3/14 => 1/14)

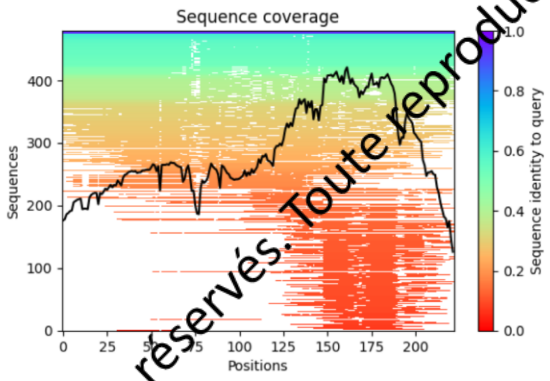
Mutations

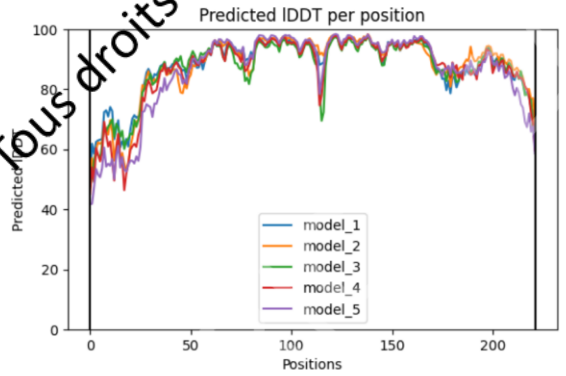
POSITION	EFFECT	LOCUS_TAG	ANNOTATED PRODUCT	PREDICTION HHPRED/HMMER/Phyre
41566	missense_variant c.1745A>C p.Lys582Thr	HOQ69_gp039	Structural protein (ESI-MS)	Putative carbohydrate-binding domain protein (Staphylococcus virus K)
43215	missense_variant c.1067G>T p.Lys356Asn	HOQ69_gp041	Structural protein (ESI-MS)	Putative receptor binding protein (Staphylococcus virus K)
60502	missense_variant c.91T>C p.Ser31Pro	HOQ69_gp060	Putative DNA polymerase	Uracil-DNA glycosylase
82393	missense_variant c.170G>A p.Ser57Asn	HOQ69_gp095	Hypothetical protein	/

Example 2 of “phage training”









Patient with *Klebsiella pneumoniae* osteomyelytis

Missense mutation in the loop region of the hinge connector of the distal tail fiber protein

Eskenazi *et al*. Nat. Comm. In press.

Defined Phage Products RCTs

- Evaluated **definded phage products** as **stand-alone therapy** – as if they were new ABs
- Showed **disappointing efficacy**, in contrast with:
  - Historical phage therapy applications (e.g. Georgia, Poland,..)
  - Recent “western” case studies
    - Positive result publication bias
    - Phages as **adjunctive therapy** (e.g. phage-antibiotic synergy)
    - **Selected** phages (personalized)
    - **Pre-adapted (“trained”)**, or even engineered to be more effective

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Note: Until the 1990s, Institut Pasteur delivered phage preparations in France

Two decades of PT "renaissance"

nature biotechnology

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nature > nature biotechnology > features > article

Published: 01 January 2004

Old dogma, new tricks—21st Century phage therapy

Karl Thiel

Nature Biotechnology **22**, 31–36(2004) | Cite this article

↓

No phage medicines on EU/US markets!

.be

High profile PT cases in the Media

Pressure on MDs to apply PT in desperate cases

Belgian magistral phage medicine concept

PHAGE BANK

Seed lot

- Characterized phages
- Stored using a (tiered) banking system

CONTROLLED ENVIRONMENT

Active Pharmaceutical Ingredient (API)

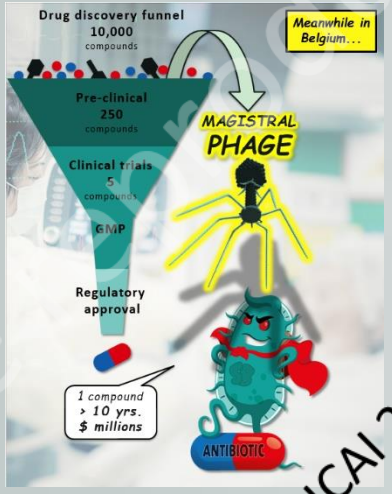
- A phage API (single phage) is produced using a suitable host
- Produced according to a **monograph**
- External quality testing based on a monograph, performed by a **"Belgian Approved Laboratory"**

HOSPITAL PHARMACY

Magistral preparation

- Upon **prescription**
- One or more phage APIs are selected (phagogram)
- Phage APIs are mixed with a carrier (e.g. a hydrogel)

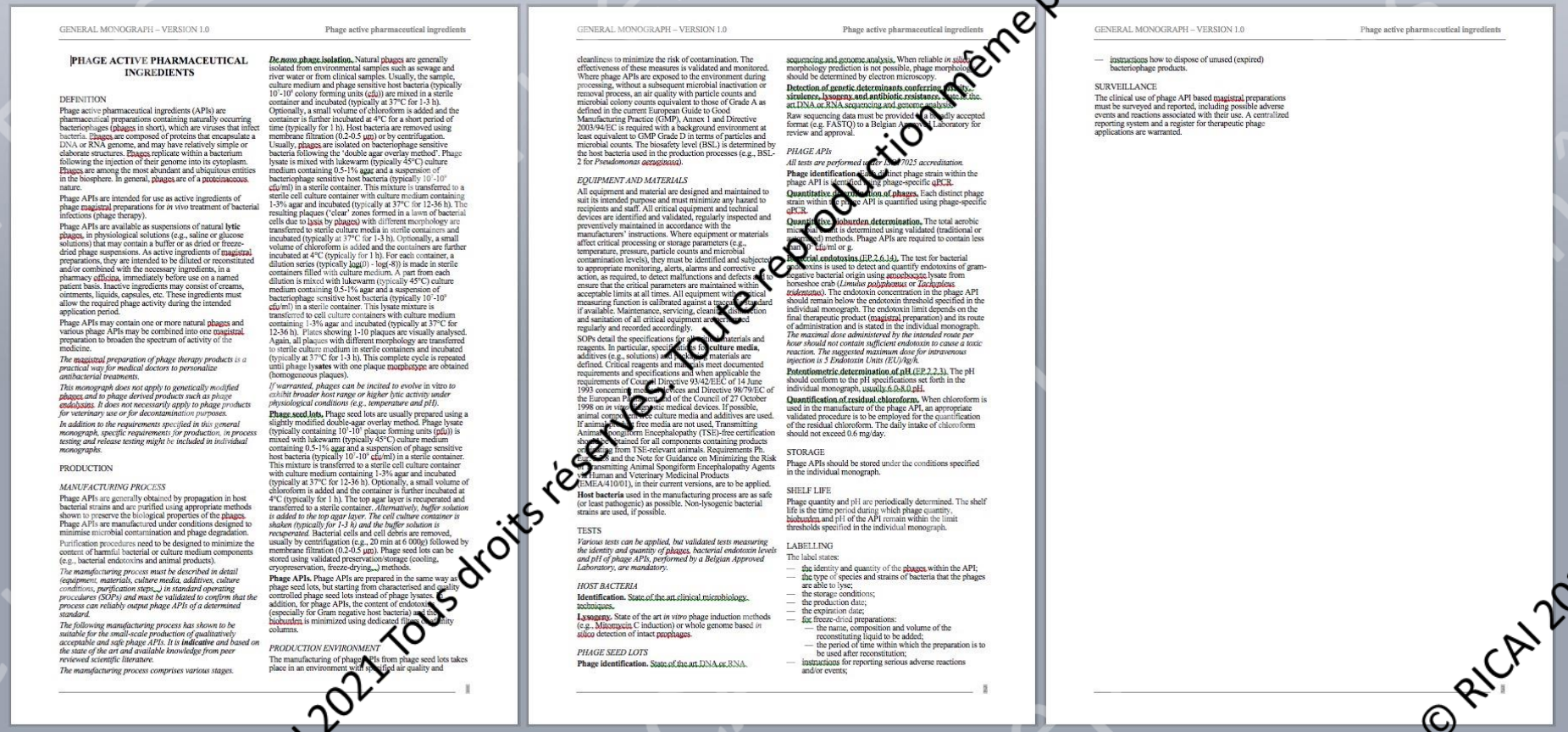
Patient



Pirnay *et al.* **The Magistral Phage**
Viruses. 2018;10(2). pii: E64

Phage Active Pharmaceutical Ingredient (API) Monograph

A “recipe”, which describes how phage APIs are produced and tested



GENERAL MONOGRAPH – VERSION 1.0 — Phage active pharmaceutical ingredients

PHAGE ACTIVE PHARMACEUTICAL INGREDIENTS

DEFINITION

Phage active pharmaceutical ingredients (APIs) are pharmaceutical preparations containing naturally occurring bacteriophages (phages in short), which are viruses that infect bacteria. Phages are composed of proteins that encapsulate a DNA or RNA genome, and may have relatively simple or elaborate structures. Phages replicate within a bacterium following the injection of their genome into its cytoplasm. Phages are among the most abundant and ubiquitous entities in the biosphere. In general, phages are of a proteinaceous nature.

Phage APIs are intended for use as active ingredients of phage magistral preparations for *in vivo* treatment of bacterial infections (phage therapy).

Phage APIs are available as suspensions of natural **lytic** phages, in physiological solutions (e.g., saline or glucose solutions) that may contain a buffer or as dried or freeze-dried phage suspensions. As active ingredients of magistral preparations, they are intended to be diluted or reconstituted and/or combined with the necessary ingredients, in a pharmacy officina, immediately before use on a named patient basis. Inactive ingredients may consist of creams, ointments, liquids, capsules, etc. These ingredients must allow the required phage activity during the intended application period.

Phage APIs may contain one or more natural phages and various phage APIs may be combined into one magistral preparation to broaden the spectrum of activity of the medicine.

The magistral preparation of phage therapy products is a practical way for medical doctors to personalize antibacterial treatments.

This monograph does not apply to genetically modified phages and to phage derived products such as phage endolysins. It does not necessarily apply to phage products for veterinary use or for decontamination purposes.

In addition to the requirements specified in this general monograph, specific requirements for production, in process testing and release testing might be included in individual monographs.

PRODUCTION

MANUFACTURING PROCESS

Phage APIs are generally obtained by propagation in host bacterial strains and are purified using appropriate methods shown to preserve the biological properties of the phages. Phage APIs are manufactured under conditions designed to minimise microbial contamination and phage degradation.

Purification procedures need to be designed to minimize the content of harmful bacterial or culture medium components (e.g., bacterial endotoxins and animal products).

The manufacturing process must be described in detail (equipment, materials, culture media, additives, culture conditions, purification steps,..) in standard operating procedures (SOPs) and must be validated to confirm that the process can reliably output phage APIs of a determined standard.

The following manufacturing process has shown to be suitable for the small-scale production of qualitatively acceptable and safe phage APIs. It is **indicative** *and based on the state of the art and available knowledge from peer reviewed scientific literature.*

The manufacturing process comprises various stages.

***De novo* phage isolation.** Natural phages are generally isolated from environmental samples such as sewage and river water or from clinical samples. Usually, the sample, culture medium and phage sensitive host bacteria (typically 10^7-10^9 colony forming units (cfu)) are mixed in a sterile container and incubated (typically at 37°C for 1-3 h). Optionally, a small volume of chloroform is added and the container is further incubated at 4°C for a short period of time (typically for 1 h). Host bacteria are removed using membrane filtration (0.2-0.5 µm) or by centrifugation. Usually, phages are isolated on bacteriophage sensitive bacteria following the ‘double agar overlay method’. Phage lysate is mixed with lukewarm (typically 45°C) culture medium containing 0.5-1% agar and a suspension of bacteriophage sensitive host bacteria (typically 10^7-10^9 cfu/ml) in a sterile container. This mixture is transferred to a sterile cell culture container with culture medium containing 1-3% agar and incubated (typically at 37°C for 12-36 h). The resulting plaques (‘clear’ zones formed in a lawn of bacterial cells due to lysis by phages) with different morphology are transferred to sterile culture media in sterile containers and incubated (typically at 37°C for 1-3 h). Optionally, a small volume of chloroform is added and the containers are further incubated at 4°C (typically for 1 h). For each container, a dilution series (typically $log(0) - log(-8)$) is made in sterile containers filled with culture medium. A part from each dilution is mixed with lukewarm (typically 45°C) culture medium containing 0.5-1% agar and a suspension of bacteriophage sensitive host bacteria (typically 10^7-10^9 cfu/ml) in a sterile container. This lysate mixture is transferred to cell culture containers with culture medium containing 1-3% agar and incubated (typically at 37°C for 12-36 h). Plates showing 1-10 plaques are visually analysed. Again, all plaques with different morphology are transferred to sterile culture medium in sterile containers and incubated (typically at 37°C for 1-3 h). This complete cycle is repeated until phage lysates with one plaque morphotype are obtained (homogeneous plaques).

If warranted, phages can be incited to evolve in vitro to exhibit broader host range or higher lytic activity under physiological conditions (e.g., temperature and pH).

Phage seed lots. Phage seed lots are usually prepared using a slightly modified double-agar overlay method. Phage lysate (typically containing 10^7-10^9 plaque forming units (pfu)) is mixed with lukewarm (typically 45°C) culture medium containing 0.5-1% agar and a suspension of phage sensitive host bacteria (typically 10^7-10^9 cfu/ml) in a sterile container. This mixture is transferred to a sterile cell culture container with culture medium containing 1-3% agar and incubated (typically at 37°C for 12-36 h). Optionally, a small volume of chloroform is added and the container is further incubated at 4°C (typically for 1 h). The top agar layer is recuperated and transferred to a sterile container. *Alternatively, buffer solution is added to the top agar layer. The cell culture container is shaken (typically for 1-3 h) and the buffer solution is recuperated.* Bacterial cells and cell debris are removed, usually by centrifugation (e.g., 20 min at 6 000*g*) followed by membrane filtration (0.2-0.5 µm). Phage seed lots can be stored using validated preservation/storage (cooling, cryopreservation, freeze-drying,..) methods.

Phage APIs. Phage APIs are prepared in the same way as phage seed lots, but starting from characterised and quality controlled phage seed lots instead of phage lysates. In addition, for phage APIs, the content of endotoxins (especially for Gram negative host bacteria) and the bioburden is minimized using dedicated filtration and purity columns.

PRODUCTION ENVIRONMENT

The manufacturing of phage APIs from phage seed lots takes place in an environment with a qualified air quality and cleanliness to minimize the risk of contamination. The effectiveness of these measures is validated and monitored. Where phage APIs are exposed to the environment during processing, without a subsequent microbial inactivation or removal process, an air quality with particle counts and microbial colony counts equivalent to those of Grade A as defined in the current European Guide to Good Manufacturing Practice (GMP), Annex 1 and Directive 2003/94/EC is required with a background environment at least equivalent to GMP Grade D in terms of particles and microbial counts. The biosafety level (BSL) is determined by the host bacteria used in the production processes (e.g., BSL-2 for *Pseudomonas aeruginosa*).

EQUIPMENT AND MATERIALS

All equipment and material are designed and maintained to suit its intended purpose and must minimize any hazard to recipients and staff. All critical equipment and technical devices are identified and validated, regularly inspected and preventively maintained in accordance with the manufacturers’ instructions. Where equipment or materials affect critical processing or storage parameters (e.g., temperature, pressure, particle counts and microbial contamination levels), they must be identified and subjected to appropriate monitoring, alerts, alarms and corrective action, as required, to detect malfunctions and defects and to ensure that the critical parameters are maintained within acceptable limits at all times. All equipment with a critical measuring function is calibrated against a traceable standard if available. Maintenance, servicing, cleaning, disinfection and sanitation of all critical equipment are performed regularly and recorded accordingly.

SOPs detail the specifications for all critical materials and reagents. In particular, specifications for **culture media**, additives (e.g., solutions) and gases for culture materials are defined. Critical reagents and materials meet documented requirements and specifications and when applicable the requirements of Council Directive 93/42/EEC of 14 June 1993 concerning medical devices and Directive 98/79/EC of the European Parliament and of the Council of 27 October 1998 on *in vitro* diagnostic medical devices. If possible, animal component free culture media and additives are used. If animal component free media are not used, Transmitting Animal Spongiform Encephalopathy (TSE)-free certification should be obtained for all components containing products or derivatives from TSE-relevant animals. Requirements Ph. Eur. 5.2.8 and the Note for Guidance on Minimizing the Risk of Transmitting Animal Spongiform Encephalopathy Agents via Human and Veterinary Medicinal Products (EMEA/410/01), in their current versions, are to be applied.

Host bacteria used in the manufacturing process are as safe (or least pathogenic) as possible. Non-lysogenic bacterial strains are used, if possible.

TESTS

Various tests can be applied, but validated tests measuring the identity and quantity of phages, bacterial endotoxin levels and pH of phage APIs, performed by a Belgian Approved Laboratory, are mandatory.

HOST BACTERIA

Identification. State of the art clinical microbiology techniques.

Lysogeny. State of the art *in vitro* phage induction methods (e.g., Mitomycin C induction) or whole genome based *in silico* detection of intact prophages.

PHAGE SEED LOTS

Phage identification. State of the art DNA or RNA sequencing and genome analysis. When reliable *in silico* morphology prediction is not possible, phage morphology should be determined by electron microscopy.

Detection of genetic determinants conferring to virulence, lysogeny and antibiotic resistance. State of the art DNA or RNA sequencing and genome analysis.

Raw sequencing data must be provided in a locally accepted format (e.g. FASTQ) to a Belgian Approved Laboratory for review and approval.

PHAGE APIs

All tests are performed under ISO 17025 accreditation.

Phage identification. Each distinct phage strain within the phage API is identified using phage-specific qPCR.

Quantitative detection of phages. Each distinct phage strain within the phage API is quantified using phage-specific qPCR.

Quantitative bioburden determination. The total aerobic microbial count is determined using validated (traditional or rapid) methods. Phage APIs are required to contain less than 100 cfu/ml or g.

Bacterial endotoxins (EP 2.6.14). The test for bacterial endotoxins is used to detect and quantify endotoxins of gram-negative bacterial origin using amoebocyte lysate from horseshoe crab (*Limulus polyphemus* or *Tachypleus tridentatus*). The endotoxin concentration in the phage API should remain below the endotoxin threshold specified in the individual monograph. The endotoxin limit depends on the final therapeutic product (magistral preparation) and its route of administration and is stated in the individual monograph. *The maximal dose administered by the intended route per hour should not contain sufficient endotoxin to cause a toxic reaction. The suggested maximum dose for intravenous injection is 5 Endotoxin Units (EU)/kg/h.*

Potentiometric determination of pH (EP 2.2.3). The pH should conform to the pH specifications set forth in the individual monograph, usually 6.0-8.0 pH.

Quantification of residual chloroform. When chloroform is used in the manufacture of the phage API, an appropriate validated procedure is to be employed for the quantification of the residual chloroform. The daily intake of chloroform should not exceed 0.6 mg/day.

STORAGE

Phage APIs should be stored under the conditions specified in the individual monograph.

SHELF LIFE

Phage quantity and pH are periodically determined. The shelf life is the time period during which phage quantity, bioburden and pH of the API remain within the limit thresholds specified in the individual monograph.

LABELLING

The label states:

- the identity and quantity of the phages within the API;
- the type of species and strains of bacteria that the phages are able to lyse;
- the storage conditions;
- the production date;
- the expiration date;
- for freeze-dried preparations:
  - the name, composition and volume of the reconstituting liquid to be added;
  - the period of time within which the preparation is to be used after reconstitution;
- instructions for reporting serious adverse reactions and/or events;
- instructions how to dispose of unused (expired) bacteriophage products.

SURVEILLANCE

The clinical use of phage API based magistral preparations must be surveyed and reported, including possible adverse events and reactions associated with their use. A centralized reporting system and a register for therapeutic phage applications are warranted.

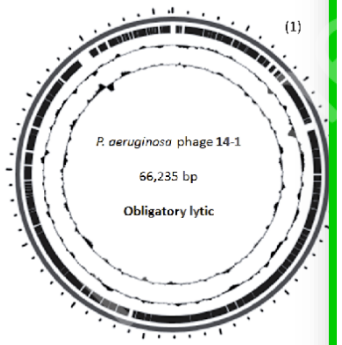
sciensano

Report date 15/09/2018

Genetic analysis of Seed lot phage - Report

Pseudomonas phage 14-1

Date of submission	07/07/2018
Submitting client	MHQA
Natural isolate	YES
Sequence provided	YES



CONCLUSION

Based on analysis of its genome and according to criteria set in the general monograph v1.0 for the production of active pharmaceutical ingredients, Pseudomonas phage 14-1 can be used as Seed Lot. The phage is obligatory lytic, and lacks transducing capacity due its non-redundant genome ends. No potential malignant proteins are predicted. The phage is been reported to be long-term stable during storage at 4°C. No intact prophages were predicted in the propagation host, which was only partially sequenced. A specific assay for absolute quantification by digital PCR has been validated targeting genome region 21,225-21,329.

Phage Identification and Classification

Full Name (proposed)	vB_PaeM_14-1
Genus	*Pbunavirus*
Family	Myoviridae
Order	Caudovirales
Predicted lytic properties	Obligatory lytic

NGS[2]

Sequence run details :

Genome extraction	UNK
Library construction kit	TruSeq paired-end
Sequencing platform	Illumina MiSeq
Read length	2X300
Facility	IBIS, Laval University (CAN)

NA, not applicable. UNK, unknown

Sample details:

BioSample	
Procedure	
Total Read Numbe	
% phage reads	
Median phage cov	

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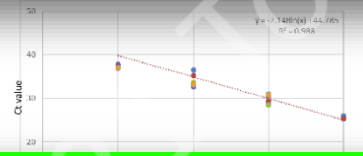
Report date 15/09/2018

Genetic analysis of Seed lot phage - Report

Key phage properties[3]

Nucleid acid type	Double-stranded DNA
Genome size	66,238 bp
Open reading frames	90
%GC	55.6%
Reported genome ends[4]	Non-permuted
Transducing capacity	Limited to none[5]
Predicted coding potential	
Lysogeny related genes	Absent
Genes related to antibiotic resistance	Absent
Insertion Sequences / Transposons	Absent
Genes related to virulence	Absent
Endonucleases	Absent
Additional features inferred from literature[4]	
Receptors	LPS
Reported *P. aeruginosa* host range (%)	UNK



qPCR details:

Procedure	SOP 12/AB/01/E
Targeted ORF	031
Chemistry	Taqman
RT-PCR device	Quantstudio 5 (Thermo Scientific)
R^2	0.988

Bacterial host properties[3]

Propagation host	*Pseudomonas aeruginosa* CN573
Prophages predicted in propagation host	YES

Name	Region length (kb)	GC (%)	Genes	INTACT	Induced
YMC11/02/R656	18.7	64.2	22	NO	NA
phi2	4.7	63.4	8	NO	NA

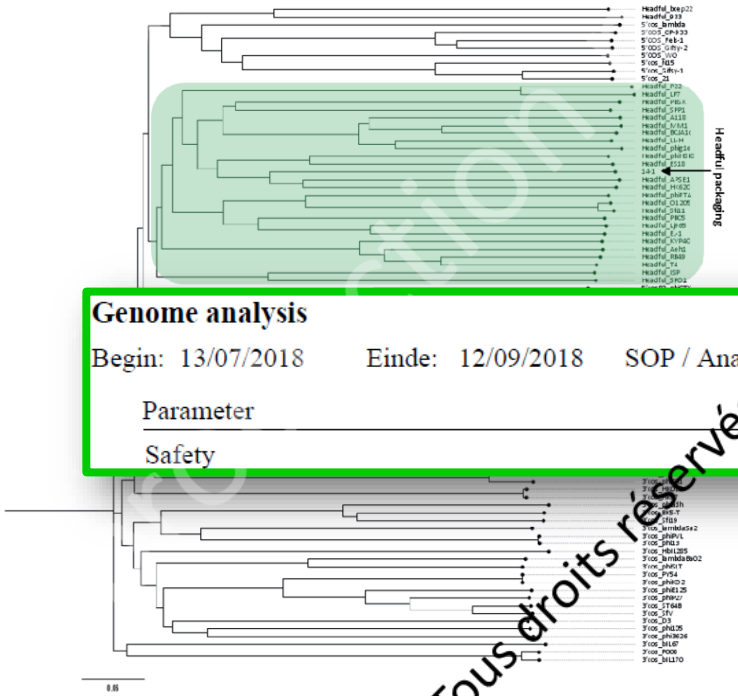
[1] The 14-1 genome is circularized for visualization. From outwards to inwards, the predicted open reading frames, the GC plot and GC skew are shown.

[2] Based on publicly available raw NGS data. No raw data found for phage 14-1.

[3] The following algorithms were used. PHASTER for prophage detection. PROKKA (Galaxy Version 1.11) for gene prediction, web versions of HHPRED and BlastP for prediction of gene function, RESFINDER v3.0 and VIRULENCEFINDER v1.5 for prediction of genes involved in antibiotic resistance and virulence. Phage receptors are inferred from literature and the Phage Receptor Database (https://phred.herokuapp.com). Insertion sequences and Transposon are inferred at https://www-is.biotoul.fr/index.php.

[4] Accessed literature: Ceyssens et al., Environ Microbiol. 2009 Nov;11(11):2874-83. Jarrell, K., and Kropinski, A.M. (1977) J Virol 23: 461–466

[5] Phylogenetic analyses of the large subunit of the phage terminases, indicative for genome end structure within the Caudovirales. The 14-1 sequence is indicated with an arrow. Intriguingly, the phage protein clusters within the headful packaging terminases, despite the experimentally verified non-redundant genome ends[4]. Likely, the stochastic composition of the phage capsid allows only very minor variations of DNA content, strongly reducing the transducing capacity of the phage.



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Dr Jean-Paul Pirnay
Hôpital Militaire Reine Astrid - Laboratoire
Lab MKT
Bravenstraat 1
1820 Neder-over-Heembeek

Analyseverslag S18BD04735

Rapportdatum: 13/09/2018

Version 01 (Nederlands)

In geval van vragen over dit rapport gelieve te contacteren:
Pieter-Jan Ceyssens (email: pieterjan.ceyssens@sciensano.be - tel:)
Wesley Mattheus (email: wesley.mattheus@sciensano.be - tel: +32 (0)2/373 32 24)

Gegevens over het staal

Sciensano staalref.:	S18BD04735	Aard van het staal:	Phage
Klant staalref.:	14	Staat van het staal:	Goede staat
		Ontvangstdatum:	03/07/2018

Labo: Bacteriophage QC

Genome analysis
Begin: 13/07/2018 Einde: 12/09/2018 SOP / Analysemethode: SOP 12/AB/02/E

Parameter	Resultaat
Safety	Approved [1]

Bijlage 1: Phage Genome Rapport.pdf

Genome analysis
Begin: 13/07/2018 Einde: 12/09/2018 SOP / Analysemethode: SOP 12/AB/02/E

Parameter	Resultaat
Safety	Approved [1]

[1] Aangeduide resultaten worden niet gedekt door het MJA attest.

[2] Aangeduide resultaten zijn gewijzigd ten opzichte van de vorige versie van het analyseverslag. Aangeduide resultaten vervangen de eerder gerapporteerde resultaten.

[3] Resultaat bekomen via onderaanneming. De code van het onderaannemend laboratorium is op het rapport boven de resultaten getoond. De volledige identificatie van het laboratorium is op aanvraag beschikbaar bij Sciensano.

APPROVED

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Dr Jean-Paul Pirnay
Hôpital Militaire Reine Astrid - Laboratoire

Analysecverslag S18BD02140 Rapportdatum: 11/04/2018

Version 01 (Nederlands)

Endotoxine

Begin: 06/04/2018 Einde: 06/04/2018 SOP / Analysemethode: SOP 42/III-25

Parameter	Resultaat	Eenheid
Endotoxine content	< 0.5 [1,3]	EU/dose

Labo: Bact. & Vir. Food Pathogens

Telling van het totaal kiemgetal bij 30°C

Begin: 05/04/2018 Einde: 09/04/2018 SOP / Analysemethode: SOP 11/VM/01/N Technische norm: ISO 4833

Parameter	Resultaat	Eenheid
totaal kiemgetal *< 1cfu/ml*	0,00 [1,3]	CFU/ml

Labo: Medicines

Bepaling van de pH

Begin: 10/04/2018 Einde: 10/04/2018 SOP / Analysemethode: SOP 21/126/N

#	Parameter	Resultaat
1	pH	7,330 [1,3]
2	pH	7,360 [1,3]
3	pH	7,370 [1,3]
1	Mean pH	7,35 [1,3]

levensmiddelen de limiet is de bovengrenswaarde ("M") zoals weergegeven in de "Europese Verordening (EG) 2073/2005 inzake microbiologische criteria voor levensmiddelen" en/of de "Actiegrenzen voor microbiologische contaminanten" van het FAVV. De in dit rapport uitgedrukte opinies/interpretaties vallen buiten de scope van het MJA attest van dit OMCL.

[1] Aangeduide resultaten worden niet gedekt door het MJA attest.

[2] Aangeduide resultaten zijn gewijzigd ten opzichte van de vorige versie van het analyseverslag. Aangeduide resultaten vervangen de eerder gerapporteerde resultaten.

[3] Resultaat bekomen via onderaanneming. De code van het onderaannemend laboratorium is op het rapport boven de resultaten getoond. De volledige identificatie van het laboratorium is op aanvraag beschikbaar bij Sciensano.

APPROVED

NOT GMP certified
NO central MA

Cleanrooms

DEFENSIE
LA DÉFENSE

VOORRANG VOOR VREDE
PRIORITÉ À LA PAIX

Magistral phage preparations

Phage monograph in Ph. Eur

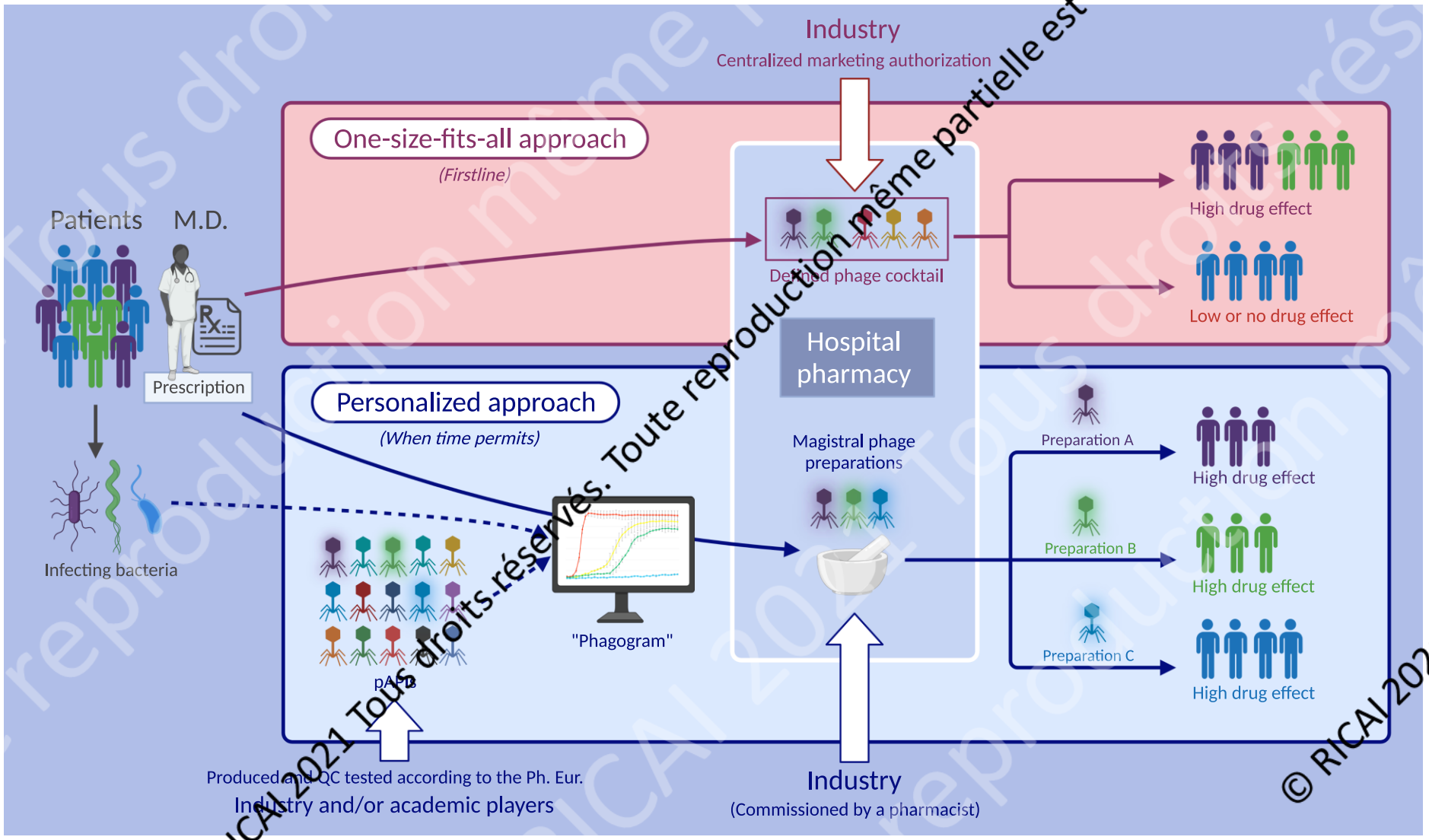
ADDITIONS TO THE WORK PROGRAMME (new texts to be elaborated)

2.6.41.	High throughput sequencing for the detection of extraneous agents	N/A
5.1.13.	Pyrogenicity	Pyrogénicité
5.31.	Phage therapy active substances and medicinal products for human and veterinary use	Substances actives et médicaments à usage humain et vétérinaire utilisés en phagothérapie

Added to the 2021 work programme for the European Pharmacopoeia

Verbeken and Pirnay. Curr. Opin. Virol. 2021 Nov 18;52:24-29.

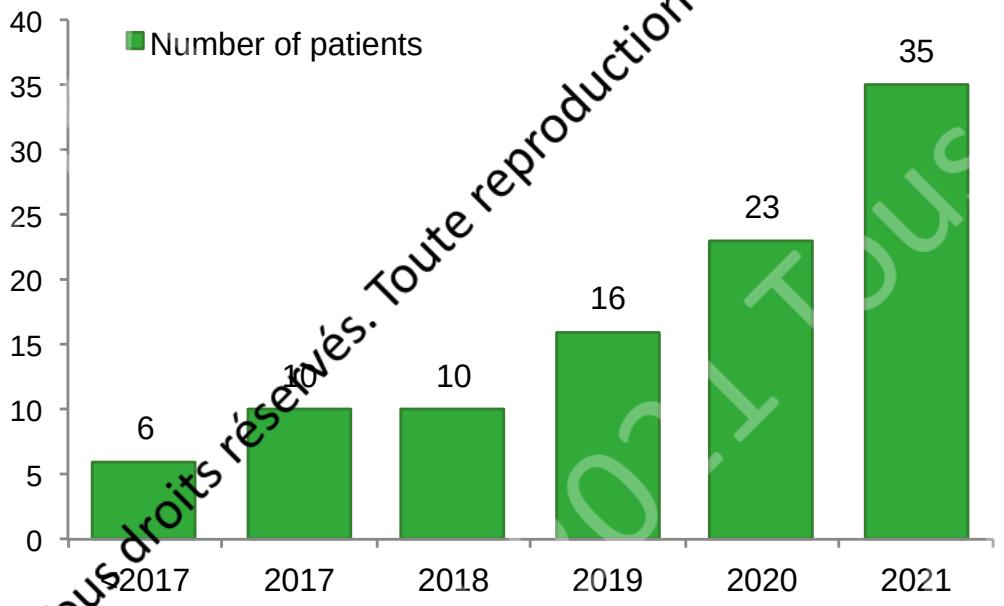
"Personalized" next to "broad spectrum"



Verbeken and Pirnay. Curr. Opin. Virol. 2021 Nov 18;52:24-29.

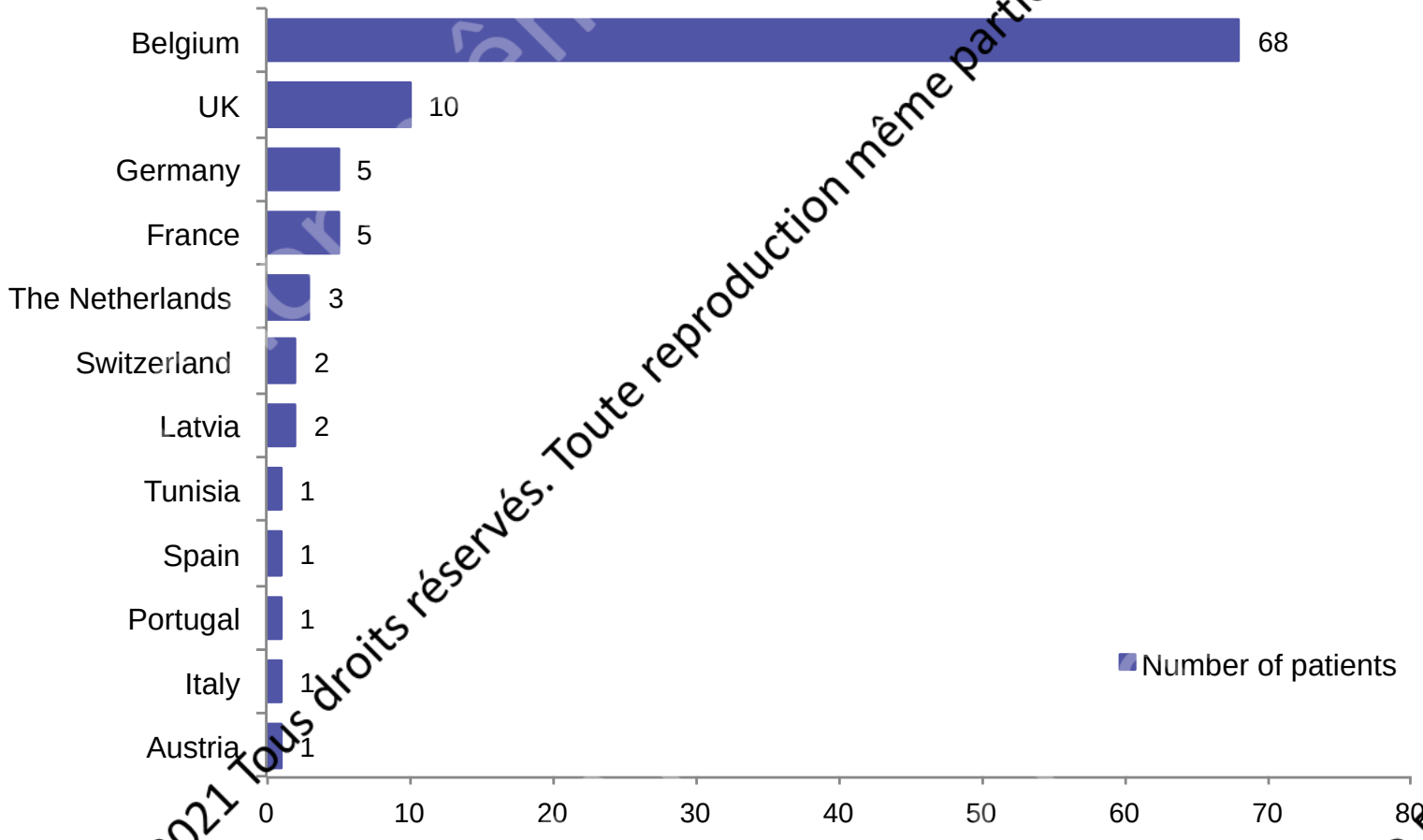
Increasing number of cases

pt@mil.be (Sarah Djebara and Patrick Soentjens)



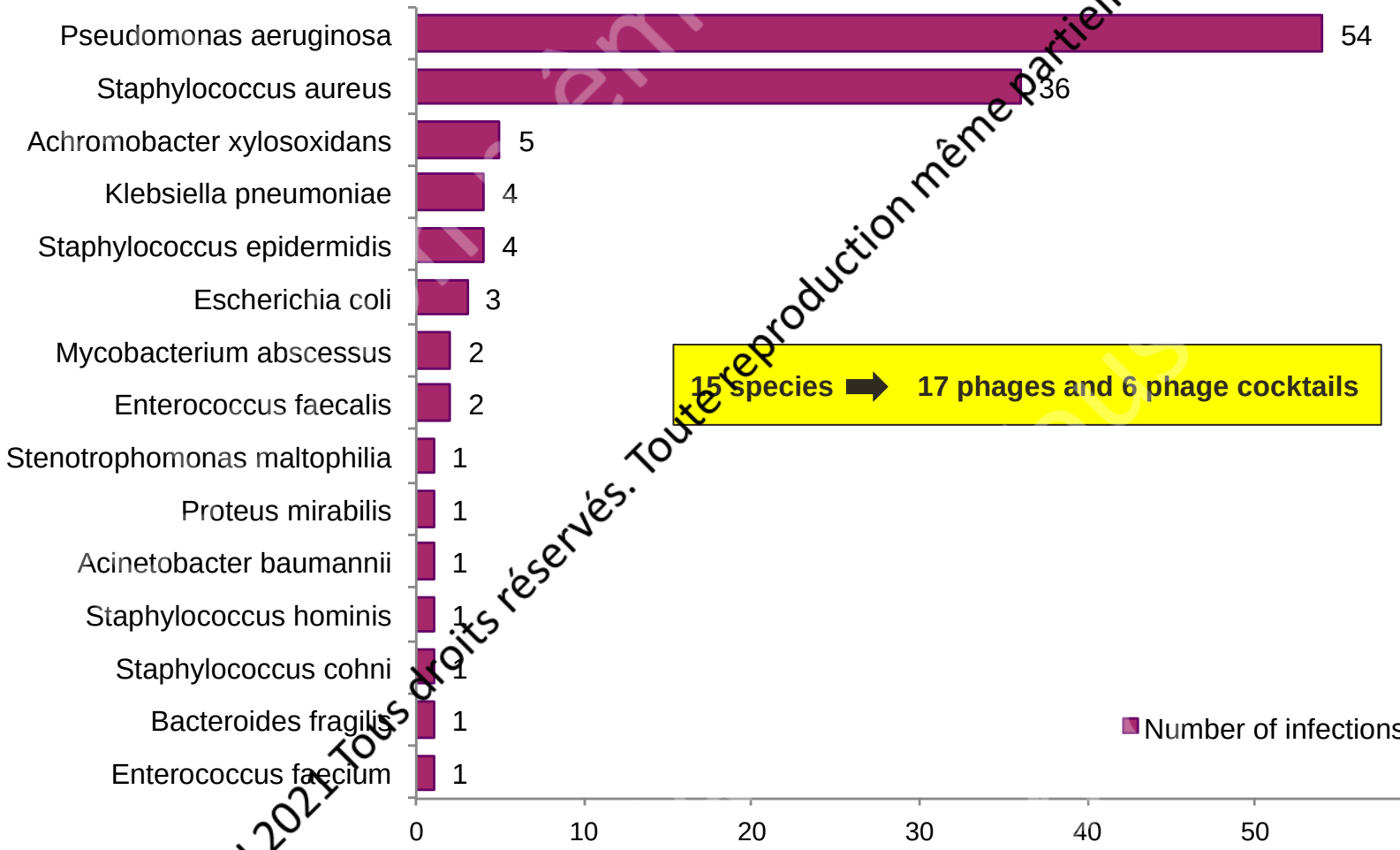
100

patients

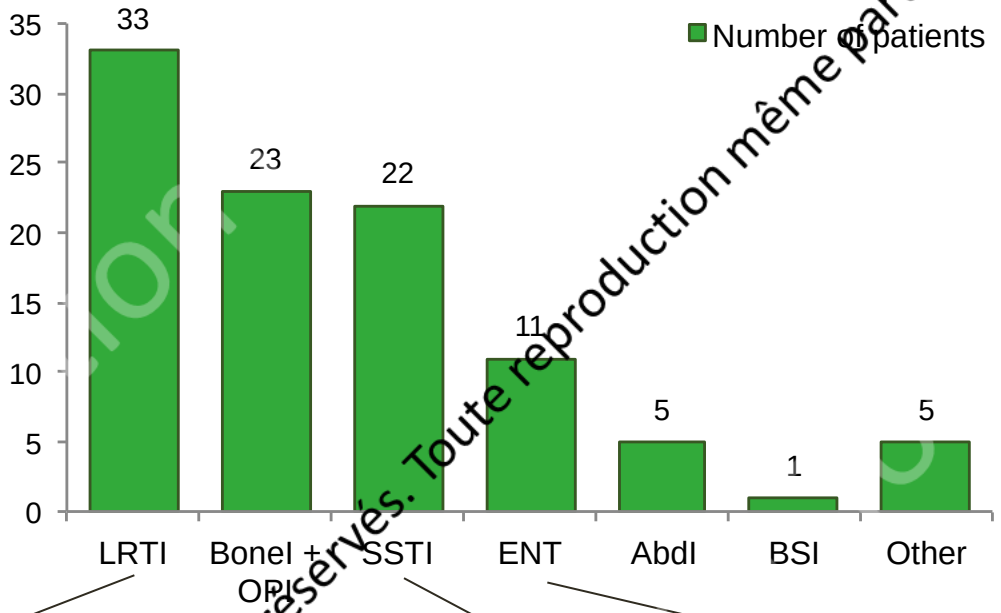


12 countries - 28 cities - 34 hospitals

Targeted bacterial species



Infection types



Nebulisation
2-4 ml
10^7 – 10^8 pfu/lm
q6h
5 days – 6 weeks

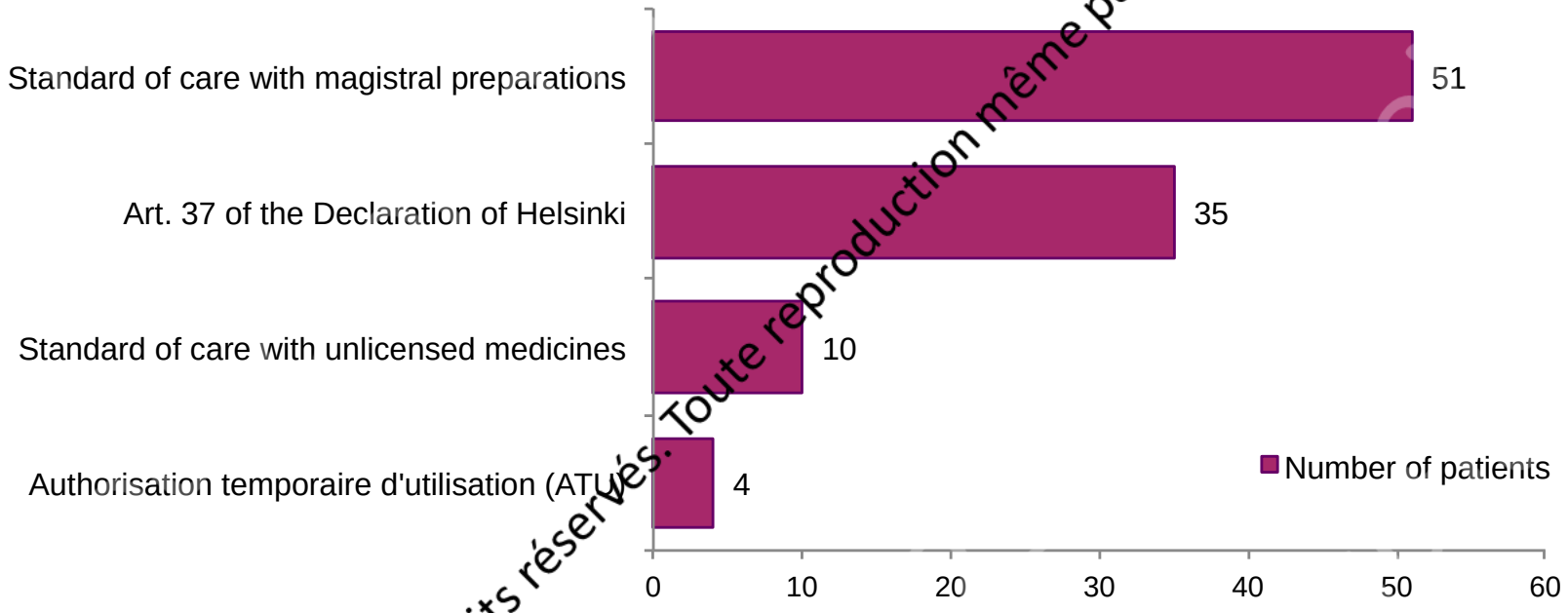
Intralesional
2-5 ml
10^7 – 10^8 pfu/lm
q24h
5 days – 3 weeks

Topical
In excess
10^7 – 10^9 pfu/lm
q24h
5 days – 3 weeks

Nasal spray
1 – 15 ml
10^7 pfu/lm
q8h
1 – 3 weeks

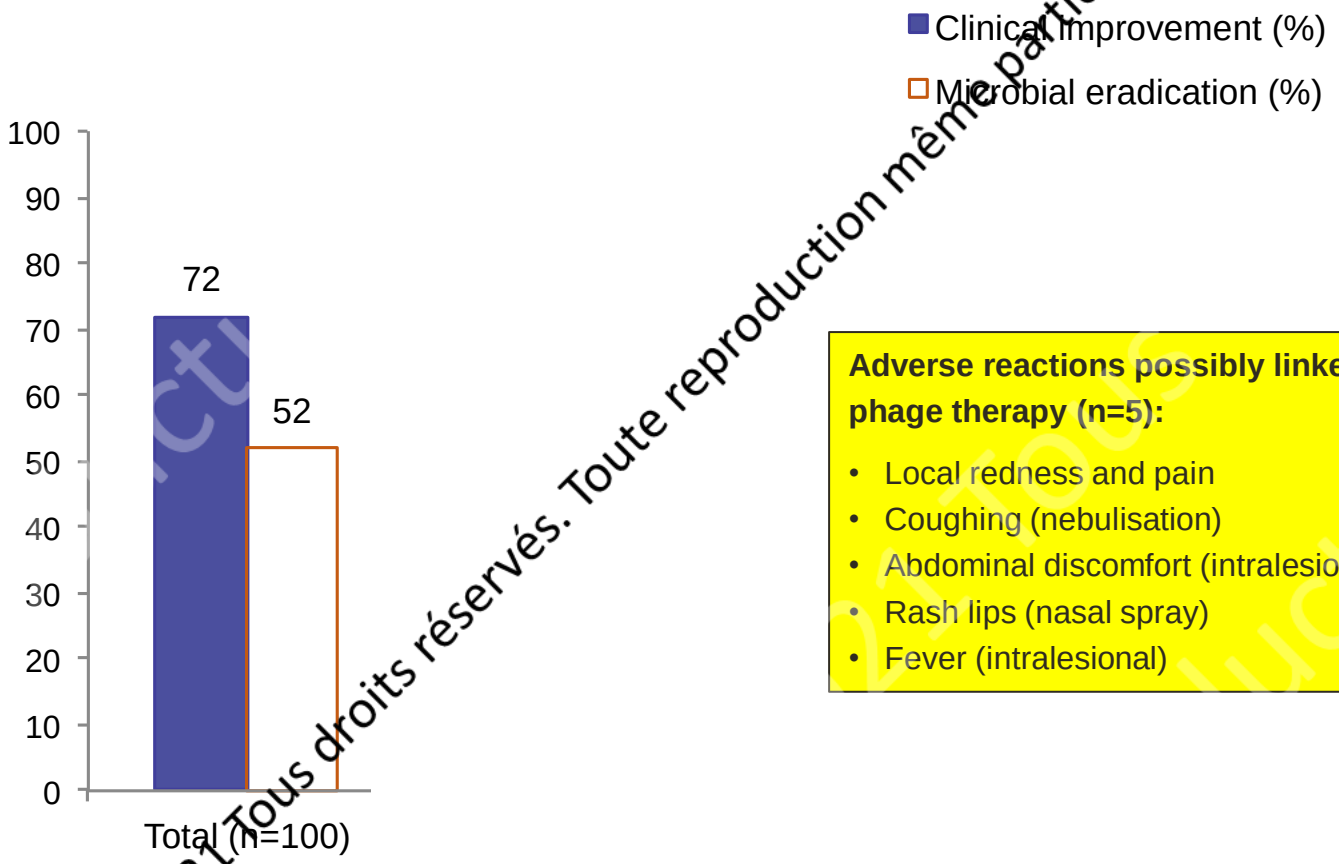
Intravenous (n=18)
50 – 100 ml (6h infusion)
10^6 – 10^7 pfu/ml
q24h
5-10 days

Regulatory context



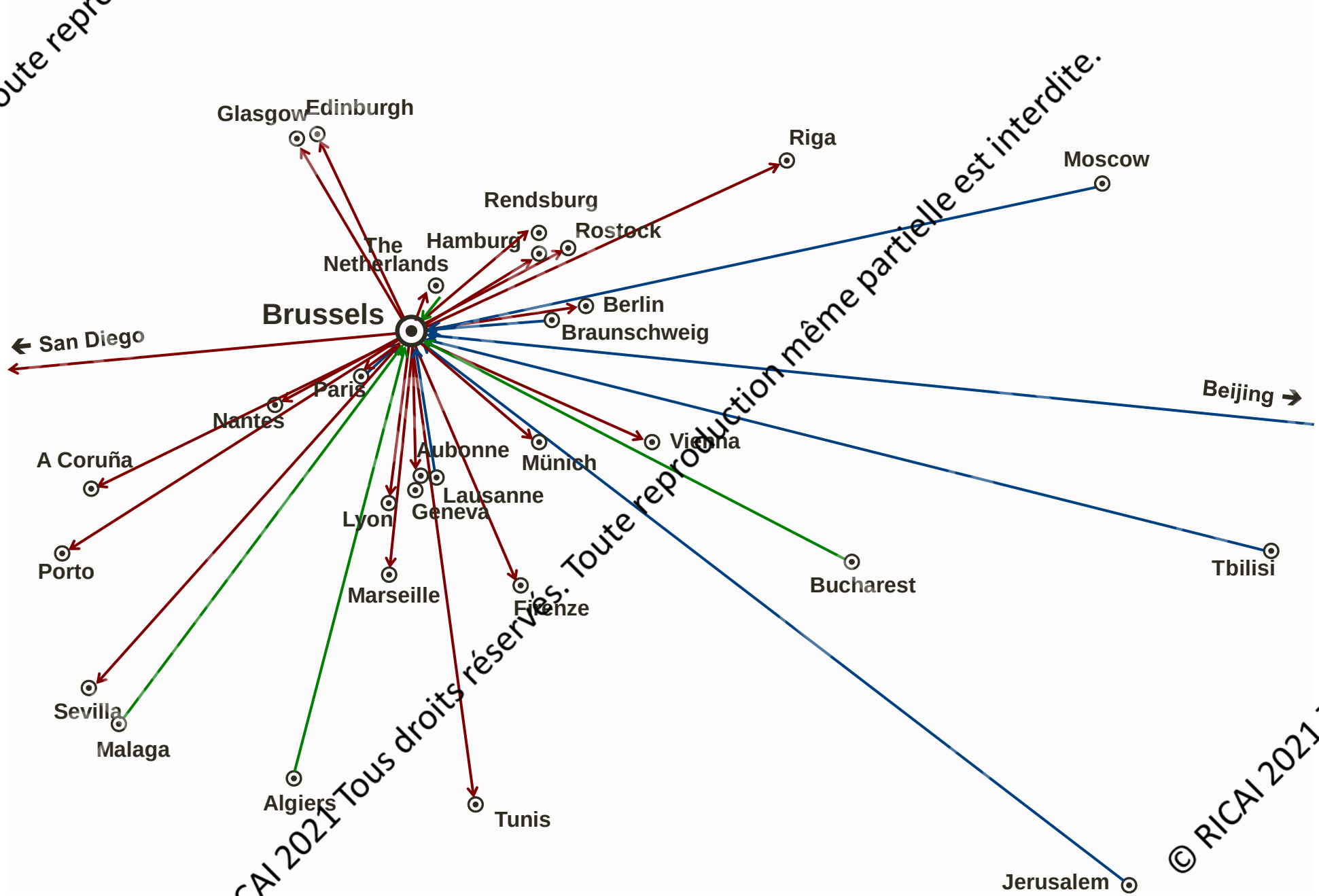
Hospitalised: 78 patients
Ambulatory: 22 patients

Safety and efficacy at a glance



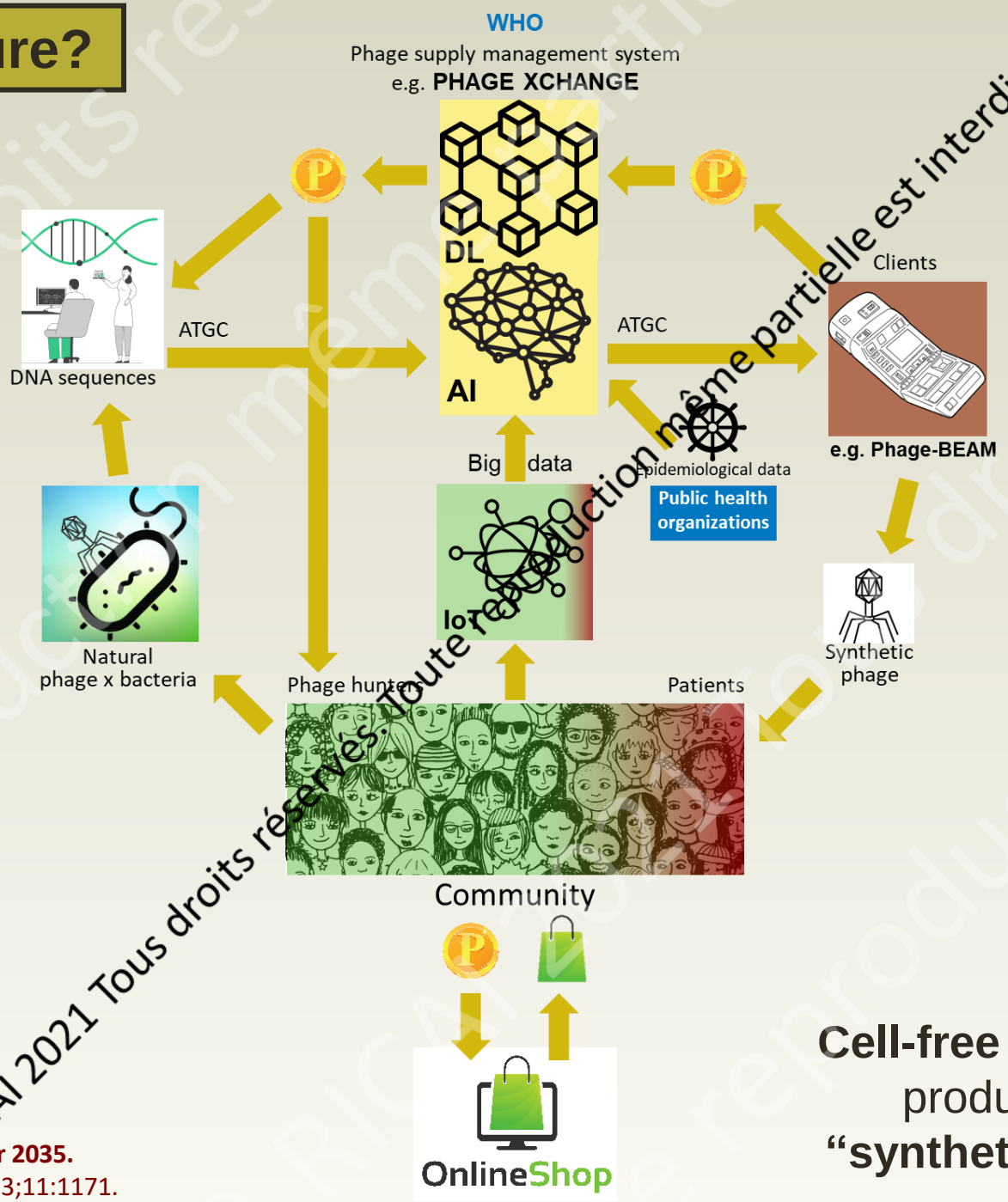
Adverse reactions possibly linked to phage therapy (n=5):

- Local redness and pain
- Coughing (nebulisation)
- Abdominal discomfort (intralesional)
- Rash lips (nasal spray)
- Fever (intralesional)



Transfer of phages or bacteria to Brussels (**blue arrows**), and from Brussels to other cities (**red arrows**). Transfer of patients to Brussels (**green arrows**)

The Phuture?



Pirnay JP.
Phage Therapy in the Year 2035.
Front Microbiol. 2020 Jun 3;11:1171.

Cell-free and **on-site** production of **"synthetic phages"**

Colleagues

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Tea Glonti
Johann Griselain
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Bruno Pascual
Thomas Rose
Patrick Soentjens
Griet Steurs
Lilo Tranchina
Gilbert Verbeken

Thank you!