

**Federal agency for medicines and health
products**

**Workshop on the therapeutic use of
bacteriophages**

Current issues on quality of phage products

Alan Fauconnier

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Disclaimer

- Alan Fauconnier is Quality assessor at the Federal Agency for Medicines and Health Products (FAMHP), the Belgian Regulatory Authority competent for Medicinal Products.
- Alan Fauconnier is also delegate at the Biologics Working Party (BWP) of the CHMP (EMA/London).
- However, this presentation represents a personal view and may not necessarily reflect the view of the FAMHP, the BWP, the CHMP, the EMA, the EDQM and/or other regulatory bodies.



Medicinal product (MP): definition

According to Directive 2001/83/EC on the Community code relating to medicinal products for human use as amended

- *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 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.*



Directive 2001 / 83 / EC

Article 2

This Directive shall apply to medicinal products for human use intended to be placed on the market in Member States and either prepared industrially or manufactured by a method involving an industrial process.

(≈ proprietary medicinal products)

Article 6

No medicinal product may be placed on the market of a Member State unless a marketing authorisation has been issued by the competent authorities...



Directive 2001 / 83 / EC

Article 8

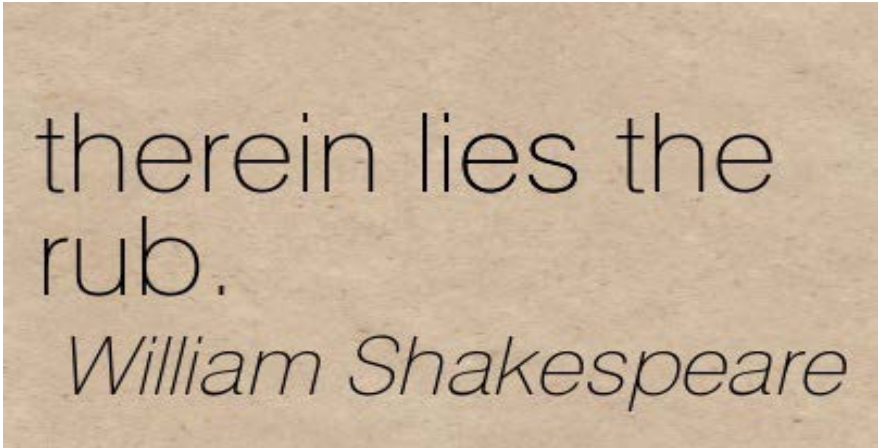
The application (for a marketing authorisation) shall be accompanied by the following particulars and documents...

(a) Name of the applicant

(b) Name of the medicinal product

(c) Qualitative and quantitative particulars of all the constituents of the medicinal product,

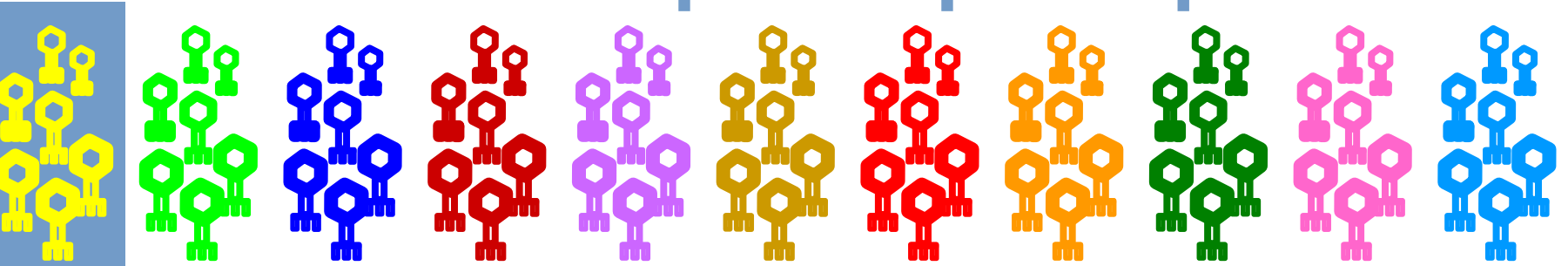
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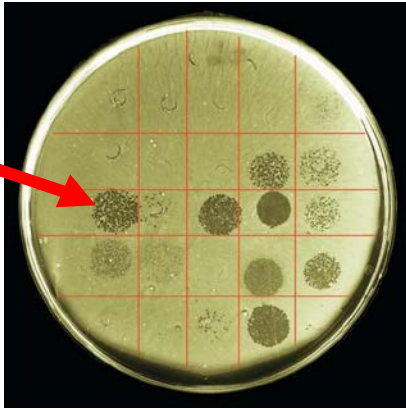
therein lies the
rub.
William Shakespeare



Anticipated principle



Collection of phages: each phage is tested on the bacteria isolated from the patient



On the basis of the « phagogram », a cocktail of phages will be formulated in a medicinal product for treating an infection with the corresponding bacterial isolate.

meaning that:

- cocktails customized for patient
- adapted over time if the phage resistance profile of bacteria is evolving.
- new phages could be added to the collection

NO PREDETERMINED COMPOSITION



Quality issues of phage products

- Regulatory quality requirements

SmPC section 2 Qualitative and Quantitative Composition

CTD Module 3

3.2.P.1 Description and Composition of the Drug Product

- Scientific quality points for consideration



Directive 2001 / 83 / EC

How to reconcile the moving target composition with the regulatory requirement on quality, i.e. providing a predetermined “qualitative and quantitative particulars” for proprietary medicinal products?



Regulatory quality requirements

Inspiration from the veterinary regulation 1 :

Autogenous vaccines

excluded from the scope of – but defined by - Directive 2001/82/EC on the Community code relating to veterinary medicinal products :

“... immunological veterinary medicinal products which are manufactured from pathogens and antigens obtained from an animal or animals from a holding and used for the treatment of that animal or the animals of that holding in the same locality”



Regulatory quality requirements

Autogenous vaccines

Licensure requirements (if any) within the remit of MS

Well developed French regulation

- on veterinary prescription
- manufacturers must be authorised (QP)
- GMP for autogenous vaccines
- labelling must include a.o. :
 - denomination of the pathogen
 - qualitative composition



Regulatory quality requirements

Inspiration from the veterinary regulation 2 :

Multi-strain dossier

*Vaccines against avian influenza, Bluetongue and Foot-and-Mouth disease represent a special case in terms of the **need for rapid and frequent change in the strains included and therefore do not fit well within the general regulatory model for vaccines***



Regulatory quality requirements

Inspiration from the veterinary regulation 2 :

Multi-strain dossier

- **II.A. Qualitative and quantitative particulars**

*The applicant has to define the **maximum number of antigens** that can be included in the vaccine and specify **the quantity for each antigen**. If a fixed amount of antigen is not targeted during the formulation process, minimum and maximum quantities for each antigen should be specified.*



Regulatory quality requirements

Conclusion

Regulatory examples of

- approval of a generic composition stating a maximum number of active ingredients.
- release of a customized labeling which specifies the qualitative and quantitative composition of a given lot.

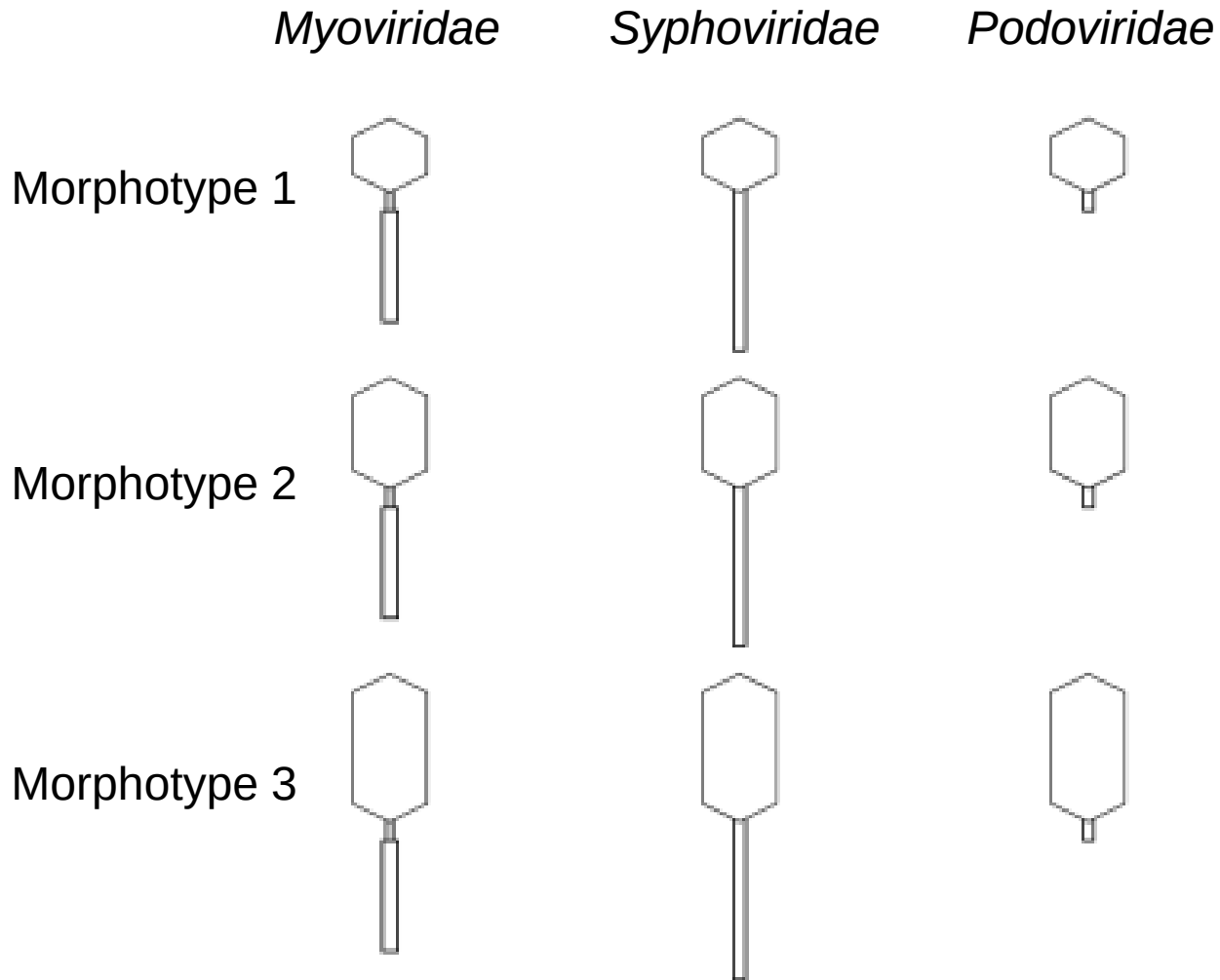


Scientific quality points for consideration

Setting the scene

Order	Family	Genome	Host
Caudovirales 96% of all characterised phages	<i>Myoviridae</i>	dsDNA	Bacteria
	<i>Siphoviridae</i>	dsDNA	Bacteria
	<i>Podoviridae</i>	dsDNA	Bacteria
Unassigned	<i>Corticoviridae</i>	dsDNA	Bacteria
Unassigned	<i>Cystoviridae</i>	dsRNA	Bacteria
Unassigned	<i>Inoviridae</i>	ssDNA	Bacteria
Unassigned	<i>Leviviridae</i>	ssRNA(+)	Bacteria
Unassigned	<i>Microviridae</i>	ssDNA	Bacteria
Unassigned	<i>Plasmaviridae</i>	dsDNA	Bacteria
Unassigned	<i>Tectiviridae</i>	dsDNA	Bacteria & Archaea
Unassigned	8 to 9 additional families	dsDNA and ssDNA	Archaea

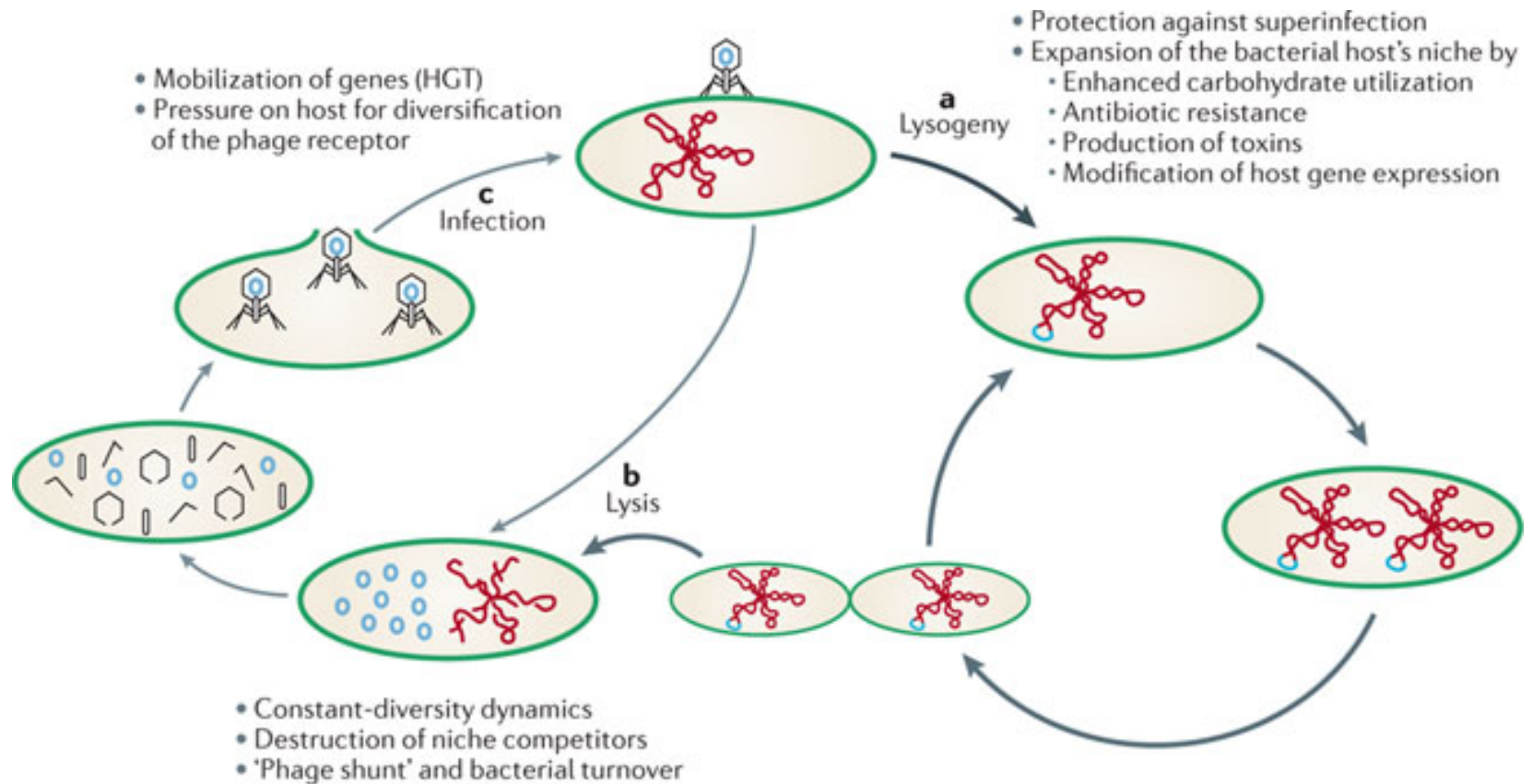
Scientific quality points for consideration



Adapted from Ackermann, Arch. Virol; 2001, 146: 843-857

Scientific quality points for consideration

Lytic versus lysogenic pathways



Nature Reviews | Microbiology

From Reyes *et al*, Nat. Rev. Micro. 2012; 10: 607-617

Scientific quality points for consideration

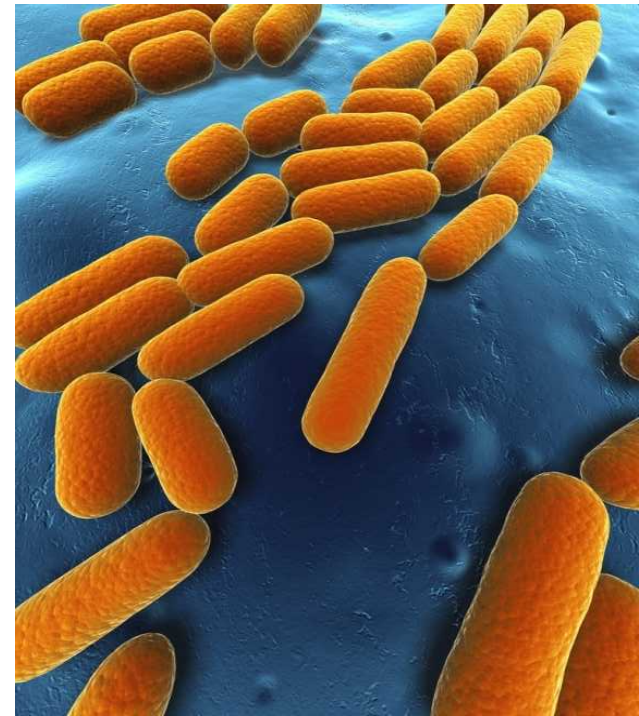
No specific guidelines but...

- *ICH Q5D Derivation and characterization of cell substrates used for production of biotechnological/biological products*
 - *Cell banking system*
- *FDA: Characterization and Qualification of Cell Substrates and Other Biological Materials Used in the Production of Viral Vaccines for Infectious Disease Indications*
 - *Virus seeds*



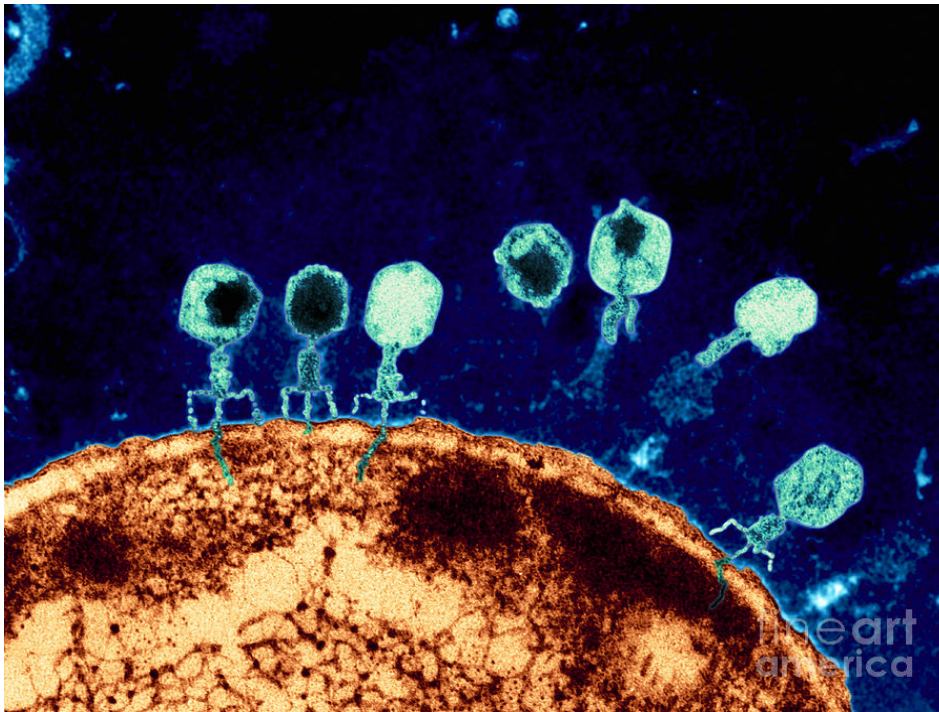
Cell banking systems

- Multiple cell substrates
- Classical two-tiered versus one-tiered
- Identity (discrimination between putatively closely related strains)
- Absence of prophages (e.g. by mitomycin C induction)
- Absence of antibiotic resistance
- Absence of virulence factors



Viral seeds

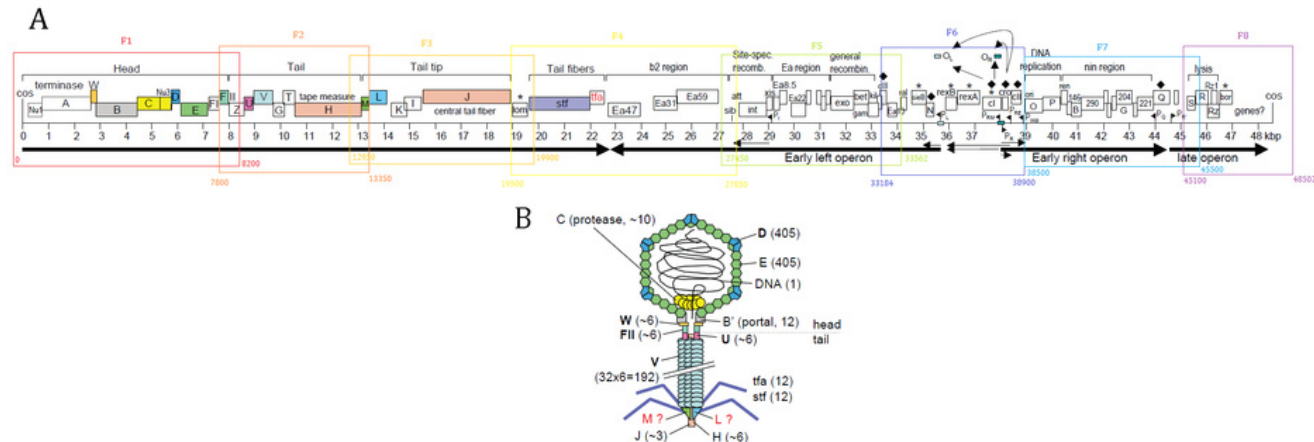
- Multiple seeds (dozens if not hundreds)
- Growing collection
- Restrict to Caudovirales ?
- Classical two-tiered versus one-tiered



Characterization

Genotypic, i.e. genome sequencing

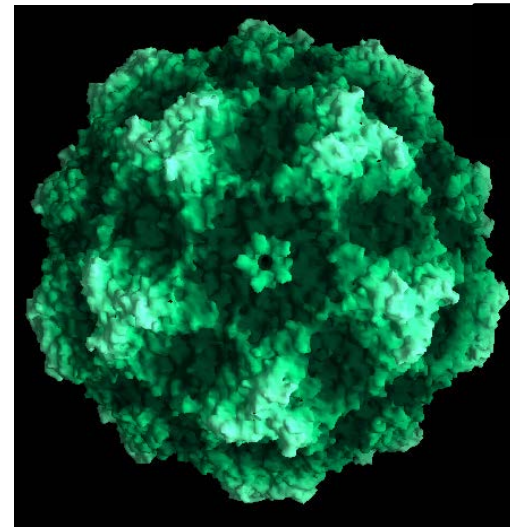
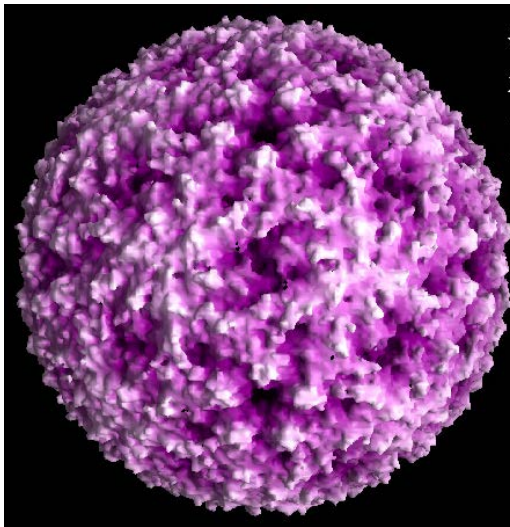
- Identity
- Absence of virulence factors/bacterial toxins
- Absence of genes necessary for temperate lifestyle (e.g. integrase gene, Sie systems, lysogeny mechanisms...)
- Genetic stability: « end of production » phages



Characterization

Genotype versus phenotype

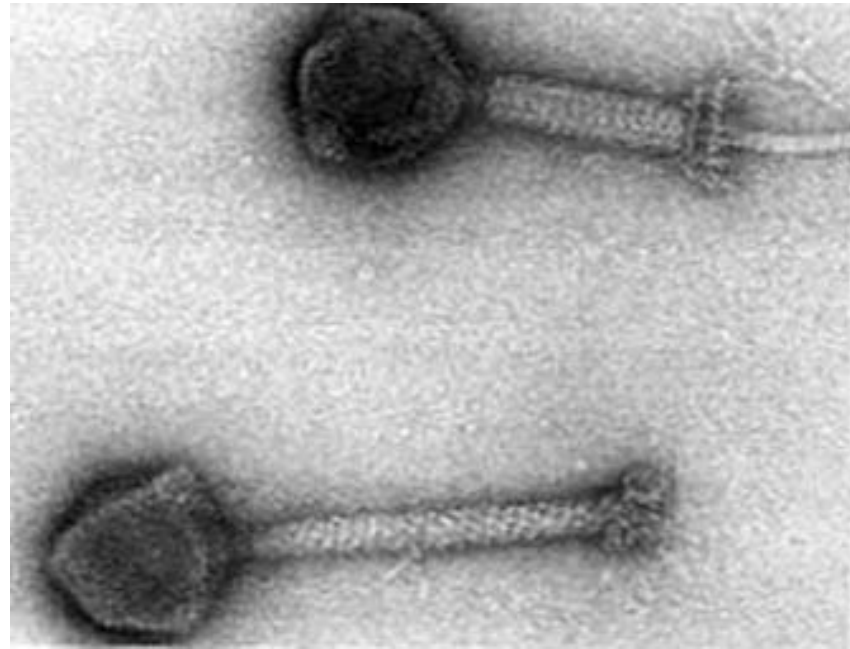
- Foot-and-mouth disease virus : same disease although significant genome variability
- Parvoviruses of cats and dogs, only minor differences in sequences but major differences in biologic properties



Characterization

Phenotypic

- Specificity: bacteriophages should specifically infect the bacterial isolate (or limited number of closely related strains).
- Lytic only (no temperate phages). Lytic growth curve.
- TEM: morphology.



Impurities

- Host Cell Proteins
- Host Cell DNA
- Residual reagents (e.g. Benzonase, solvents)
- Endotoxin (depending of the route of administration)
- Pyrogenic exotoxins (rabbit pyrogen test)
- Hemolysins
- ...



Control of Drug Substance

DS specification

Potency:

- plaque count (PFU determination) (routine)
- Enumeration by negative staining TEM (orthogonal)
- Sensitivity of the bacterial substrate for titration: efficacy of plating. Not necessarily the same as the cell production substrate.

Purity

- Absence of adventitious phages/plasmids by PCR
- Bioburden/Sterility



Description and composition of the DP

VT multi-strain application model ?

Flexibility regarding the addition and exchange of phage strains in the Drug Product...

...in order to adapt the product to the diversity of bacterial strains occurring as disease-causing pathogens



Control of Drug Product

Ideally, identification and quantification of each individual phage in the Drug Product (e.g. phage titration, qPCR), however, could prove to be challenging

Flexible approach could be considered (formulation based release ?)

Endotoxin/pyrogens (if applicable)

Sterility



Stability of the Drug Product

Shelf life is not expected to be long for **custom-made anti-bacterial** medicinal product. But this is not a reason for overlooking stability assessment (e.g. cell-based medicinal product stability needs to be addressed)

Ideally, depends on the availability of a quantitative assay for each individual phage mixed in the DP.

Alternatively, demonstration of the stability of each individual phage **formulated as mono-strain product**. The shelf-life of the multi-strain product containing different strains corresponds to the shelf-life of the formulated strain which has the shortest stability (from the guideline on multi-strain VT vaccines)



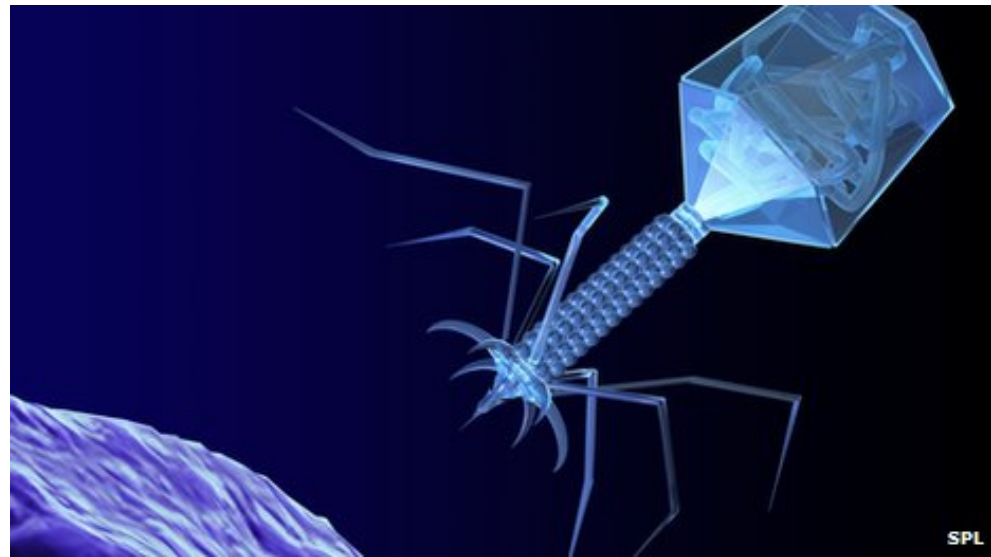
Adventitious agents

- Avoid material of animal/human origin
- Steam sterilization wherever possible



GMO issue

- It is not excluded to have a collection of recombinant phages.
- In such case, the medicinal product would also be submitted to the environmental regulation applicable to the deliberate release of GMO (Directive 2001/18/EC)



Homologous group-like approach

- The concept of *Homologous groups* originally developed for the allergen products (EMEA/CHMP/BWP/304831/2007) could possibly be applied to several parameters (e.g. validation of manufacturing process, stability data, safety assessment in non-clinical studies, ERA for GMO...).
- Full set of data could be presented for only **one phage representative** of a **homologous group/taxonomic family**.
- This could make easier the inclusion of new phages or variants/mutants within the collection.



Concluding remark

- Phage therapy has a place within the therapeutic armamentarium against bacterial infections.
- Regulation needs to be adapted to phage therapy and not vice versa.
- No specific guideline is available (yet) but several medicinal product guidelines may represent a source of inspiration
- Inspiration may also be found in other regulated industries. For instance, phages were approved as food additive by the FDA



Phages as a model

Phages were generally recognised as valuable **models** during pioneering molecular biology

Today, they could regain their model position:

- **custom-made / personalized medicine** products.
- “often discussed but never established” concept of “**Biological Master File**” putatively applicable to each individual phage entering the phage collection.



Max Delbrück and Salvador Luria



Thank you

