Experience of the Eliava Institute of Bacteriophage, Microbiology and Virology in development of the innovative bio-preparations and their commercialization

Nina Chanishvili
The Eliava Institute of Bacteriophage, Microbiology and Virology, Tbilisi, Georgia

Innovative Drug Discovery Workshop, ISTC,
Toronto, Canada,
6-10 August, 2011

The first evidences

“The first evidence for a viral-like agent with antibacterial properties was reported by M. E. Hankin in 1896. Found in the Ganges river in India, it was temperature sensitive, capable of passing through a porcelain filter, and could reduce titres of the bacterium *Vibrio cholerae* in laboratory culture. Hankin suggested that it might help to decrease the incidence of cholera in people using water from the Ganges.” Sankar Adhya, Carl Merrill, *The road to phage therapy*, Nature, 2006, 443:754-55.

Troubled waters? Bathers in the Ganges were thought to be protected from cholera by phage.

Discovery of Bacteriophages

1898

Nnikolai Gamalea

Russia

Russ Arch Pathol Clin Med Bacteriol 6 (1898), pp. 607–613.

1915

Frederik Twort

UK

An investigation on the nature of ultramicroscopic viruses (1915), Lancet 11: 1241

1917F

Feliqs D'Herelle

France

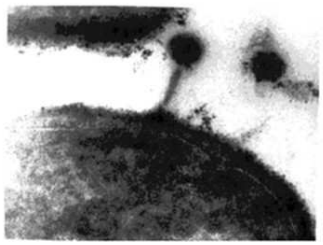
Sur un microbe invisible antagoniste des bacilles dysentériques (Comptes rendus de l'Académie des Sciences, Paris, 1917. 165 :p.373-5.

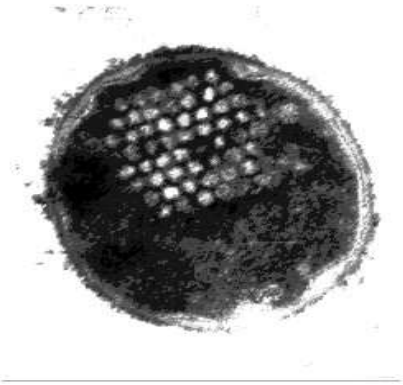
1918 ?

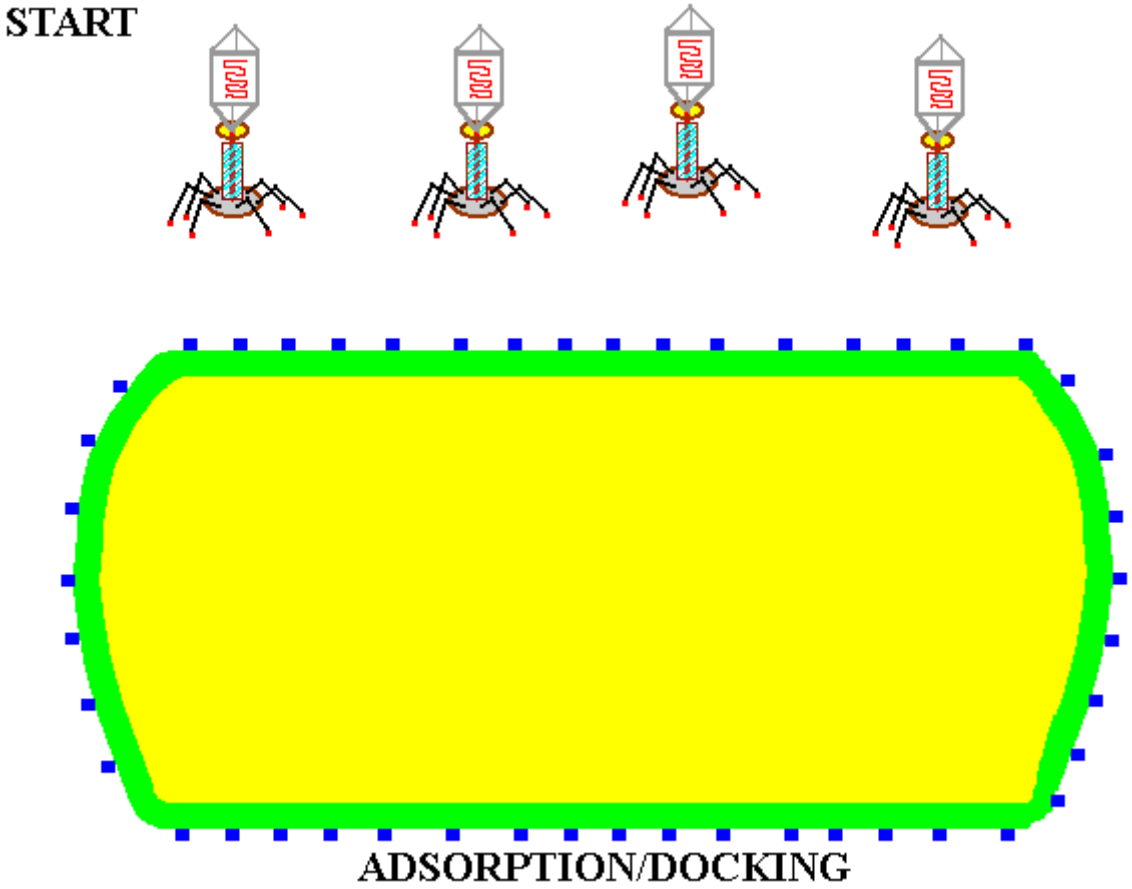
George Eliava

Georgia

Phage Reproduction on the Bacterial Cell







Bacteriophage in action - to find the target and neutralize

History of Phage Therapy

1919 – The first clinical trial - Hôpital des Enfants-Malades, felix D'Herelle, Paris, France.

1921 – The first publication on phage therapy: Richard Bruynoghe and Joseph Maisin, Essais de thérapeutique au moyen du bacteriophage. C. R. Soc. Biol. 85:1120-1121.

1927 – The first mass application of phages, Campbell Hospital, Calcutta, India, Felix D'Herelle.

"Your enemy's foe is your friend." **Alexander the Great**

Past Experience in the World

The oldest phage preparations from the Eliava collection dated by 1930's.

- D'Herelle's commercial laboratory at the **Pasteur Institute** (Today **L'Oreal**) (5 preps)
- **German Bacteriophage Society** (dried phages in tablet forms)
- **German company Antipiol** (Enterofagos)
- **Eli Lilly Company** (USA) (7 phage-based products)
- **Squibb and Sons** (USA) (Today **Bristol-Meyers Squibb** & **Swan-Myers** of Abbot Laboratories)
- **Parke, Davis and Company** (now part of **Pfizer**)

G.Eliava Institute of Bacteriophage, Microbiology and Virology, Tbilisi, Georgia (1923-2010)

Eliava IBMV was founded in 1923 in Tbilisi, Georgia by Professor George Eliava.

George Eliava and a French-Canadian Professor Felix D'Herelle came across in 1920's in the Pasteur Institute in Paris.

The main idea of G. Eliava and F. D'Herelle was creation of the World Centre of Phage Research.

Professor Eliava was executed in 1937.

The First Mass Application of Phages - Field Trials

Finnish Campaign 1938-1940

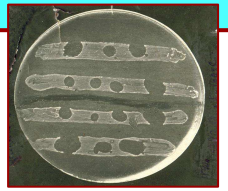
Krestnikova, 1947

Experience of phage prophylaxis based on the results of 3 mobile brigades.

I brigade applied phages for 2,500 solders, among them gangrene symptoms were revealed in 35 (1,4%) cases. In the control group of 7,918 solders gangrene was registered in 342 (4.3%) cases.

II brigade applied phage treatment for 941 solders, only 14 (1,5%) got sick. In the control group 6,8% were infected.

III brigade applied phage treatment for 2,584 soders, gangrene was developed in 18 (0,7%) cases, while in the control group incidence of infection was 2,3%



ISTC Project G-1467

Preparation of a detailed review article/ monograph on the practical application of bacteriophages in medicine, veterinary, environmental

research, based on old documents and publications.

Screening results:

- **> 5000 volumes of scientific journals, selected articles, books and thesis of dissertations have been screened. About 10% of the total contain information on phages.**
- **The gathered information was included into:**

"A Literature Review of the Practical Application of Bacteriophage Research".

Main conclusions:

- ***Success outcome of phage therapy is up to 95%.***
- ***Rapid improvement and cure within 3-5-7 days.***
- ***Positive cosmetic effect (no scars left).***
- ***No relapsed cases.***
- ***No side effects.***
- ***Minimization of the mortality rate among children.***
- ***Due to prophylactic "phaging" number of actually registered disease cases in comparison with expected rates (i.e EI) is reduces 3-6 times.***
- ***Mild disease cases in a "phaged" group.***
- ***Effect similar to vaccination.***
- ***Reduced hospital days.***

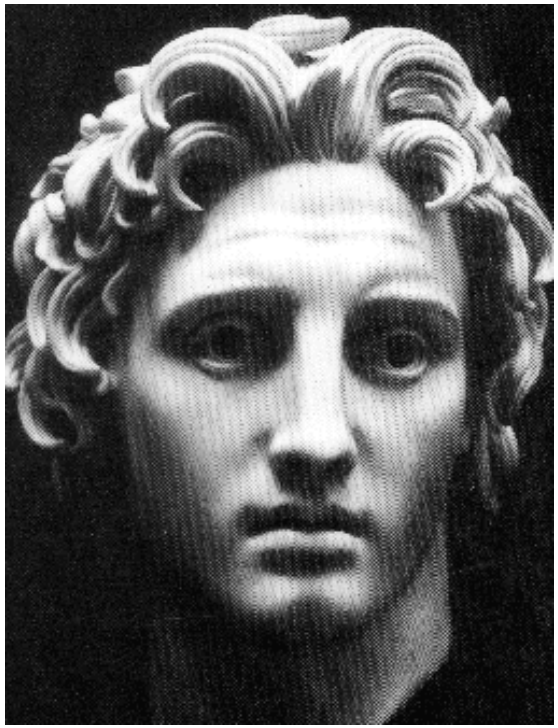
Publications dated by 1930s-1950s.

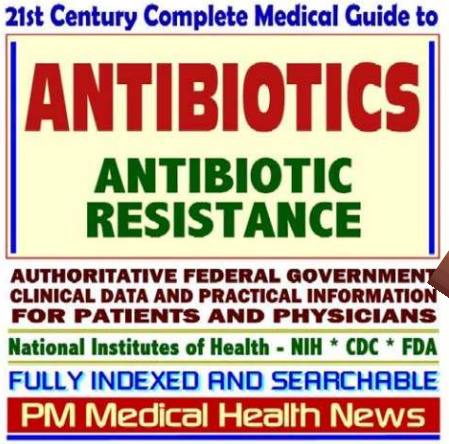
2009


Thanks to PENICILLIN
...He Will Come Home!
Thanks to PENICILLIN
Home!
1929
1943
PENICILLIN
CURES
GONORRHEA
IN 4 HOURS
SEE YOUR DOCTOR TODAY
1945
SCHENLEY LABORATORIES, INC.
Producers of PENICILLIN-Schenley
Producers of PENICILLIN-Schenley

"I am dying from the treatment of too many physicians."

Alexander the Great





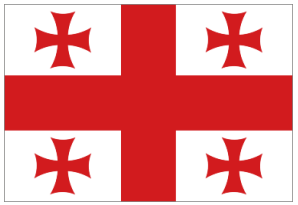
Comparison of bacteriophages vs. antibiotics

- Live bacterial viruses
- Viable
- Specific action
- Self reproducible
- No evidence
- Effective against drug-resistant bacteria
- N/A
- Non-hazardous industry
- Inexpensive

- Chemical substances
- Standard
- General action
- N/A
- Side effects (e.g. allergy)
- Spread of drug-resistance
- Yeast, fungal infections
- Hazardous industry
- Expensive

Bacteriophage production in the Former Soviet republics

In 1988 Scientific-Industrial Union "Bacteriophage" has been formed with the branches in:



Georgia, Tbilisi

SIU "Bacteriophage" => Eliava IBMV, Ministry of Education of Georgia

After privatization in 1994-1995 10 small companies have been formed, two out of them with the phage profile:

- **ChemBioPharm Ltd.**
- **BioPharm Ltd.**

- **Eliava BioPreparations Ltd. (formed in 2008)**

Russia

- **Perm (Filial of "Microgen Ltd." => "Biomed Ltd")**
- **Ufa (Filial of "Microgen Ltd" => "Immunopreparat Ltd.")**
- **Nizhni Novgorod (GPU production "BactPreparations, Russia)**
- **Khabarovsk (GPU production "BactPreparations", Russia)**
- **Saratov (Anti-plague Institute "Microbe" => Biofon Ltd.)**

Phage preparations presently in use in the former Soviet republics

Monovalent phage preparations:

- **Staphyloccocal**
- **Streptococcal**
- **E.coli**
- **Proteus**
- **Pseudomonas aeruginosa**
- **Klebsiella (active against *K.pneumoniae, K.ozena, K. rhinoscleroma)***
- **Typhoid (active against *Salmonella typhi A,B,C,D,E*)**
- **Dysenetrial**
- **Salmonlla sp.**

Polyvalent (combined) phage preparations:

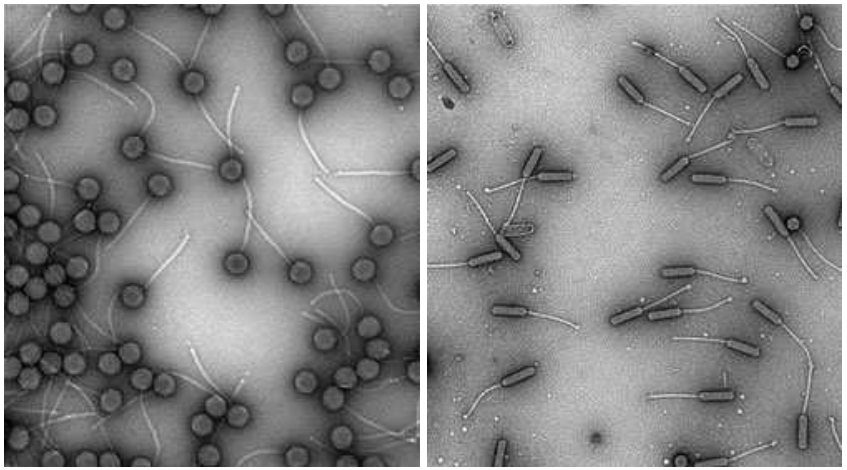
Intesti-bacteriophage

Sh.flexneri serotypes 1,2,3,4,5, 5; Sh. sonnei;
Salmonella paratyphi A & B; S. enteritidis, S.typhimurium.S. cholera-suis, S.oranienburg, enteropatogenic
E.coli,
Proteus vulgaris, P. mirabilis,
Staphylococcus aureus,
Streptococcus sp.
pathogenic Enterococcus.

Pio-bacteriophage

Staphylococcus,
Streptococcus,
Ps. aeruginosa,
E.coli,
Proteus (in some caaes Klebsiella pneumoniae)

Coli-Proteus



Diagnostic phages:

- **Saratov**
  5 types of *Cholera* phages

Commercial Companies Involved in Bacteriophage Research and Development

Exponential Biotherapies (USA) 1994
- Closed the phage program in 2005

Phage Therapeutics Inc (USA) 1997
- Closed the phage program in 2003

Intralytix (USA) 1997
- ListShieldTM , EcoShieldTM

- **New Horizons Diagnostic Corporation (USA)**
  - Treatment of chronic patients with phages in Georgia, Tijuana, Mexico
- **Phage International, Inc (USA)**
  - AgriPhage for treatment of tomatoes, pepper
- **OmniLytics, Inc (USA)**
  - Diagnostic kits for detection MRSA & MSSA
- **Microphage Inc (USA)**
- **Phage Pharmaceuticals, Inc i.e. Phage Biotechnology Corporation .(USA)**
- **Phage Genomics**
  - Finalyse for treatment of animals
- **Eli Lilly (USA)**
  - Information not found

Phage Solutions (USA)

Novophage (USA) 2011
- Healthcare, Pharmaceuticals, & Biotech

Biophage Inc. (Canada)
- Research on the veterinary and environmental applications

Targanta Therapeutics Inc. (Canada)
- Listex P100 for control of *Listeria*

GangaGen Life Sciences Inc. (Canada)

- **Phagetech (Canada)**

Hexal Genentech (Germany)

EBI Food Safety (Netherlands)
- Staph-TAME (i.e. P128) for control of MRSA

GangaGen Biotechnologies Ltd (India) 2001
- Healthcare solutions from naturally occurring phages

Novolytics Ltd. (UK)
- Phage-based therapeutics against drug-resistant bacteria

Biocontrol Limited and Biocontrol International Inc. (UK)
- Treatment and prevention of infection & bacterial contamination in medicine, food safety, and environmental sanitation

Sarum Biosciences (UK)

Fixed Phage Limited (UK) 2010

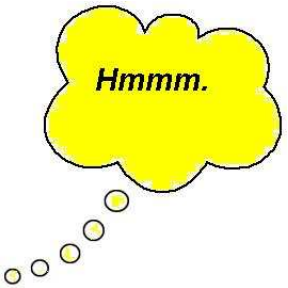
PHAGE BIOTECH LTD. (Israel)
- Phages solutions for Environment, Cosmetic and Medical bacteria infections (ino)

Micropeace (Australia)

Special Phage Holdings (Services) Pty Ltd. (Australia)

InnoPhage, Ltd (Portugal)

Why phage therapy is not recognized in the West?



"Unfortunately, these clinical applications were initiated before certain microbiological aspects of phage strains, such as their narrow host range, were fully appreciated." **Sankar Adhya, Carl Merrill,** ***The road to phage therapy,*** **Nature, 2006, 443:754-55.**

"In the U.S., the regulatory system is designed to handle one-size-fits-all drugs, not quickly changing individual meds. The FDA would require every phage to go through a multi-year testing process – by which time the bug may have evolved again." **Andrew Cantor, Cyber Speak, 2006,** ***"US needs open eye on phage therapy"***

"In the United States, the FDA would want the phages in each new concoction to be gene sequenced, because regulations require every component of a drug to be identified."
http://gojomo.blogspot.com/2006/06/team-human-and-still-more-on.html

"*Phage therapy is not a new concept, and it is important to ask why it is not part of the current repertoire of western medicine despite the fact that it has been continuously and extensively used in Eastern Europe for almost a century. Answering this question successfully will, largely, determine whether phage therapy can gain the credibility needed to overcome the scientific, financial and regulatory hurdles facing its adoption in mainstream clinical practice. Despite a paucity of such information from human studies, pharmacokinetic data and clinical outcomes from animal studies are currently providing convincing evidence for the safety and efficacy of phage therapy."* **John N. Housby, Nicholas H. Mann,** ***Phage therapy,*** **Drug discovery today, 2009, 14(11-12):536-540**

FDA approved products

(GRAS status i.e. Generally Recognized as Safe)

- 2006 August - **ListShieldTM** ready-to-eat meat and poultry products, Intralytix, USA
- 2006 October - **Listex P100**, EBI Food Safety, Netherlands
- 2011- **EcoShield** against *E.coli O157*

2006 - AgriPhage, Omnilytics, EPA registration

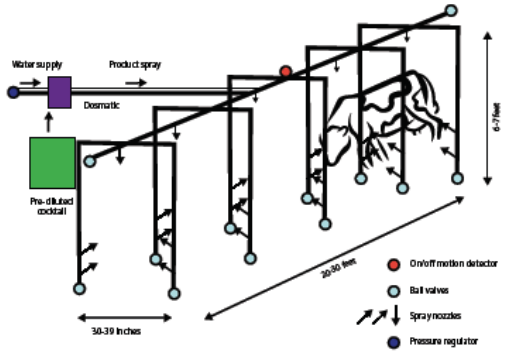
Eli Lilly, Indiana, USA
Elanco,Animal Health Division (2010)

Finalyse®

Topical biological control of *E. coli* O157 found on the hide of cattle at harvest facilities.

Active ingredients:	Contains a mixture of bacteriophage active against *E. coli*	0.0046%
Other ingredients:		99.9954%

Elanco



Pre-harvest hide wash used to aid in the reduction of *E. coli* O157:H7 in cattle

Ongoing clinical trials in Europe

UK

Biocontrol Ltd.

OBJECTIVES:

- To evaluate the efficacy and safety of a therapeutic bacteriophage preparation (Biophage-PA) targeting antibiotic-resistant *Pseudomonas aeruginosa* in chronic otitis.
- DESIGN:
- Randomised, double-blind, placebo-controlled Phase I/II clinical trial approved by UK Medicines and Healthcare products Regulatory Agency (MHRA) and the Central Office for Research Ethics Committees (COREC) ethical review process.

Poland

Institute of Immunology and Experimental Therapy of the Polish Academy of Sciences

Experimental Phage Therapy of Bacterial Infections, NCT00945087, started in 2009.

Bacteriophage lysates containing phages lytic for *Staphylococcus, Enterococcus, Escherichia, Citrobacter, Enterobacter, Klebsiella, Shigella, Salmonella, Serratia, Proteus, Pseudomonas, Stenotrophomonas, Acinetobacter, or Burkholderia* strains are used.

Switzerland

Nestlé Nutrition Corporate, 2009-2012

Randomized, Double Blind Placebo-controlled Studies to Evaluate the Effect of an Orally-fed Escherichia Coli (E. Coli) Phage in the Management of ETEC and EPEC Induced Diarrhea in Children NCT00937274, commercial T4 phage mixture is used. The study is performed in Bangladesh.

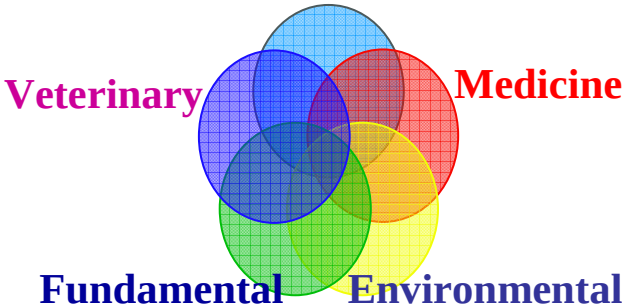
The Elaiva Institute Today

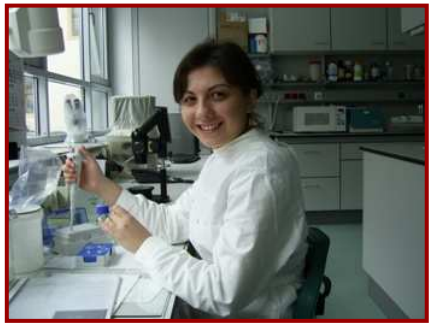
"The rumors about my death are greatly exaggerated"

Marc Twain

Current areas of phage research

Soft rot/ Black Leg
- *Erwinia carotovora*
Brown rot /Bacterial wilt
Ralstonia solanacearum
Diseases of rice and cotton
- *Xanthomonas spp.*

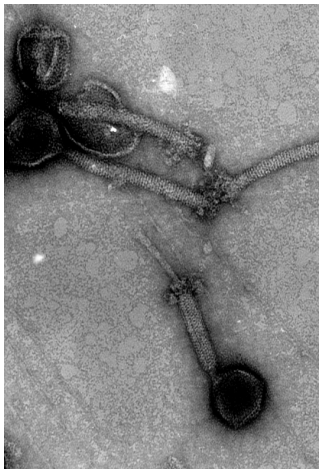




Staphylococcus phage for intravenous use

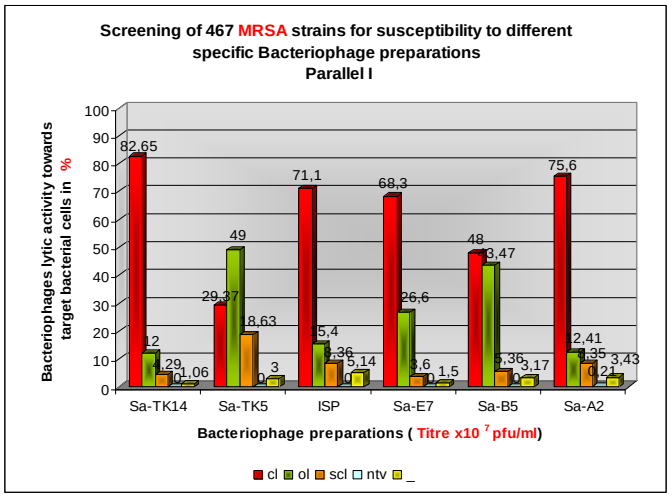
Screening Results of MRSA strains - The UK Study, 2007

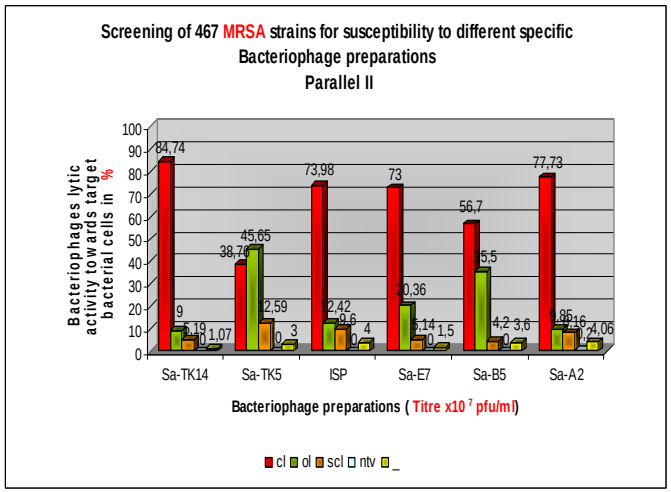
Participants: The Eliava Institute, Georgia (N. Chanishvili, M. Tediashvili)
Cardiff University, UK (Prof. Stephen Denyer),
University of Brighton, UK (Prof. Geoff Hanlon)

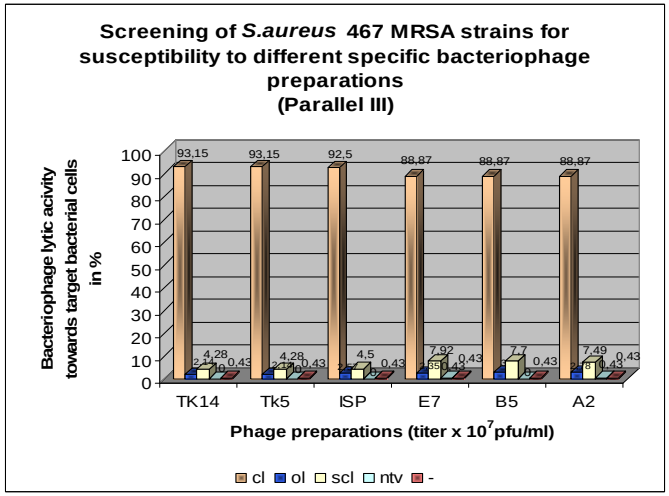


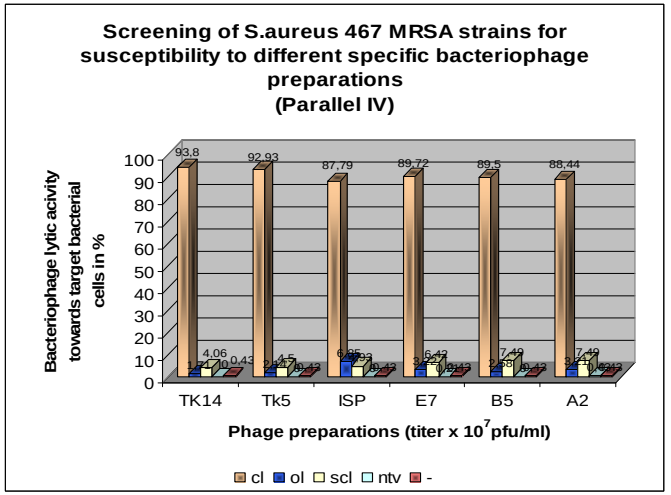
467 MRSA strains obtained from the UK hospitals were screened against 6 S.aureus phages from the Eliava IBMV collection: ISP, A2, B5, E7, TK5 and TK 14.

High activity (99.5%) against standard MRSA strains and clinical isolates has been demonstrated







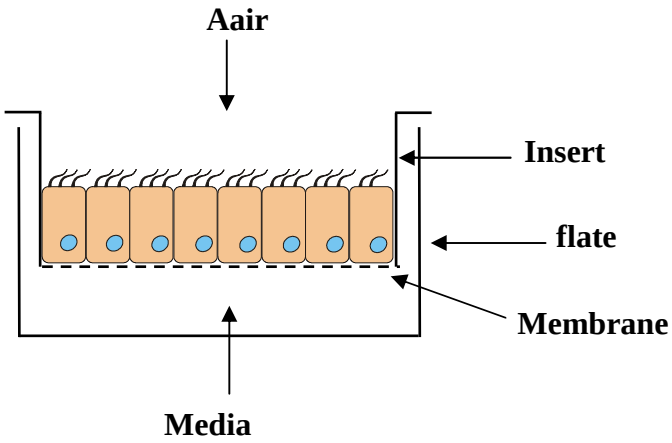


Project funded by Mr. Robert Patton, Ireland

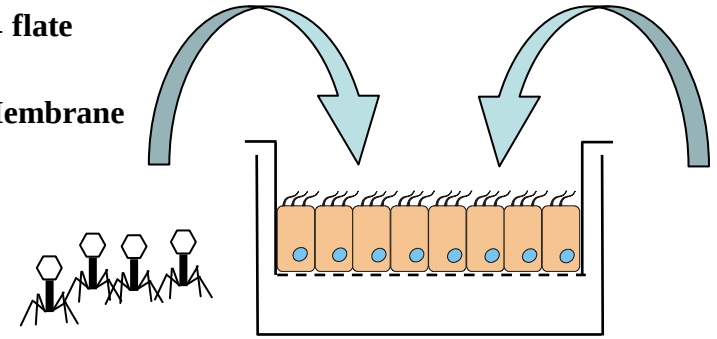
Application of phage therapy against lung infections:

In vitro infectious model

(INTAS -6610)



Infectious model



Cell line: A549
Bacteria: *Pseudomonas* (573)
phage: pT6

Simulation:

- **phage 1 h prior to bacterial infection**
- **phage + bacteria simutaneous infection**
- **phage 1 h after bacterial infection**

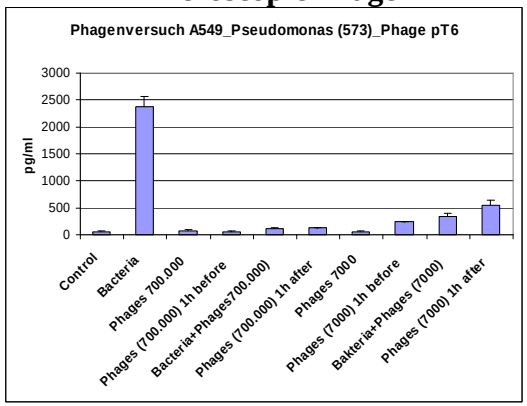
Results:

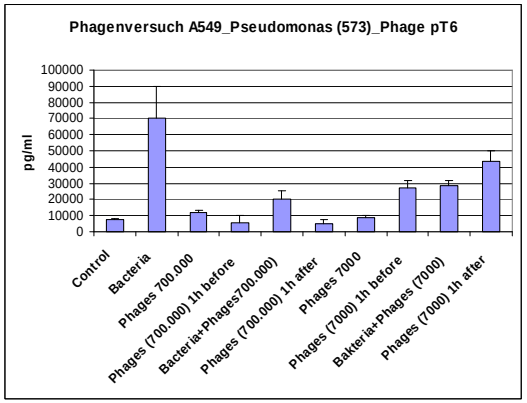
IL-6
IL-8
CFU

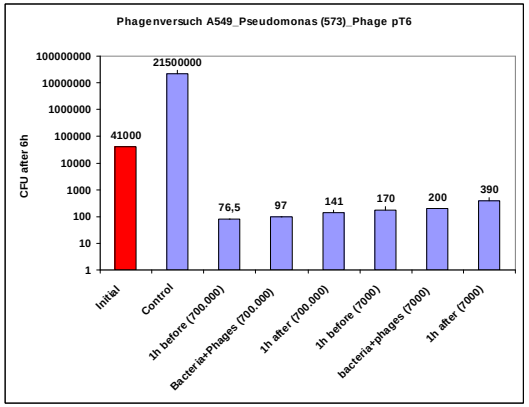
- Air-Liquid-Interface culture System (ALI)

ALI - **microscopic image**

Phage does not cause inflammation!!!



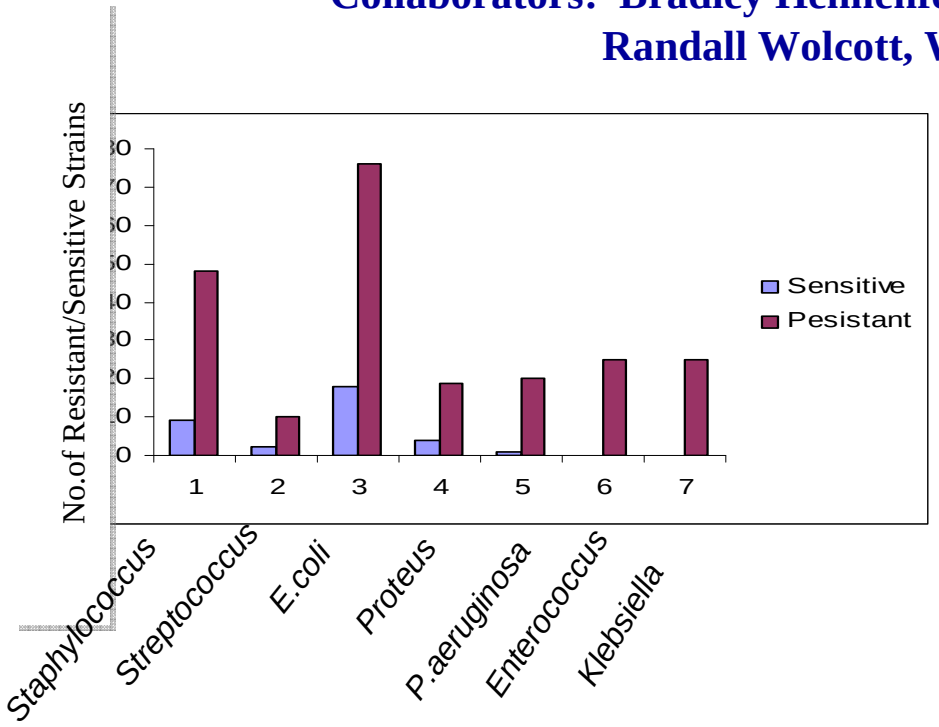


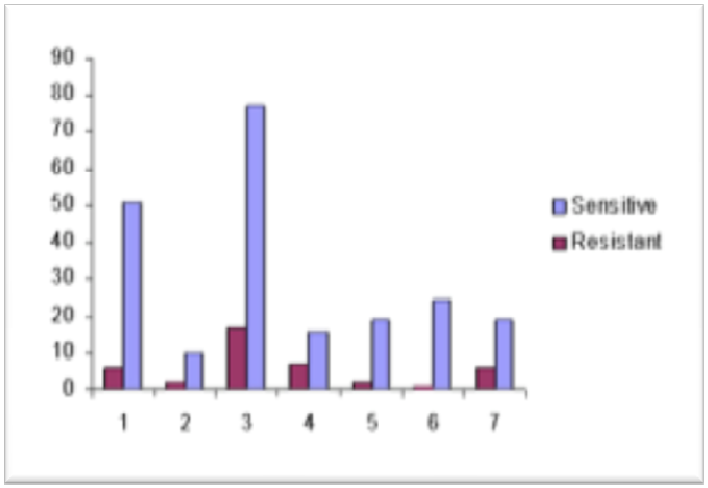


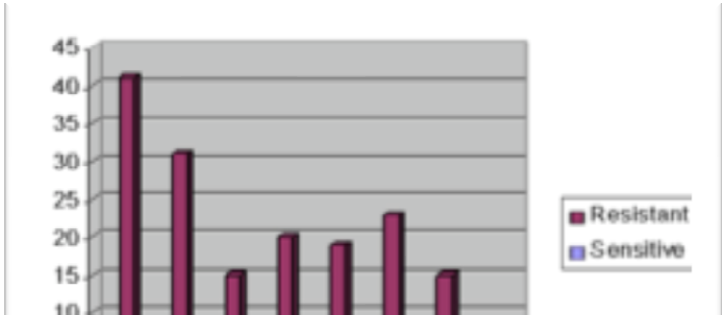
Project: ISTC- G 1369 Chronic prostatitis and phage therapy prospectes

Collaborators: Bradley Hennenfent, University of Illinois, USA

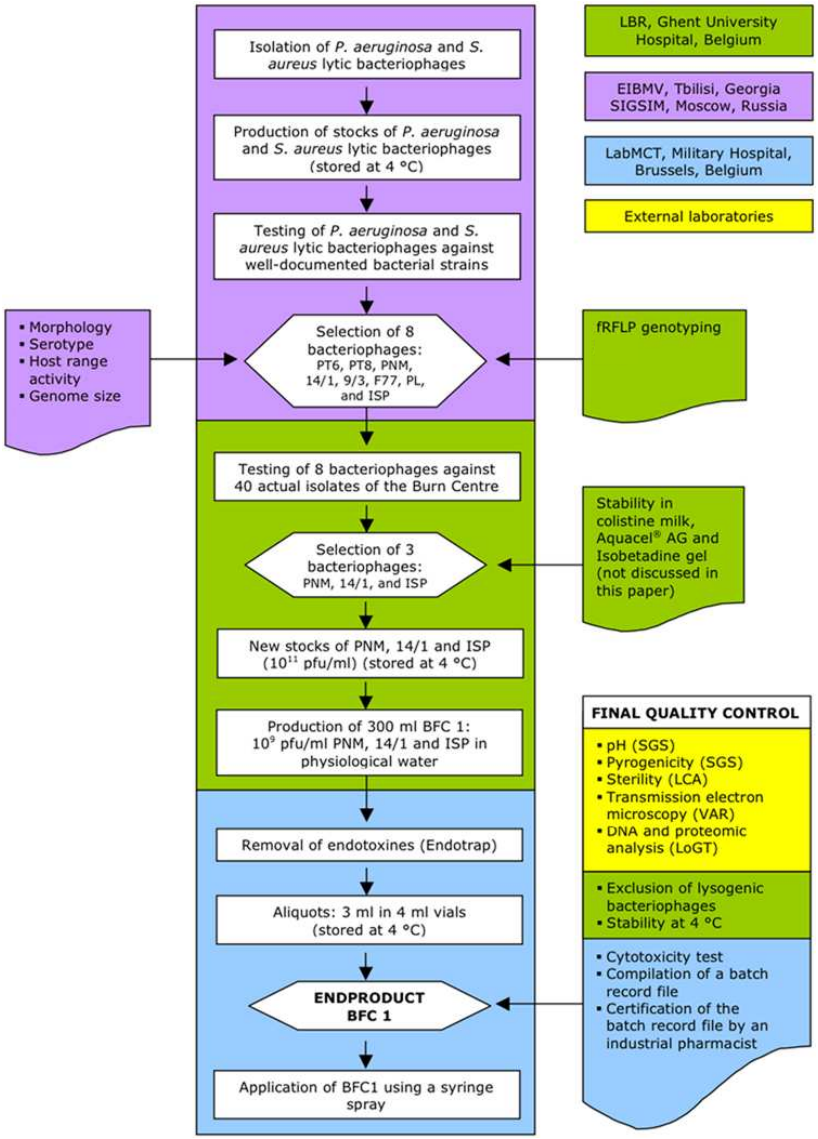
Randall Wolcott, Wound Care Center, TX, USA



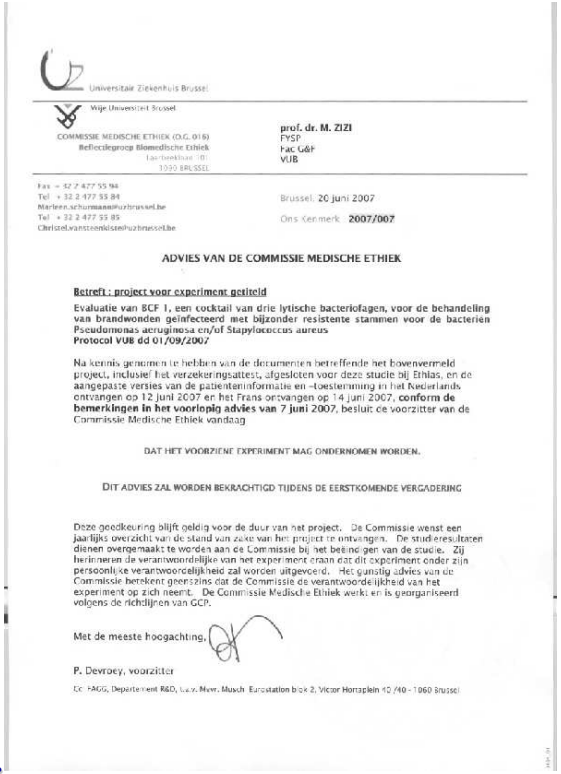




GMP Production and Clinical Trials in Belgium (Georgian-Belgian collaboration)



File
Product information
Study protocol
"No fault" insurance
Informed consent
...
Submitted Dec 06
False perception
Biosafety council
Insurance x10
...
Approval 20 Jun 07
Belgian leading ethical committee (University Hospital Brussels)


Universitair Ziekenhuis Brussel
Vrije Universiteit Brussel
COMMISSIE MEDISCHE ETHIEK (O.G. 016)
Reflectiegroep Biomedische Ethiek
prof. dr. M. ZIZI
PVSP
Fac G&P
VUB
Brussel, 20 juni 2007
Ons Kenmerk: 2007/007

ADVIES VAN DE COMMISSIE MEDISCHE ETHIEK

Betreft : project voor experiment getiteld

Evaluatie van BCF 1, een cocktail van drie lytische bacteriofagen, voor de behandeling van brandwonden geïnfecteerd met bijzonder resistente stammen voor de bacteriën Pseudomonas aeruginosa en/of Stapylococcus aureus
Protocol VUB dd 01/09/2007

Na kennis genomen te hebben van de documenten betreffende het bovenvermeld project, inclusief het verzekeringsattest, afgesloten voor deze studie bij Ethias, en de aangepaste versies van de patiënteninformatie en -toestemming in het Nederlands ontvangen op 12 juni 2007 en het Frans ontvangen op 14 juni 2007, **conform de bemerkingen in het voorlopig advies van 7 juni 2007**, besluit de voorzitter van de Commissie Medische Ethiek vandaag

DAT HET VOORZIENE EXPERIMENT MAG ONDERNOMEN WORDEN.

DIT ADVIES ZAL WORDEN BEKRACHTIGD TIJDENS DE EERSTKOMENDE VERGADERING

Deze goedkeuring blijft geldig voor de duur van het project. De Commissie wenst een jaarlijks overzicht van de stand van zake van het project te ontvangen. De studieresultaten dienen overgemaakt te worden aan de Commissie bij het beëindigen van de studie. Zij herinnert de verantwoordelijke van het experiment eraan dat dit experiment onder zijn persoonlijke verantwoordelijkheid zal worden uitgevoerd. Het gunstig advies van de Commissie betekent geenszins dat de Commissie de verantwoordelijkheid van het experiment op zich neemt. De Commissie Medische Ethiek werkt en is georganiseerd volgens de richtlijnen van GCP.

Met de meeste hoogachting,

P. Devroey, voorzitter

cc: FAGG, Departement R&D, t.a.v. Mevr. Musch Eurostation blok 2, Victor Hortaplein 40 /40 - 1060 Brussel

BFC 1 Production Process

Selection of phages

***P.a.*: 82 phages/115 strains**

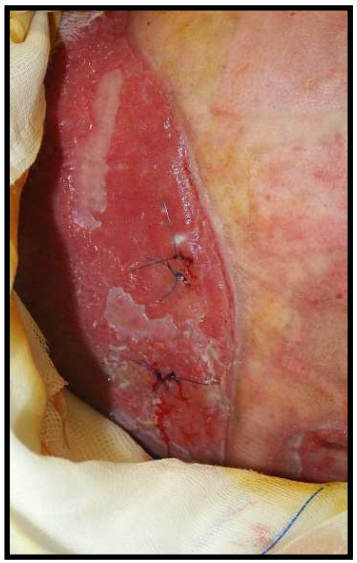
***S.a.*: 8 phages/99 strains**

Quality Control

pH
Sterility
Pyrogenicity
Cytotoxicity
EM
Sequence
Exclusion of lysogeny

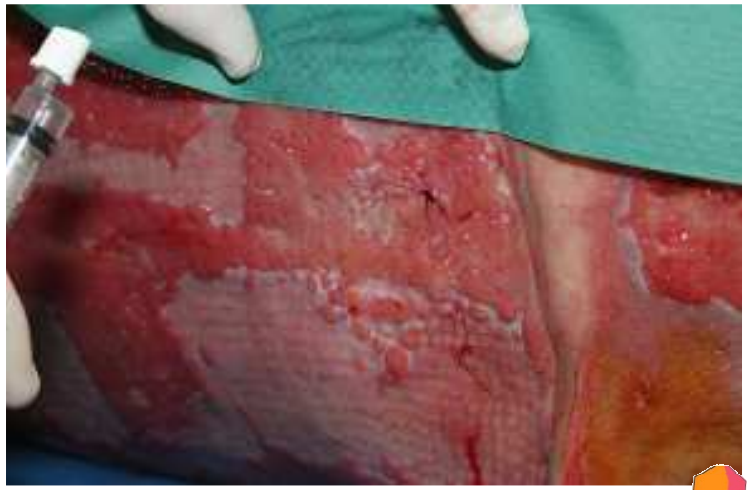
Results of the Clinical Trial

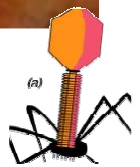
Eight out of ten applications (80%) biopsies and swabs taken before and after BFC 1 or standard treatment were negative!



In 2 cases no significant differences in bacterial load were observed before and after standard or BFC 1 treatment.

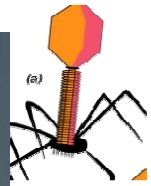
No clinical or laboratory test abnormalities have been observed!





Future developments:

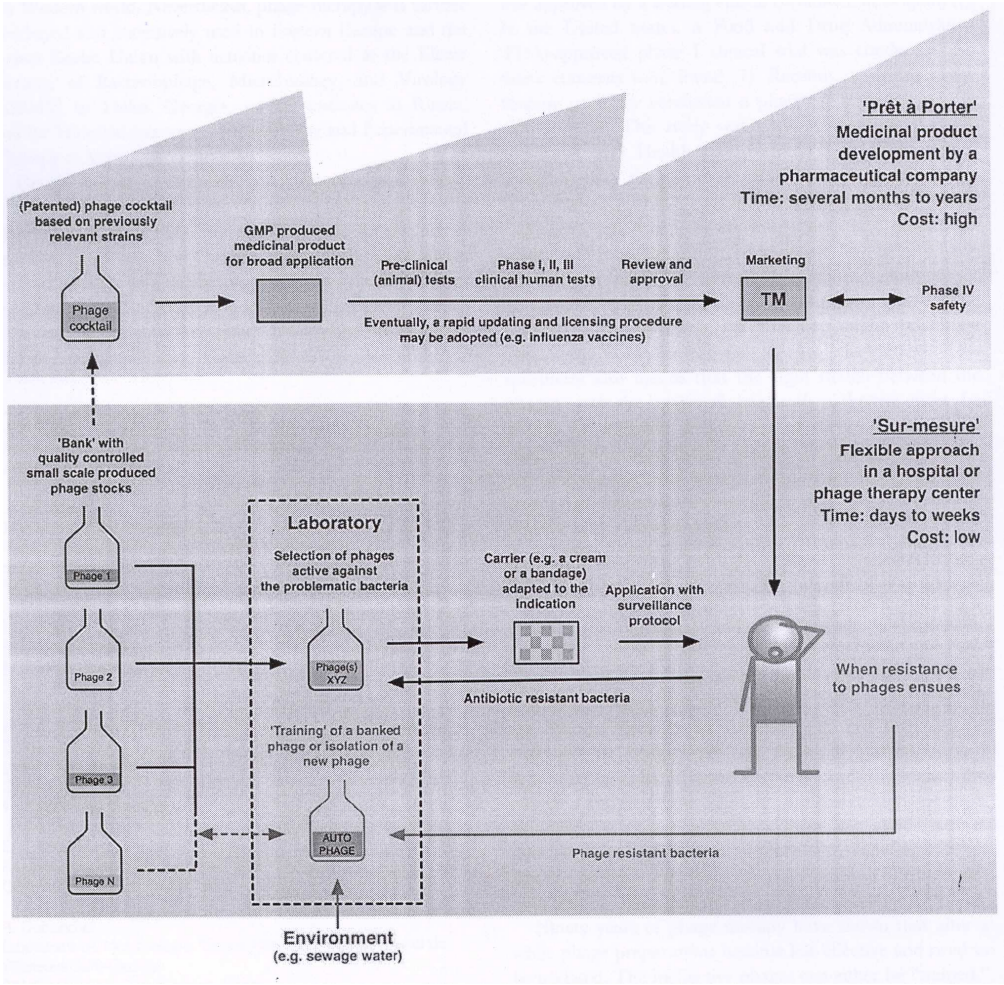
- To simplify patients access to phage treatment
- To design the new protocol aiming to prove effectiveness of phage treatment in comparison to standard treatment with antibiotics



D.De Vos
J.-P. Pirnay
M. Merabishvili
M. Vanehoutte
T.Rose
R. Lavigne
G. Verbeken

MARTIN RAMELOT PRIZE 2009

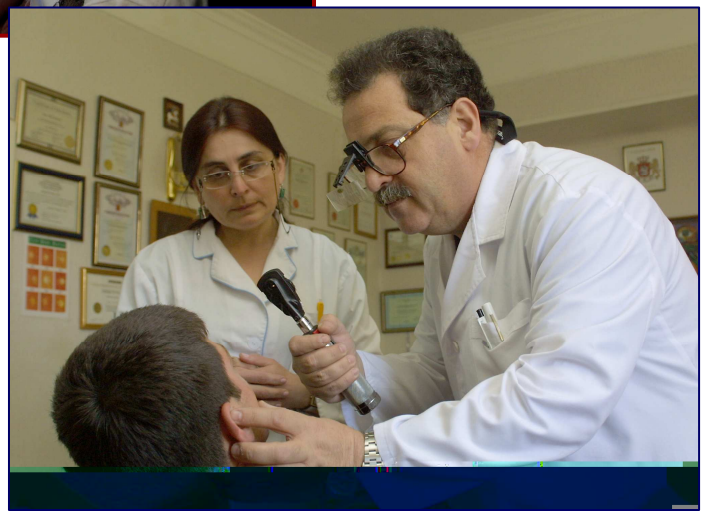
The phage paradigm: *Prét-a-porter* or *Sur masure*?
J.P.Pirney et al., Pharm. Res., 2011, 28:934-937

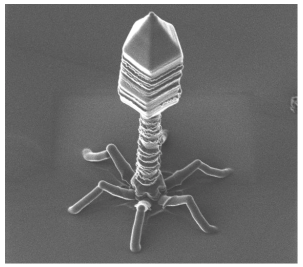


While others are still thinking...

Eliava spin-off companies:

- **Eliava Diagnostics**
- **Eliava MediaProduction**
- **Eliava BioPreparations**
- **Eliava Phage Therapy Center, Tbilisi, Georgia**
- **Eliava Phage Therapy Center, New York, USA**





“There is nothing impossible to him who will try.”

“Remember upon the conduct of each depends the fate of all.”

Alexander the Great

Thank you