



Project n° FP7-HEALTH-2013-INNOVATION-2-601857

PhagoBurn

Evaluation of phage therapy for the treatment of *Escherichia coli* and *Pseudomonas aeruginosa* burn wound infections (Phase I-II clinical trial)

www.phagoburn.eu

Thematic priority: HEALTH-2013.2.3.1-1 – Drugs and vaccines for infections that have developed or are at the risk of developing significant anti-microbial resistance

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1. **EXECUTIVE SUMMARY**

In the context of a worldwide growing antibiotic resistance threat, notably the emergence of multi-drug resistant, even pan-resistant, bacterial strains, PhagoBurn was launched to evaluate the clinical potential of bacteriophages (phages) as a novel and innovative strategy to fight this critical issue.

Launched in 2013 and achieved in 2017, PhagoBurn was the world first prospective multicentric, randomised, single blind and controlled clinical trial of phage therapy ever performed according to both Good Manufacturing (GMP) and Good Clinical Practices (GCP)

Several results of major significance for the future of phage therapy stemmed from this pioneer project.

First, significant advancements were achieved regarding the regulatory framework of phage therapy. At the beginning of the project, no clear legal framework regulating the use of therapeutic phages was available in all EU member states but Poland, where the Helsinki treaty prevails at the Institute of Immunology and Experimental Therapy (Wroclaw) for using phage therapy. Consequently, their compassionate use was not allowed in these countries and patients facing therapeutic dead-end due to antibiotic resistance often travelled to the Eliava Institute (Georgia) where they received handmade cocktails without western standard medical control. It is to the credit of the PhagoBurn study that discussions were first opened in 2013 with the French (ANSM) and Belgium (AFMPS) and then in 2014 with the Swiss (Swissmedic) regulators. These exchanges supported the organisation in June 2015 of the first regulatory workshop on phage therapy in London at the European Medicine Agency (EMA). **In particular, a close, strong and trustful cooperation was developed throughout the project with the team of the ANSM Anti-Infectious Department.** Such collaboration led to temporary authorisations for use (ATUs) for two patients. Indeed, a dedicated committee of experts was quickly created by the ANSM to evaluate ATU requests and both were granted in France, one ATU for each phage cocktail.

Second, PhagoBurn led to the world first GMP-like production of bacteriophage drug products. This achievement implied overcoming several highly challenging steps and dedicating strong manpower and extensive financial resources to the phage cocktails bio-production. Accordingly, two years were necessary to produce both cocktails according to GMP standards.

Third, the PhagoBurn clinical study, representing the world first phage therapy clinical trial using GMP produced phage cocktails, was launched in July 2015 and ended in January 2017. Due to an extended period for overcoming manufacturing challenges and consequently obtaining the necessary authorisations to launch the clinical study, only 13 months remained for patient inclusions instead of the 24 months initially planned. Accordingly, the number of inclusions was lower than expected: 27 patients were included and 25 analysed. Moreover, additional factors contributed to decrease the total number of inclusions, including i) the variability of local ecology in burn centres and ii) the fact that while most burn infections are polymicrobial (induced by several bacterial species), our drug products were mono-specific (targeting only one bacterial species), which in many cases prevented their use to treat such infections and therefore to include corresponding patients.

Despite this limited number of inclusions, along with stability monitoring and shelf life issues, **the trial yielded highly stimulating and informative results**, which are in the process of being published in detail in an appropriate scientific journal. For that reason, they cannot be developed here.

Most importantly, no serious adverse event was observed in the GMP phage cocktail arm, which is in line with the expected high level of safety for such treatments. This provides a first answer to the main question of drug agencies. Efficacy results will be reported in the scientific publication underway.

To conclude, PhagoBurn paved the road to the re-implementation of bacteriophages in the occidental therapeutic arsenal. Indeed, the work achieved in this project demonstrated that in the present settings, i) phage-based products could be produced as for any other human medicine,

according to GMP standard, ii) GMP phage products were devoid of unwanted side effects and iii) GMP phage products significantly decreased the pathogen load in burn wounds.

Pursuing the evaluation of phage therapy as a complementary strategy to the classical antibiotic therapy remains of utter importance, as the World Health Organisation predicts that **bacterial infectious diseases will kill 10 million people a year by 2050**. In this critical context, research and innovation are key to develop new tools and strategies. Close cooperation between public, private, civil or military partners, such as in PhagoBurn, is the only way to achieve global breakthroughs.

2. **SUMMARY DESCRIPTION OF PROJECT CONTEXT & OBJECTIVES**

PhagoBurn context – an urgent need for an original alternative to fight antimicrobial resistance application in burn trauma

Sepsis is a major problem for burn patients and their predominant cause of death. In high-income countries, more than 50% of the mortality-morbidity rate of burn trauma is related to infection. Patients are at high risk of suffering from multidrug resistant (MDR) microbes. Hard-to-treat MDR Gram-negative bacteria such as *Escherichia coli* (*E. coli*) or *Pseudomonas aeruginosa* (*P. aeruginosa*) are highly prevalent in many burn centres.

Such infections cannot be eradicated efficiently with conventional antibiotics (AB). Moreover, the number of new antimicrobials under clinical development is extremely low and their introduction to the market is disappointingly slow, leaving physicians with very few alternatives.

There is only little hope for any new product in the coming years. MDR Gram-negative infections will soon become orphan diseases without effective therapeutics available. Finding innovative ways to counter multidrug resistance will have a very broad impact on infected burn wounds but also on other bacterial infections due to MDR microorganisms.

In this context, bacteriophage (phage-) therapy represents a highly promising alternative to antibiotics.

Natural and lytic bacteriophages (phages) are viruses that specifically infect and kill bacterial cells during their life cycle. Shortly after their discovery in the beginning of the 20th century, these natural predators of bacteria have been widely used for the treatment of human infectious diseases and referred to as “phage therapy”, but were subsequently abandoned following the advent of AB (except in some parts of Eastern Europe, notably Russia, Georgia and Poland).

Nowadays, and mainly in response to the constant increase of bacterial resistance to AB, phage therapy encounters a high renewed interest because of its potentially high efficacy combined with an attractive safety profile. This alternative therapy is therefore increasingly considered as a promising way to fight MDR bacterial infections.

Although, phage therapy is currently available in some countries (Georgia, Poland, Russia, based on historical use), very few controlled, well-powered and well-designed clinical trials have tested its efficacy and safety regarding evidence based medicine criteria. This situation is a clear limiting step for implementing phage therapy outside Georgia, Poland and Russia in other European countries in spite of its numerous advantages.

As the European Medicines Agency (EMA) tends to consider that this therapy should go through the same authorisation procedures as any new human medication, conducting clinical trials to validate the relevance of phages in the treatment of human infectious diseases has thus become utterly important. This is even truer as a number of governments (as the US Food and Drug Administration) seem willing to initiate research programmes on phage therapy.

*PhagoBurn objectives – Evaluation of phage therapy for the treatment of *E. coli* and *P. aeruginosa* burn wound infections (Phase I-II clinical trial)*

In this context, the **PHAGOBURN** project was initiated by PHERECYDES-PHARMA a 2006-founded French Small and Medium-sized Enterprise (SME) with strong expertise and extensive know-how in lytic bacteriophages isolation, characterisation and production and Percy Military Hospital (Percy)..

The core objective of PhagoBurn, launched in June 2013 and achieved in February 2017, was **to assess the efficacy and safety of two therapeutic bacteriophage cocktails to treat *E. coli* and *P. aeruginosa* burn wound infections in a prospective randomised multicentric single blind and open clinical trial (phase I-II)** including burn patients hospitalised in France, Belgium and Switzerland.

The following table provides a detailed overview of PhagoBurn objectives:

1	Bioproduction of anti <i>E. coli</i> and anti <i>P. aeruginosa</i> phage cocktails in GMP conditions
2	Preparation, implementation and coordination of a phase I-II multicentric clinical trial: <ul style="list-style-type: none"> - Total duration: 19 months; - 7 initial clinical centres and up to 11 at the beginning of the trial, in three European countries (France, Belgium and Switzerland), <i>see below</i>; - Planned inclusion of around 40 patients (lower than initially envisaged due to recruitment issues).
3	Proof of safety and local tolerance of the bacteriophage cocktails
4	Proof of efficacy of the bacteriophage cocktails as topical agents
5	Phase III clinical trial preparation (as continuation of the Phase I-II clinical trial if successful)
6	Contribution towards the standardization of phage therapy at a regulatory level (EMA) Contribution to an EMA-compatible standardized regulatory process to authorize phage therapy at the European level
7	Business plan for the exploitation of both phage cocktails

These objectives were in complete accordance with the targeted topic of the FP7-2013 Health Programme (i.e. *HEALTH-2013.2.3.1-1 – Drugs and vaccines for infections that have developed or are at the risk of developing significant anti-microbial resistance*).

Indeed:

- PhagoBurn was designed **to assess the efficacy and safety of phage therapy** as an innovative and alternative medical approach to treat *E. coli* and *P. aeruginosa* burn wound infections;
- PhagoBurn aimed at **boosting the development of alternative solutions against pathogenic bacteria** for which treatments are becoming increasingly limited due to the spread of MDR microorganisms;
- PhagoBurn planned and **conducted a prospective, randomised, phase I-II clinical trial in three different European countries involving several reference burn centres** (*see below*). This trial was carried out according to the highest national/international quality and ethical standards (Guidelines of the International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH), Good Manufacturing Practices (GMP), Good Clinical Practices (GCP), etc.) under the promotion of PHERECYDES-PHARMA and the control of an independent Data and Safety Monitoring Board (DSMB);
- **PHARECYDES PHARMA**, an innovative French SME, co-led PhagoBurn as sponsor of the clinical trial, alongside with the overall project coordinator Percy, with the long-term objective to translate the gathered results into a **genuine innovative market application**;
- **A clearer EU regulatory path forward relevant for phage therapy** should emerge from the PhagoBurn project.

PhagoBurn partners and subcontractors – a complementary set of expert actors

To achieve the planned objectives, the PhagoBurn project gathered a Consortium of **5 partners from France, Belgium and Switzerland**:

- **Two French SMEs**: Pherecydes Pharma (www.pherecydes-pharma.com) and Clean Cells (www.clean-cells.com);

- **Three hospital burn units** located within:

- o Percy Military Hospital (France, Military Health Service, French Ministry of Defence);
- o Queen Astrid Military Hospital (Belgium, Royal Military Academy);
- o Centre Hospitalier Universitaire Vaudois in association with the University of Lausanne (Switzerland, CHUV & UNIL, respectively).

Apart from Consortium members, PhagoBurn included **8 additional burn units** (subcontractors) located within:

- Centre Hospitalier Saint Joseph & Saint Luc (France), acting as co-lead investigator with Percy military hospital;
- Centre Hospitalier Universitaire (CHU) Nantes (France);
- Centre Hospitalier Universitaire (CHU) Bordeaux (France);
- Centre Hospitalier Régional (CHR) Metz-Thionville (France);
- Military Hospital Sainte-Anne Toulon (France);
- Hôpital de la Conception Marseille (France) ;
- Centre Hospitalier Universitaire (CHU) Liège (Belgium);
- Grand Hôpital de Charleroi-Loverval (Belgium).

Finally, **3 other actors** were involved (subcontractors):

- **Statitec**, a French Clinical Research Organisation (CRO);
- **Laboratoires Brothier**, a French Company providing ALGOSTERIL™ dressings for the clinical trial;
- **France Europe Innovation**, a French SME assisting the coordinator and co-coordinator in all administrative and financial management aspects of the project.

These Consortium partners and subcontractors gathered the necessary complementary expertise, experience and networks to achieve the project objectives. Indeed, PhagoBurn involved **very complementary participants**, and notably:

- **Two innovative complementary French SMEs**: Pherecydes Pharma, with extensive skills and experience in bacteriophage isolation, characterisation and production for R&D, as well as Clean Cells, specialised in pharmaceutical products production and associated quality control in compliance with GMP standards and aiming at transforming the Research & Development (R&D) manufacturing process of Pherecydes Pharma into a GMP one;
- **The French Ministry of Defence**, whose medical corps is especially interested in alternative non-antibiotic treatment of infections, not only in the context of burns but also in all other combat-related injuries;
- Other European clinical centres with similar interest.

Finally, most partners of PhagoBurn had already previously worked together (i.e. Pherecydes with Clean Cells and with Percy). PhagoBurn therefore represented the formalisation pre-existing cooperation.

3. DESCRIPTION OF THE MAIN S&T RESULTS / FOREGROUND

The PhagoBurn project was divided into **7 matching Work Packages (WP)**: 5 related to R&D activities, 1 dedicated to Project Management & Ethics and 1 to Dissemination and Communication activities.

In details:

- **WP1** was related to the bioproduction of both anti *E. coli* and anti *P. aeruginosa* phage cocktails. The aim was to determine the optimal processes to produce and control both cocktails in order to comply with GMP standards before certification by national regulators.
- **WP2 & WP3** focused on implementing and conducting the PhagoBurn clinical trial.
- Subsequent analyses of biological samples as well as biological and clinical data were carried out within **WP4 & WP5**. Both packages included also all required and relevant statistical analyses.
- **WP6** aimed at ensuring the efficient administrative and financial management of PhagoBurn, including relevant ethical questions.
- Finally, **WP7** addressed activities related to communication, dissemination and future exploitation of project results.

The main Scientific and Technical (S&T) results and foreground obtained during Work Package (WP) 1 to WP5 are detailed below. Results related to WP7 are presented in section 4 – Potential impact and main dissemination activities and exploitation of results. At last, WP6 related to the management and the coordination of the project.

3.1 WP1 – BIOPRODUCTION PROCESS OF ASSEMBLY OF TWO PHAGE COCKTAILS ACCORDING TO GMP

WP1 aimed at preparing the two phage cocktails (anti *E. coli* and anti *P. aeruginosa*), in accordance to GMP standards. To this end, two main tasks were performed:

- Task 1.1 – Optimisation and validation of the phage bioproduction and purification process;
- Task 1.2 – Methods and tests of Quality Control (QC).

Synthesis of work performed

3.1.1 Optimisation and validation of the phage bioproduction and purification process

First, discussions were conducted at project initiation to define the needs to go from research-grade to manufacturing-grade processes. Several technical improvements appeared necessary. Notably the process had to be optimised to improve the scale of production, and to reduce the manufacturing duration stability. The formulation was also changed to increase the stability of the drug substances and drug product. The principle of purifying the phages using a simple hollow-fibre process was maintained, as it appeared to be applicable to all sorts of bacteriophages. In parallel, a manufacturing QC plan was established.

At the beginning of WP1, while the production of most of the 28 phages (for both cocktails, i.e. 15 for the anti *E. coli* phages cocktail and 13 for the anti *P. aeruginosa* cocktail) worked well, the composition of each cocktail was slightly modified as listed below due to different observations:

- In the anti *E. coli* cocktail: 1 phage was removed due to inconstant production yield, a second phage was removed due to its similarity with another one, and a third phage had reduced concentration due to limiting production yield but was kept in the cocktail because of its interesting complementary host range;
- In the anti *P. aeruginosa* cocktail: 1 phage was removed due to insufficient production yield.

The impact of these changes on the efficacy spectrum of targeted bacteria species proved to be negligible given some redundancy (deliberately wanted) between the host ranges of the phages composing each cocktail.

In addition to the optimisation and validation of the process, several intermediate tasks were performed. The bacteria used in the production were expanded, stored and organised as cell banks, and the phages were also expanded, stored and organised as phage stocks.

Furthermore, several exchanges took place between Pherecydes Pharma and the three national medical agencies in the countries in which the clinical trial was planned to be conducted (ANSM in France, AFMPS in Belgium and Swissmedic in Switzerland). Notably, the ANSM required the phage cocktails to be produced under the constraint of a sterile product (according to the European Pharmacopeia monograph 2008:0927) in aseptic conditions. This request was unforeseen given that:

- the clinical trial targeted topical applications, where current practices do not necessarily require sterile products,
- the proposed plan to ensure the safety of the products was already at a high level, given the early stage of development of the products,
- the benefit/risk ratio was thought to be well-balanced (while the infection can be extremely severe, possible side effects were expected to be likely insignificants).

Due to this increased level of manufacturing constraints, it became necessary to produce the bacteriophage cocktails in a specific class A containment environment. For that, a class A isolator was used in a GMP class B at Clean Cells. The consortium supported the strategy of buying and installing a dedicated isolator at Clean Cells manufacturing site and to implement its GMP use to produce the phages therapeutic products. Setting-up this change in the manufacturing process led to a 9 months delay in the project production plan.

Furthermore, since products were made of 12 to 13 individual phage strains (i.e. active substances), the ANSM recommended providing hospitals with single drug products containing either 12 or 13 mixed “equimolar” phages. Indeed, the agency wanted to reduce the risk of mixing errors, in case products would be provided as 12 or 13 individual vials that would be mixed together at the Point of Care (POC). Although logical and agreed by the Consortium members, this recommendation had significant impact on the clinical trial, as explained in other WP reports (WP3 and WP4, see below).

Following these recommendations, manufacturing Critical Process Parameters (CPP) were identified. On this basis, Pherecydes Pharma independently worked to improve its original process to decrease or eliminate the risks associated with such CPP, in further manufacturing campaigns. Specifically, the process was optimised to:

- Limit open phases during manufacturing and ensure better sterility standards;
- Significantly reduce:
 - o Endotoxin levels in the final dose of drug product;
 - o Host Cell DNA (HCD) contaminant level in the final dose of drug product;
 - o Host Cell Protein (HCP) contaminant level in the final dose of drug product;
- Improve phage stability through formulation changes;
- Increase phage yield, including those of poor yielding phage strains;
- Reduce the number of production bacteria strains;
- Improve QC measures in order:
 - o To reduce the risk of phage cross-contaminations;
 - o Eliminate the risk of contamination by other unexpected phages.

Overall, the aims were i) to broaden the range of bacteriophage-based drug products that could be produced in the future through an optimised manufacturing process and; ii) to expand therapeutic applications in which phages could be used (e.g. nebulisation or systemic injections) and in which the concentrations of bacterial contaminants should be even lower.

3.1.2 Method and test of Quality Control

The goal was to develop and validate the methods for the characterisation of each phage batch, notably in terms of identity, purity, safety and stability.

Accordingly, the strategy for QC was set up for:

- The phages banks, belonging to the final products,
- The cell banks,
- The production intermediates or bulks,
- The drug substances,
- The drug product.

As part of the GMP-grade, some of the QC needed to fulfil the guidelines had to be available at Clean Cells facility. These assays were already validated according to the ICH Q2(R1) text, or were compendial to specific sections of the European Pharmacopoeia.

GMP was not required for assays used to characterise the starting biological materials, i.e. the bacterial cell banks and the phage stocks produced under R&D grade, prior to establishing the manufacturing master/working bacterial lines and phage stocks. On this basis, some assays were developed and validated according to the pharmaceutical requirements (notably, ICH Q2(R1) and European Pharmacopoeia 5.14). In addition, some assays were used only for characterisation, so a full validation was not performed. These assays were informative in terms of routine product characterisation (e.g.: RFLP or host range analysis).

The following tables summarise the different QC methods that were set up, developed, and/or validated for QC of phages or bacteria all along the project.

QC for Phages	Methods	Description	Status of the method
Viability	Titration	Titration of bacteriophage on LB agar plate inoculated with specific bacteria. The enumeration of bacteriophage corresponds to the count of lysis plaque on serial dilution of the product.	Developed and validated (GMP-grade: release test for phages banks, drug substances and pharmaceutical products)
Identity	Host range	Evaluation of the range of bacteria that each bacteriophage could infect.	Developed w/o validation (supporting characterisation)
	Full genome sequence	Determination of the whole sequence of each phage by High-Throughput Sequencing	Outsourced without validation (supporting characterisation)
	DNA restriction profile	Digestion by specific enzyme of the DNA bacteriophage and migration on the bioanalyzer system to obtain digestion restriction profile.	Developed and validated (GMP-grade: release test for phages banks and drug substances)
	Genotyping – RAPD-PCR based method	Random Amplified Polymorphic DNA assay performed by end point PCR. A single primer hybridized randomly on the DNA sequence to generate a specific PCR products profile observed by migration on the bioanalyzer system.	Developed and validated (GMP-grade: release test for phages banks and drug substances)
	Protein profile	Determination of the protein profile related to the bacteriophage by migration of protein extract on the bioanalyzer system	Developed w/o validation (supporting characterisation)
	Morphotype by e.m.	Observation of the morphology and the size of each bacteriophage by electronic microscopy	Outsourced w/o validation (supporting characterisation)
Purity	Sterility	Automated contaminant microbial detection with the Bac T/Alert system.	Validated method available before project start (GMP-grade: release test for phages banks, drug substances and pharmaceutical products)
	Bioburden	Quantitative measurement of the bacterial load	Validated method available before project start (GMP-grade: release test for drug substances and pharmaceutical products)
	Endotoxins	Chromogenic quantitative and kinetic assay for the detection of Gram negative bacterial endotoxins	Validated method available before project start (GMP-grade: release test for drug substances and pharmaceutical products)
	Host Cell DNA	Quantification by real time PCR of host cell DNA (Assay <i>E. Coli</i>)	Validated method available before project start (GMP-grade: release test for drug substances and pharmaceutical products)
		Quantification by real time PCR of host cell DNA (Assay <i>P. Aeruginosa</i>)	Developed and validated (GMP-grade: release test for drug substances and pharmaceutical products)
	Total proteins	Measurement of total protein concentration by spectrophotometry with a BCA assay	Developed w/o validation (supporting characterisation)
Visual aspect	Observation by two operators of the visual aspect of the product	Developed and validated (GMP-grade: release test for pharmaceutical products)	
Chemistry	pH	Determination with a pH-meter of the pH of the product	Developed and validated (GMP-grade: release test for pharmaceutical products)

QC for bacteria	Methods	Description	Status of the method
Viability	Titration	Titration of bacteria on LB agar plate. The enumeration of bacteria corresponds to the count of colony on serial dilution of the product.	Developed and validated (GMP-grade: release test for bacteria banks)
Identity	Full genome sequence	Determination of the whole sequence of each bacterium by High-Throughput Sequencing	Outsourced w/o validation (supporting characterisation)
	Strain Characterization	Analyse by sequencing of the 16S DNA sequence of each bacteria and comparison to available public databases	Outsourced w/o validation (GMP-grade: release test for bacteria banks)
	Genotyping – RAPD-PCR based method	Random Amplified Polymorphic DNA assay performed by end point PCR. A single primer hybridized randomly on the DNA sequence to generate a specific PCR products profile observed by migration on the bioanalyzer system	Developed and validated (GMP-grade: release test for bacteria banks)
	Plating	After isolation on LB agar plate, macroscopic observation of the colonies.	Developed and validated (GMP-grade: release test for pharmaceutical products)
Purity	Prophages detection	After induction with Mitomycin C	Developed w/o validation (supporting characterisation)

All QC methods that were initially planned for the release of the phages as pharmaceutical products were thus made available and fulfilled the requirements of GMP-regulated products.

Furthermore, some questions were raised by national Agencies during the project course, mainly related to the development of an analytical method for the quantification of the biological activity of each cocktail as a whole, and of each active substance of a cocktail. Unfortunately, the development of analytical methods for measuring the biological activity of a cocktail (i.e. its global activity) and of each of its active components (each phage strain activity) was particularly challenging, due to the high number of phage strains per cocktail and the overlaps of phage tropisms for each bacterial strain(s) used for single phage titration.

Indeed, the stability overtime of a particular phage, necessary to determine the shelve life of a phage cocktail, may be assessed by a handful of techniques such as:

- Detecting the presence of a phage strain (its capsid) using a monoclonal antibody (Mab),
- Following its biological activity by titration on a bacterial strain,
- Measuring the concentration of its particular DNA, for instance using oligonucleotide primers.

However, no one has ever tried to implement them for cocktail with a high phage type number.

At first, these measures require to have a reagent or a bacterial strain that is highly specific to the phage. Then, the detection methods need to detect the active bacteriophages and not some of its components (DNA, proteins, etc.) that do not fully reflect the integrity of the organism and its biological activity. Following discussions with P.A.R.I.S. (a SME specialised in Mab development), the strategy of developing Mabs was quickly abandoned due to its high cost (5 to 10 000 € per antibody multiplied by 25 different Mabs) and the lack of specificity of each Mab, which could have led to detecting not only the targeted phage but others in the cocktail mix. In addition, Mabs are not suited to characterize infectious viral particles.

Furthermore, the strategy of detecting a phage through Polymorphism Chain Reaction (PCR), using specific primers, was not selective enough and led to detecting the DNA of phages with related DNA sequences in the cocktail mix. In addition, the amount of phage DNA detected did not match with the number of viable phage particles since it included degraded and inactive phages in the form of capsids without tails, for instance. Therefore and despite elegant, this method would have led to an overestimation of the viable phage particle count, if conclusive.

At last, when a phage is mixed with several other phages, which target the same bacterial strains, single phage titration is accurate if different bacteria are available to titer each single phage. Since our phages presented overlapping host ranges, such specific bacteria could not have been identified although many strains were tested.

The purpose of a cocktail strategy containing multiple phages was i) to make a product with a antibacterial species spectrum as wide as possible and ii) to alleviate the risk of bacteria phage-resistance. Indeed, bacteria turning resistant to a specific phage would be lysed by another phage present in the same cocktail. Nevertheless, it has been also recently described in the literature that phages can cooperate or compete to lyse bacteria. Even more, depending on the relative ratio between bacteriophages and bacteria, killing from without can occur with bacteria being lysed by multiple phage without being able to generate new phage progeny.

GMPs require to track the shelf life of a drug product through the stability of its components. These phenomena, alone or in conjunction, including the technical challenges described above, made it impossible for us to follow the activity of a precise phage in a complex cocktail, and the activity of a whole cocktail. These data confirm the fact that phage cocktails with multiple phage strains are to be avoided.

Finally, the risk assessment of the first manufacturing process developed for making the clinical batches has prompted Pherecydes Pharma to develop new relevant QC methods:

- For the quantification of excipients used to improve the process,
- And to measure or improve the dosage of:
 - o Residual HCDNA,
 - o Residual HCP,
 - o Raw materials (enzymes and chemicals) traces used by Pherecydes Pharma to improve the manufacturing process.

Synthesis of results / foreground obtained at the end of the project

All requirements to meet the expectancies of GMPs were conducted, notably in terms of:

- Organisation of the production Company (Clean Cells),
- Qualification of the process,
- Qualification of the equipment,
- Qualification of raw material,
- Staff training,
- Documentation.

Clean Cells, with the support of Pherecydes Pharma, carried out all the necessary steps to finalise and validate both phage cocktails clinical batches, including, QC methods and tests. Altogether, the compliance with GMP requirements was certified by the relevant national agency, i.e. ANSM. Notably, Clean Cells was granted the “pharmaceutical laboratory” status by ANSM. The SME obtained on April 29, 2015 the full authorisation to produce both Investigational Medicinal Products (IMPs) related to the project, which were released for the clinical trial launching in July 2015.

The process for authorisation involved three rounds of inspection and continuous interactions, with the three agencies, especially because phages appeared as a new type of product, without a pre-established European pharmacopeia framework. Due to this absence of pre-existing regulatory context, the project attracted a lot of interest, as well as, a lot of questions and expectancies. While this work was crucial to obtain the authorisation of manufacturing IMPs, it took much more efforts than initially planned.

To conclude, the production process was optimised and the dedicated QC developed. Nevertheless, two aspects were unachieved (*see explanations above*):

- The development of validated methods for titrating the global activity of a cocktail overtime.
- The development of validated methods for titrating each individual phage in the final product overtime.

Yet, this did not prevent the successful launching of the PhagoBurn clinical trial. However, the lack of a validated method of measurement of the phage cocktail shelf life led to the interruption of the clinical trial between January and June 2016 (see WP3 below) and may also explain some of the study results.

3.2 **WP2 – PREPARATION OF THE PHASE I-II CLINICAL STUDY**

WP2 aimed at efficiently preparing the phase I-II clinical study of the PhagoBurn project. To this end, the following tasks were performed (WP3):

- Finalisation and validation of the clinical trial protocol at the Consortium level,
- Completion of all ethical aspects necessary to launch the trial,
- Preparation of the Investigation Drug Brochure (IDB) and of all additional necessary documentation,
- Submission of all relevant documents (protocol, IDB...) to the national authorities,
- Discussions with the national authorities before launching the trial, following the submissions of all necessary documents and finalisation of these regulatory documents following the remarks of national authorities.

Synthesis of work performed

3.2.1 Finalisation and validation of the clinical trial protocol at the Consortium level

Numerous discussions and exchanges took place to obtain a finalised version of the clinical trial protocol, ready to be submitted for validation to the national authorities:

- Constant exchanges between the Principal investigator (Percy) and all other investigators involved in PhagoBurn (partners and subcontractors);
- Exchanges with partners involved in WP1 (Pherecydes and Clean Cells) to adapt the clinical trial protocol to the bacteriophage cocktails production process and GCPs;
- Discussions with the regulatory agencies of the three countries and subsequent adaptation of the protocol to comply with their requests.

The following table presents the list of meetings, which took place with the regulatory agencies to finalise a first version of the protocol:

Date	Place	Participants
5 December 2013	ANSM	Meeting with ANSM (Percy, Pherecydes, Clean Cells, ASPE Conseil)
29 January 2014	Swissmedic	Meeting with Swissmedic (Pherecydes, Clean Cells, CHUV, Percy)
3 March 2014	ANSM	Meeting with ANSM (Percy, Pherecydes, Clean Cells, SD pharmaconsulting)
5 May 2014	AFMPS	Meeting with AFMPS (Percy, Pherecydes, Percy, RMA)

Based on these meetings outcomes, a first version of the protocol, delivered in November 2013 by the Consortium (project month 6) was amended.

The following elements correspond to a non-exhaustive synthesis of the discussions:

- Products application should be standardised using:
 - o The same dressing across all investigational centres: Algosteril™ from Brothier Laboratories,
 - o The same solvent (0.9% NaCl for irrigation) used to dilute 1000-fold the genuine treatment.
- Bacteriological samples should be standardised using the same swabs (eSwabs for collection and preservation of aerobic, anaerobic and fastidious bacteria from COPAN was provided by Pherecydes Pharma to all participating centres);
- A maximum of 1000 cm² skin surface may be treated (i.e. 55% of an adult man skin), with each phage preparation after product dilution at the hospital burn unit;
- Patients should be included in the trial after they show up a positive preliminary diagnosis to *E. coli* or *P. aeruginosa* infection (2 days before inclusion);

- As bacteriophages may be sensitive to local antiseptics, products containing silver having a negative interaction with phages must be especially avoid. Therefore, Betadine™ cream was applied onto the patient burn surface, at the time of microbiological diagnostic sampling , i.e. 2 days before inclusion);
- Randomisation, stratified according to presence/absence of antibiotic treatment at inclusion, should take place when the strain sensibility and the ongoing antibiotics are known;
- Only in-hospitalised patients should be included in the trial;
- Only patients with a mono infection by *E. coli* or *P. aeruginosa* should be included in the trial;
- The impact of phages on intestinal microbiome should be documented by bibliographic data;
- Bacteriophages cocktails to be used during the trial should be sterile;
- Concerning dose, a rationale should be provided to explain why the final dose and regimen has been retained.

Following these meetings, a new version of the clinical protocol was prepared and the steps described below were carried out:

- Synopsis of the clinical trial was filed online by Statitec on www.clinicaltrial.gov in June 2014;
- In November 2014, Percy noted that there were still some imprecisions remaining within the protocol. Thus slight modifications of the clinical protocol were decided, requiring the amendment of the clinical protocol;

This new finalised version, issued in March 2015, was submitted by Statitec to the ethical committees (see below) and to the regulatory agencies in France, Switzerland and Belgium, and updated on www.clinicaltrial.gov.

3.2.2 Completion of all ethical aspects necessary to launch the clinical trial

To be able to launch the clinical trial, it was necessary to seek and obtain, in each participating country, all requested national authorisations from relevant ethical committees.

The Consortium, with the support of the CRO Statitec, implemented all necessary actions towards the achievement of this objective, and authorisations were ultimately obtained from the three concerned ethical committees after careful examination of the clinical protocol and of all other necessary documentation (information and consent forms, Investigator Brochure, investigator list, insurance certificate, etc.):

- The French ethical committee (Comité de protection des personnes Ile-de-France III) granted its approval on May 20th, 2014;
- The Swiss ethical committee (Commission cantonale de Vaux d'éthique de la recherche sur l'être humain) granted its approval on October 15th, 2014;
- The Belgium ethical committee (Comité d'éthique hospitalo-facultaire universitaire de Liège [707]) granted its approval on November 18th, 2014.

The new version of the protocol, issued in March 2015, did not change the clinical trial original process. Therefore, this amendment to the original protocol was accepted by the three ethical committees.

3.2.3 Preparation of the Investigation Drug Brochure (IDB) and all additional necessary documentation

Apart from the IDB, the following documentation had to be prepared prior launching the clinical trial:

- Investigator Medicine Product Dossier (IMPD);
- Randomisation guide;
- Data management guide;
- Data validation plan.

The IDB was prepared in a 1st version, with the support of CRO Statitec, then slightly modified, as explained, to comply with the ultimate modifications of the clinical protocol (see above).

With the support of specialised outsourced pharmacists (SD-Pharma Consulting and ASPE), identified at first by the CRO and then directly contracted by Pherecydes Pharma for practical reasons, two IMPDs were prepared following the progression of WP1, and finalised in March 2015.

Finally, documents related to randomisation and data (randomisation guide, data management guide and data validation plan) were prepared and validated before the actual launching of the clinical trial, as determined in the project work plan.

3.2.4 Submission, discussions with the national authorities and finalisation of regulatory filing necessary to launch the clinical trial

The final version of the clinical trial protocol, along with the IMPDs (one for each bacteriophage cocktail) and the final version of the IDB were submitted to the French, Belgian and Swiss regulatory authorities in March 2015. Following the submission of these documents, numerous exchanges took place with these agencies between April and July 2015 (through a set of questions/answers). The Consortium provided all necessary relevant information, enabling obtaining the clinical trial authorisations on June 24th, 2015 for France and Belgium and on July 7th, 2015 for Switzerland, with the commitment to provide an updated version of the regulatory documents in the following months.

In December 2015, the Consortium submitted a new version of the regulatory documents (clinical protocol and IDB). These documents did not change the clinical trial original process, except on one minor point: inclusion of the bacteriological samples study at visit D14 (not planned in the first version of the protocol).

3.2.5 Additional work performed during the clinical trial implementation

The clinical trial had to be stopped in January 2016 (see WP3). In this context and due to the new project orientation (regarding the clinical trial implementation, see WP3 below), it was necessary to submit an updated version of the clinical protocol and an updated version of the IMPD, to allow restarting the clinical trial. This regulatory procedure was implemented for the cocktail *P. aeruginosa* only. Indeed, because of a very poor inclusion rate of patient infected by *E. coli* (1 only during the first part of the study), the clinical evaluation of the anti-*E. coli* phage cocktail was stopped. Regulatory authorities were informed of that decision.

Finally, the authorisation to relaunch the trial with the anti-*P. aeruginosa* cocktail was obtained on May 31st, 2016 in France and on July 7th, 2016 in Belgium. Because no patient had been included at CHUV since the beginning of the trial (due to a major internal reorganisation of the burn unit and the departure of the principal investigator), it was decided to cancel restarting the trial in Switzerland. This decision was announced to Swissmedic in September 2016 and confirmed in December 2016.

The table below synthesises all regulatory documents and their versions, as prepared during the course of PhagoBurn.

	IMPD	IDB	Clinical protocol
<p><i>E. coli</i> cocktail PP0121 clinical batch</p>	<p>version 001 release date: 2015-04-02: version sent to obtain the authorisation for the clinical trial</p>	<p>version 001 release date: 2014-06-05</p> <p>version 002 release date: 2015-03-20: sent to get approval of the clinical trial</p> <p>version 003 release date: 2015-12-22: updated following questions received from the authorities</p>	<p>version 1 2013-2014 working project</p> <p>version 2 release date: 2015-03-20: version sent to obtain the authorisation for the clinical trial</p> <p>version 3 release date: 2015-11: updated version following questions received from the authorities</p>
<p><i>P. aeruginosa</i> cocktail PP1131 clinical batch</p>	<p>version 001 release date: 2015-04-01: version sent to obtain the authorisation for the clinical trial</p> <p>version 002 release date: 2016-05-20: version sent to obtain the authorisation to relaunch the clinical trial</p>	<p>version 001 release date: 2014-06-05</p> <p>version 002 release date: 2015-03-20: sent to get approval of the clinical trial</p> <p>version 003 release date: 2015-12-22: updated following questions received from the authorities</p>	<p>Version 4 release date: 2016-5: sent to obtain the authorisation to relaunch the clinical trial but <u>only with PP1131 product</u></p>

Synthesis of results / foreground obtained at the end of the project

Pherecydes Pharma and the clinical partners (Percy, RMA and CHUV) carried out the necessary steps to efficiently prepare the phase I-II clinical study. These steps included:

- The finalisation and validation of the clinical protocol;
- The completion of all ethical aspects necessary to launch the trial: all requested national authorisations from relevant ethical committees were obtained by the Consortium;
- The preparation of the IDB and of all additional necessary documentation (IMPD, Randomisation guide, Data management guide and validation plan).

To conclude, the authorisation to launch the clinical trial was obtained (June and July 2015), thus demonstrating the successful achievement of this second Work Package.

3.3 WP3 – IMPLEMENTATION OF THE PHASE I-II CLINICAL STUDY

WP3 aimed at ensuring the implementation of the PhagoBurn clinical study. To this end, the following tasks were performed:

- Organisation of the clinical trial,
- Opening of the clinical centres,
- Recruitments and patients' follow-up,
- Management of the Development Safety Update Report (DSUR).

Synthesis of work performed

3.3.1 Organisation of the clinical trial

In accordance to PhagoBurn Consortium agreement, the following roles were attributed:

- Clinical Sponsor: Pherecydes Pharma,
- Principal Investigator: HIA Percy (French Military Health Service, project coordinator),
- Clinical Investigators: several clinical centres took part in the study, either as project beneficiaries (PhagoBurn partners) or as subcontractors of some beneficiaries (four clinical centres were added to the project during its course to increase the recruitment capacity).

Briefly:

HIA Percy – SSA Clamart (France)	Principal investigator
CHUV Lausanne (Switzerland)	Clinical partner
Queen Astrid – RMA Brussels (Belgium)	Clinical partner
St Joseph St Luc hospital Lyon (France)	Subcontractor (Pherecydes)
CHU Nantes (France)	Subcontractor (Pherecydes)
CHU Liège (Belgium)	Subcontractor (RMA)
Grand Hôpital Charleroi-Loverval (Belgium)	Subcontractor (RMA)
Notre-Dame de Bon Secours Metz (France)	Subcontractor (Pherecydes)
Hôpital de la Conception Marseille (France)	Subcontractor (Pherecydes)
CHU Bordeaux (France)	Subcontractor (Pherecydes)
HIA Toulon – SSA Toulon (France)	Member of SSA (coordinator)

- Clinical Research Organisation (CRO): Statitec (France, Pherecydes subcontractor) was selected for the day-to-day coordination of the clinical trial (the list of its activities is introduced in the below table);
- Clinical Research Assistant (CRA): a specific CRA was hired by Pherecydes Pharma (through Statitec) to follow-up inclusions, notably at Percy military hospital (support in the e-CRF data inclusion);
- Data and Safety Monitoring Board (DSMB): an independent DSMB was gathered, composed of 2 clinicians, 1 statistician and 1 microbiologist;
- Logistics: several contacts were implemented by Pherecydes Pharma for:
 - o The transport of biological samples: **OCT Santé** was selected;
 - o The labelling, storage, transport and destruction of the IMP: **Amatsi group**;

- The providing of dressings: **Laboratoires Brothier**;
- The clinical insurances in 3 countries: **CHUBB**.

In addition, at the request of regulatory agencies, a specific agreement was concluded between Pherecydes Pharma (Sponsor) and Clean Cells (provider of the clinical batches) to define the role and responsibilities of each organisation in manufacturing.

The roles of each entity in the trial can be synthesised as follow:

Pherecydes Pharma (as Sponsor)	Percy (as Principal Investigator)	All clinical centres	CRO Statitec (Pherecydes subcontractor)
<ul style="list-style-type: none"> - Recruitment & follow up - Support to centres for data filing in e-CRF - Centre support for clinical trial (ordering shipping, etc.) - Microbiological procedures - Management of IMPD - Collect of swabs for ancillary endpoint - Clinical insurances - Management of DSMB through CRO - Pharmacovigilance through SD and Aspe 	<ul style="list-style-type: none"> - Global coordination of the clinical trial - Clinical centre initiation - Recruitments for the clinical centre - Recruitment & follow up for all centres - Management of serious events - Support in calculating unit cost per patient 	<ul style="list-style-type: none"> - Staff training - Clinical protocol set up & driving - Recruitment - Management of serious events - e-crf reporting 	<ul style="list-style-type: none"> - Preparation of the trial master file, investigator files - Contract management with each clinical centre - Initiation, monitoring and close out visits - Site management and reporting - SAE management - Global trial management - Set-up of pharmacovigilance - Set up of DSMB meetings - e-CRF set-up, monitoring and closing - Centre closing - Statistics - Clinical study reporting

3.3.2 Opening of the clinical centres

Before being able to recruit, all clinical centres were “opened”, which included the following actions:

- Training of the centre personnel for the use of the e-CRF;
- Detailed introduction to the clinical trial protocol, including treatment, dressing and e-swab orders;
- Detailed presentation of the microbiological procedure for testing the primary endpoint, including swab orders and for specific centres bacteria selective media orders;
- Preparation of hospital conventions, with the calculation of a unit cost per patient.

Opening visits all occurred in June and July 2015, except for CHU Bordeaux, which occurred in September 2015. Regarding CHU Marseille, the conditions imposed by the microbiological department of the hospital to the hospital management led to the impossibility to set up an agreement between Pherecydes and the hospital. As a consequence, no patients could be included by this hospital.

In addition, the consortium took advantage of the SFETB meeting in June 2015 (<http://tmsevents.fr/NOUVEAUSITE/evenement/mainframe/sfetb2015/>) to take part in a common e-CRF training realised by Statitec.

3.3.3 Recruitments and patients' follow-up

The PhagoBurn clinical study was launched in July 2015 in three countries: France, Belgium and Switzerland. The first recruitment occurred on July, 22nd, 2015.

For the first time at world level, a multicentric clinical trial, designed to evaluate blindly the efficacy and safety of phage products in the treatment of bacterial infections, was initiated.

In the framework of the PhagoBurn clinical study (n° EUDRACT 2014-000714-65), a major issue encountered was related to the speed of patients' inclusion, much slower than expected. As of January 15th, 2016 (i.e. 6 months after the launching of the trial and right before the trial interruption), recruitment status was as described in the table below:

	<i>E. coli</i> arm	<i>P. aeruginosa</i> arm
CHUV	0	0
HIA Percy	1	5
St Luc / St Joseph	0	1
CHU Nantes	0	3
RMA (Reine Astrid)	0	4
Grand Hôpital Charleroi-Loverval	0	0
CHU Liège	0	2
CHU Bordeaux	0	0
CHR Metz Thionville	0	2
Marseille Conception Hospital	0	0
Toulon Military Hospital	0	0
TOTAL Recruitments	1	17

Unfortunately, these figures are in line with the experience reported by other Companies at the Stockholm IMI meeting of January 22nd, 2016. Indeed, trials targeting antibacterial products are facing a significant recruitment issues.

In addition, the PhagoBurn clinical trial had to be stopped on January 15th, 2016, during 5 months. Indeed, and as a reminder, when the study was launched in July 2015, the Consortium was facing two technological challenges (*linked to WP1*):

- Providing a reliable quality controlled method for titrating the global activity of the cocktail, overtime,
- Providing a reliable quality controlled method for titrating each individual phage (a drug substance or DS) in the final product (DP: a cocktail), overtime.

Because such methods were not ready yet, national agencies agreed that half a dozen reference phages (RP) that belonged to each cocktail would be eligible to reflect the shelf lives of the cocktails. These RP phages had been produced and stored a year earlier than the phages of each cocktail, when the phages belonging to each cocktail had been produced in January and February 2015.

Agencies agreed that RP could mimic the shelf-life of each clinical Drug Product (DP): the assumption was that the stability overtime of RP was acceptable to estimate the stability of the DP, although each RP phage was single whereas each cocktail handled a dozen of phage strains.

RP displayed already 24 months of shelf-life stability at the time of clinical trial interruption. Accordingly, the same shelf-life period was granted to the DP, postponing shelf-life of the cocktail a year beyond. Hence, each cocktail was granted to be used until respectively Jan. 15th, 2016 and Feb. 18th, 2016.

Of course, the assumption was that quality control methods would be finalised in the coming months after the trial beginning.

Nevertheless, faced with significant technical challenges, the Consortium was unable to provide the method requested on time and thus, the decision was taken on January 15th, 2016, to freeze patients' recruitment.

Following that decision, a conference call meeting was organised with ANSM on January 28th, 2016, to inform them of the encountered difficulties. In the absence of data, the regulatory agency confirmed the "recruitment freeze" and requested additional information about the clinical batches: pH measurement, sterility and appearance. These data were generated by Clean Cells and fully provided to ANSM in March and April 2016.

In addition, the agency asked to provide shelf-life stability data about two sets of 5 and 6 different Reference Phages (RP) belonging to the cocktails. These had been produced a year ahead of the phages from the cocktails and enabled to extrapolate stability results to the phages of each cocktail. Furthermore, stability data from each individual phage of the cocktails and of the cocktails themselves (although with an unqualified method) were also given.

The results enabled Pherecydes Pharma to be granted by the ANSM the extension of the clinical batches shelf-life up to January 15th, 2017. Thus, on the basis of the work performed by Clean Cells and Pherecydes Pharma, the clinical trial was relaunched at the end of May 2016 in France and in early July in Belgium. Because of the lack of patient's recruitment in Switzerland, it was decided to stop the trial in that country.

Due to a poor recruitment pace and following this setback, it was decided to lower the recruitment plan, with the final objective of including 40 patients before January 15th, 2017.

At this clinical trial closing date, recruitments realised were as shown in the below table:

For PP1131 and <i>P. aeruginosa</i> infections		Total Included N=27	Total treated N=26
Center	N	27	26
	Missing data	0	0
	Hôpital d'Instruction des Armées Percy	11 (40.7%)	11 (42.3%)
	Hôpital Militaire Reine Astrid (Brussels)	5 (18.5%)	5 (19.2%)
	Hôpital Saint Luc/Saint Joseph	2 (7.4%)	2 (7.7%)
	CHU Nantes	5 (18.5%)	5 (19.2%)
	CHR Metz	3 (11.1%)	2 (7.7%)
	CHU Sart-Tilman (Liège)	1 (3.7%)	1 (3.8%)

Only one patient was treated with PP0121 against an *E. coli* infection at Percy Hospital.

The 28 patients were prospectively included and randomized between 21/07/2015 and 18/12/2016 (including a brake of 5 months), i.e. for 6 months during the first period and 7 months during the second one: overall, the inclusion time frame was 13 months. Seventeen patients were included during the first period. Eleven patients were included during the second one.

Several centers did not include any patient:

- CHU Marseille, because of an impossibility to contract with the hospital administration due to specific financial requests of the microbiology department in charge of rating the primary endpoint,
- CHU Bordeaux, because of issues with the e-CRF,

- CHU Lausanne, because of a restructuration in the burn unit, including the departure of the local investigator without replacement,
- Toulon military hospital and Loverval (Charleroi) for a lack of eligible patients.

3.3.4 Management of the DSUR

Four major serious safety concerns were observed during the first part of the clinical trial, all with patients enrolled in the comparator (Flammazine®) arm. The following table describes all cases of patients withdrawn from the study.

Patient Identifier	Date of Collection	Reason for Drop-out	Specification other reason	Last visit	Last date of visit	Last treatment	Last Date of treatment
01-01	02/08/2015	Death	Caused by fast cutaneous invasive fungal infection	D7	29/07/2015	D6	28/07/2015
01-03	18/08/2015	Other	Transfer in a reeducation centre	D14	16/08/2015	D6	09/08/2015
01-04	20/10/2015	Other	Transfer in a reeducation centre	D14	19/10/2015	D6	11/10/2015
01-08	29/07/2017	Cure	Full cure achieved, no further follow-up necessary	D14	29/07/2016	D6	21/07/2016
01-09	22/08/2016	Patient's decision		D1	22/08/2016	D1	22/08/2016
03-05	15/01/2016	Other	End of product shelf-life	D3	15/01/2016	D2	14/01/2016
05-01	15/10/2015	Other	Patient transfer for inclusion into another clinical trial upon decision of surgeon without investigator consent	D2	14/10/2015	D2	14/10/2015
08-01	08/11/2015	General intolerance		D14	02/11/2015	D6	25/10/2015
08-03	16/11/2015	Death	Patient died	D7	14/11/2015	D6	13/11/2015
08-04	07/10/2016	.	Investigator decision	D0	07/10/2016	D0	A

There was no safety concern with the use of the PP1131 phage cocktail in patients presenting *P. aeruginosa* burn wound infections as there was no withdrawal observed for safety reasons and no serious adverse events (SAE) occurring. 4 of 5 SAEs that were reported since the study start were concerning exclusively patients under treatment with the comparator (Flammazine®). These SAEs were related to infections (1 not controlled infection, and 3 septic shocks). One of 5 SAEs was concerning a patient under treatment with the PP1131 phage test product. This SAE – occurring after treatment end but 4 days before the study end – was not unexpected and was related to systemic and multiple organ infection in a very debilitated patient.

In addition to the study treated patients, two compassionate use treatments were requested for patients severely infected by *P. aeruginosa*:

- The first one belonged to the study and was initially treated with the comparator treatment. Because the treatment failed, the hospital requested the ANSM to be able to treat the patient with PP1131,
- The second one was not part of the study and was treated for a severe infection following amputation.

Synthesis of results / foreground obtained at the end of the project

The PhagoBurn clinical trial was effectively launched in July 2015. As such, it represented a world first: the start of a multicenter clinical study implemented according to standards of good manufacturing and clinical practices to evaluate the tolerance and efficacy of phages to treat bacterial infections in burn wounds.

Consequently and despite some issues encountered during the study implementation, the PhagoBurn trial was critical to Pherecydes Pharma (and the whole Consortium) for the acquisition of a clear understanding of essential elements to be considered for further development and clinical evaluation of phage therapy. These include the following aspects:

- Cocktail composition: rather than including too many bacteriophages in a product, leading to unsolvable shelf life and stability issues, the development of “mini-cocktails” (i.e. max. 3/5 phages) should be favoured including methods for titrating each phage strain in the drug product;
- Target population and inclusion/exclusion criteria: mono-infections may not be a majority, especially in the field of burn and open wounds. This should be carefully addressed in a clinical trial protocol. Inclusion criteria should take in consideration the standard level of bacterial colonisation of the treated site and avoid considering this background level as a factor of non-inclusion;
- Preliminary diagnosis: a preliminary diagnosis aiming at determining the efficacy of a cocktail prior to treatment is probably essential for phage therapy, thus enabling a strategy of tailored treatment.
- The effect of the treatment provided before phage therapy or comparator should be carefully evaluated to fully account for its inherent activity on targeted pathogens, as it may reduce the number of patients with evaluable study treatment efficacy, and thus influence sample size determination;

Primary evaluation criterion: in phage therapy clinical trials, whenever robust clinical primary endpoints are not available and one must rely on surrogate endpoints, the most appropriate one may not be the same as for the evaluation of antibiotics.

Thus, the PhagoBurn learning experience represents a central milestone for the successful development and clinical studying of future phage products.

3.4 WP4 – BIOLOGICAL SAMPLES ANALYSIS

WP4 aimed at analysing biological samples obtained through the implementation of WP3 (in compliance with the clinical protocol evoked in WP2).

Synthesis of work performed

3.4.1 Methodology

The clinical protocol introduced the methodology to be applied to perform microbiological analyses.

Two samples were collected. One was analysed in each hospital microbiology laboratory in a routine way. The second collected swabs were sent to Pherecydes Pharma for complementary analysis. This new analysis had three objectives: to count the number of *E. coli* or *P. aeruginosa* bacteria that grew from the samples, to count the phages and to measure the sensitivity/resistance of germs to phages cocktails and components. As an ancillary project, additional genomic analyses were also planned to be performed by partner CHUV.

To sum up:

Two swabs were collected each day on the wounds of all treated patients:

- One swab (tube n°1) was sent to the hospital microbiology laboratory for evaluating blindly the effect of the treatments (standard and phage cocktails) on *P. aeruginosa* or *E. coli* bacteria growth, on a day-to-day basis. The evaluation criterion is semi-quantitative;
- One swab (tube n°2) was properly stored.

Then, both swabs were shipped to Pherecydes Pharma:

- The tube n°1 was used by Pherecydes Pharma to count phages and bacteria;
- The tube n°2 was sent to the University of Lausanne for additional analysis (genomic analysis) in the framework of a separate project. This project aims at evaluating the impact on the skin microbiota of silver sulfadiazine or phage treatment. Lausanne University is currently looking for a financial support prior to initiate the study.

The detailed methodology is described hereafter:

Day -2: before the patient's inclusion in the clinical trial

- a) A standard swab is rubbed on the wound suspected to be infected by a bacterium, according to in-house operating procedures;
- b) Microbiological data are provided to the investigator preferably within two days after swabbing (up to three days is acceptable);
- c) If a patient is positive either to *E. coli* or *P. aeruginosa*, he/she may be included.

Days 0-7: during the patient's inclusion in the clinical trial

The ESwab model (Copan, pink cap) is provided by Pherecydes Pharma and no other swab model should be used during the clinical trial.

- a) An ESwabs (N°1) is rubbed (following a Z pattern) on the entire wound as described in accordance with the recommendations of the SFETB;
- b) The swabbing is repeated with a second ESwab (N°2);

- c) Each *ESwab* is placed in its proper tube according to the manufacturer's recommendations. The stick is broken and discarded in a standard bin dedicated to infectious material. After placing the cap, the *ESwab* is mixed gently by hand during 3 to 5 seconds;
- d) Each tube is annotated with the following information:
 - the name of the clinical trial: PhagoBurn,
 - the name of the investigator center,
 - the bacterial agent: *COLI* or *PYO*,
 - the patient n° or ID,
 - the date (day-month-year),
 - the time (HH:MM),
 - the body area where the swabbing has been done:
 - i. example if a single area: B6
 - ii. example if a larger area: B6-B8
 - the *ESwab* number: N°1 or N°2;
- e) Each day for each patient, the tubes containing the *ESwabs* are transferred to the hospital microbiology laboratory within a maximum of 2 hours after sampling at cool temperature to avoid phage multiplication.
- f) According to local laboratory practices, a sample list is provided to the laboratory including the following information: the patient's code and the collection day. Upon receipt, the laboratory checks that the samples received each day match with the list and that analyses have been performed on each of them.

A minimum of 16 ESwabs is sampled per patient during the study (without counting the swab performed at D-2)

Work to be performed at the hospital microbiology laboratory

Tubes at D-2: Standard D-2 swabs are used to identify the bacterial agent infecting the sampled wound. This identification is carried out according to the respective practices of the different investigator centres. An antibiogram is performed to check bacteria antibiotic resistance.

Eswab tubes n°1 are used for both bacterial identification and bacteria semi-quantification (through a method standardised and used by all investigation centres). Then they are stored at -80°C until shipping to Pherecydes Pharma.

Eswab tubes n°2 are stored at -80°C until shipping to Pherecydes Pharma.

Storage and shipping of Eswab tubes n°1 and n°2: at least a whole set of tubes n°1 and n°2 (supplemented with glycerol 80% and stored à -80°C) of a given patient must be grouped for shipping to Pherecydes, i.e. 16 swabs (8 tubes n°1 and 8 tubes n°2). Transportation is carried by a specialised transportation Company (OCT Santé). OCT Santé carries the samples from the centres to Pherecydes Pharma, in - 80°C temperature control containers (dry ice). Investigator centres must inform Pherecydes Pharma when samples are ready to be collected. Pherecydes Pharma is in charge of contacting OCT Santé for sample collect at each centre and delivery to its site.

Analysis of samples at Pherecydes Pharma facilities

Eswab tubes n°1: the analysis has three objectives:

- **Bacterial enumeration:** count the number of *E. coli* or *P. aeruginosa* that grow from the samples,

- **Phages enumeration:** count the phages in the samples,
- **Phages sensitivity/resistance test:** measure the sensitivity/resistance of germs to phages cocktails and components.

Eswab tubes n°2: upon reception, tubes are stored at -80°C, before shipping to Lausanne University, via a specialised transporter.

3.4.2 Biological samples analyses performed

As a reminder, the PhagoBurn clinical trial was launched in July 2015. In February 2016, i.e. after 6 months of clinical study, the Consortium decided to collect quickly all biological swab samples from treated patients, to check the efficacy of the treatments and indirectly confirm whether phages were indeed administered to patients through the clinical batches provided.

Thus, during the first part of the clinical study (July 2015 – January 2016), 17 patients were included, involving the performing of the following tasks:

- Swabs from 16 patients were collected, representing 494 eSwabs. They were sent to Pherecydes Pharma;
- Bacteria and phages were enumerated by Pherecydes Pharma in 247 eSwabs, as planned in the clinical protocol;
- When phages were identified in a sample, a phagogram was performed by Pherecydes Pharma;
- 51 phagograms using each of the individual phage (i.e. 13 phages for one cocktail and 12 phages for the other) as well as the phage cocktails were performed, representing 663 tests.

Then, during the second part of the clinical study (May 2016 – January 2017), 11 patients were included, involving the performing of the following tasks:

- Swabs from 12 patients were collected, representing 248 eSwabs. They were sent to Pherecydes Pharma;
- Bacteria and phages were enumerated by Pherecydes Pharma in 124 eSwabs, as planned in the clinical protocol;
- When phages were identified in a sample, a phagogram was performed by Pherecydes Pharma;
- 43 phagograms using each of the individual phage as well as the phage cocktail were performed.

To sum up, for the whole clinical study duration:

- Swabs from 28 patients were collected, representing 742 eSwabs;
- Bacteria and phages were enumerated by Pherecydes Pharma in 371 eSwabs;
- 94 phagograms were performed.

Synthesis of results / foreground obtained at the end of the project

Note that details of clinical data are not provided yet, because of a scientific publication being currently prepared.

All biological samples analyses were performed as planned. Semi quantitative and quantitative data led to few discordant results of the primary endpoint evaluation, and the overall rating of the primary endpoint was not affected. Although less precise, the semi quantitative method led to highly conclusive data for evaluating the microbial efficacy of the different treatments.

Performing a semi quantitative analysis directly after sampling seems more accurate than a quantitative analysis after freezing, storage and shipping. Nevertheless, the semi quantitative approach was not as rich as the quantitative one. Indeed, phage titration could only be performed with the quantitative approach. Our data show that phage quantification in the samples was not directly linked to the treatment outcome. This result could be an artefact due to technical problems, such as the freezing procedure, as well as the titration strain used for revealing the phage presence.

Although based on an incomplete dataset at the moment of writing, the strain susceptibility study strongly supported the fact that when a PP1131 treatment failed, in the product a low concentration of active phages on the patient bacterial strain was observed.

Indeed, the stability of the phage cocktail (using a non-validated QC titration method) was not as good as expected and led to using much lower concentrations of phages than expected. Assembling a cocktail with more than ten different phages is a challenge: it leads to phage interaction phenomenon that may affect the stability of some phages, leading to a degraded titer. For the future, it is wiser to target a phage cocktail containing a limited number of phage types. Titration overtime needs to be guaranteed in the first place to ensure proper shelf life monitoring. The shelve-life behavior of each single phage, which enters in the composition of the Drug product doesn't reflect the shelve-life of the cocktail.

With a complete dataset (expected for the scientific publication), it will need to be confirmed that when resistance to the phage cocktail emerged during the treatment, a bacterial control and a treatment success was nevertheless observed. In any case, these results corroborate the published observations that when a bacterium acquires resistance to a phage, it loses its virulence and can be eliminated by the patient immune system.

3.5 WP5 – BIOLOGICAL AND CLINICAL DATA ANALYSIS

WP5 aimed at performing the synthesis and analysis of all data – both biological and clinical – that would stem from the clinical trial (WP3). The ultimate objective was to draw conclusions regarding primary / secondary objectives and endpoints and to prepare the Clinical Study Report.

To this end, the following tasks were performed:

- Preparation of the statistical analysis plan;
- Analyses of data;
- Preparation of the Clinical Study Report.

Synthesis of work performed

3.5.1 Preparation of the statistical analysis plan

The statistical analysis plan was prepared by Statitec, the CRO of the clinical trial (subcontractor of Pherecydes Pharma). This analysis plan supplemented and specified the statistical analysis described in the clinical protocol according to guidelines (ICH Topic E9) and general statistical principles. It defined populations of analysis, their characterisation, as well as the evaluation methods of the principal and secondary criteria.

The statistical analysis plan determined the following elements (synthesis, non-exhaustive):

Patient datasets: a patient is considered as randomised as soon as he/she had been attributed a treatment number. Analyses should be conducted on the following patient sets:

- **Safety population (= full Intention To Treat, ITT):** all randomised patients who received at least one dressing according to study protocol;
- **Modified Intention To Treat (mITT) population:** all patients of the Safety population who received at least one dressing and without a negative microbiological result at Day 0. All dropped out patients after Day 0 should be kept into the mITT as withdrawals;
- **Per Protocol (PP) population:** all patients in the mITT without any major deviation.

The primary efficacy analysis should be based on the mITT population. A supportive analysis should be based on the PP population if more than 10% of patients are excluded from mITT population. The assessment of safety will be based on the Safety population.

Data from patients randomised and not treated should be listed but not included in any analysis. Data from patients included and not randomised should only be included in the relevant listings.

Statistical methods: the plan detailed the following aspects:

- Data processing,
- Statistical analysis strategy,
- Description of analyses,
- Significance testing and estimation,
- Multiplicity,
- Interim analysis,
- General convention on e.g. premature drop-out patients, missing data and outliers, derived data...

Disposition of patients: the plan detailed the following aspects:

- Withdrawals,
- Protocol deviations.

Demographics and other baseline characteristics: the plan detailed the following aspects:

- Subject demographic characteristics, medical history and burn history,
- Previous medications,
- Clinical / biological examination before the first treatment application.

Compliance: Safety population should be analysed. The treatment compliance should be assessed as following: statistical comparisons will be performed to assess the comparability of the compliance between groups.

Efficacy: the plan detailed the following aspects:

- Main efficacy criterion,
- Secondary efficacy criteria.

Safety: the plan detailed the following aspects:

- Extent of exposure,
- Adverse events,
- Laboratory data,
- Vital signs,
- Concomitant treatments.

3.5.2 Analyses of data & conclusions regarding primary and secondary endpoints

Several conclusions can be drawn:

- 1) **The cocktail was found to be active and this result is the first to report efficacy of a GMP-produced phage cocktail.** The treatment with the phage cocktail PP1131 significantly reduced the bacterial load in infected wounds but, reduction rate was faster in the control arm. **Several hypotheses, confirmed by ancillary analysis, can explain a lower efficacy in PP1131 than in control arm.**

We understood that modifications in the composition and application of further cocktails are now necessary. This new formulation and protocol were not conceivable when we had drawn the clinical study but are now open for discussion with regulators, even more that spectacular results were observed with 2 cases reports treated with a new version of the cocktail against *Pseudomonas aeruginosa* (Le Figaro Santé du 22/09/2017).

This result is an evidence that PHAGOBURN is a major understanding key in human phage therapy.

- 2) Based on the microbiology analysis, the phages from PP1131 cocktail, even though present at very low concentrations in the final drug product, multiplied on *P. aeruginosa* phage-susceptible strains within the infected burn wounds. This is encouraging for future phage trials, since even at very low concentrations, phages are able to infect bacteria and engage a lytic cycle. Finally, the latter observation suggests that if the cocktail could have been applied using solutions with the foreseen phage concentrations, the speed of bacterial lysis might have been faster.

Other limitations may also explain why PP1131 appeared to be less effective as expected from the pre-clinical studies:

- An alginate compress was used to apply the phages cocktail onto the infected burn wounds. This was not the case for the standard treatment: silver sulfadiazine was applied

directly onto the wound without any interface. Although at high concentrations, phages have been shown *in vitro* to be fully release by alginate, it is still possible that at low concentrations (as those of the final drug product), most of them might have been mechanically trapped into the template and thus unable to reach their bacterial target. Complementary experiments need to be performed to monitor phage release at different concentrations from alginate dressings;

- Phages are known to be susceptible to acidic environments (pH<5). Since a burn wound is a very heterogeneous area regarding pH, some local neutralization of phage activity could have occurred.
- 3) Patients of both groups were treated with antibiotics according to current standards of care after burn trauma, sometime before and/or during the start of the trial. The concomitant use of antibiotics before or during treatment had however no impact on the efficacy of both topical treatments.
- 4) The number of non-serious adverse events was not significantly different between both groups. Yet, due to the sample size (25 patients analysed) it is not possible to draw definite conclusions.
- 5) Severe adverse events were met more frequently in the control group. However, since adverse events linked to the use of silver sulfadiazine are well known, many of them were probably under declared (low level of platelets for example). Similarly, as phage is a new therapy, minor adverse events related to phages are likely unknown and difficult to observe and declare. As clinicians were not blinded, an under declaration of minor adverse events in PP0131 group is still possible.
- 6) Lower than expected phage concentrations in the final drug product might partly explain the lack of reported AE in the group of patients treated by phages. On the other hand, we could prove that high phage multiplication occurred within wounds of patients, so that patients were still exposed to significant phage concentrations. Thus, although one could postulate that concentration dependent adverse events may have been under estimated in phage treated patients in whom phage multiplication did not occur, it is likely not the case in patients in whom multiplication occurred.

PhagoBurn will be a major pillar to support modern phage therapy.

The study helps identifying the pitfall to avoid when setting up a trial or a treatment with bacteriophages. Attention should be paid to **avoid mimicking inert antibiotic evaluation clinical trial template, as phages are living reproducible medicines.**

Since PP1131 and PP0121 cocktail manufacturing, major improvements have been obtained during and after the project to **enhance product purity, stability and concentration.** New formulations sharpen GMP product composition and characterisation. Better **product purity promotes phage therapy to other clinical applications than local** (wounds), **including systemic use** (intravenous).

Major achievements have been reached from the **genuine regulatory environment at project starting (2013) to the current evolution status of phage therapy both at the national (ANSM) and international levels (EMA and FDA).** **The way to obtain phage drug marketing authorisation is on the horizon.**

3.5.3 Preparation of the Clinical Study Report

Statitec prepared the Clinical Study Report after the end of the trial and is finalising all required analyses.

4. POTENTIAL IMPACT AND MAIN DISSEMINATION ACTIVITIES AND EXPLOITATION OF RESULTS

4.1 POTENTIAL IMPACT OF PHAGO BURN – INCLUDING SOCIO-ECONOMIC / SOCIETAL IMPACT

As a reminder, by the end of PhagoBurn, the main goal was to obtain two therapeutic phage products (bacteriophages cocktails) to treat either *E. coli* or *P. aeruginosa* burn wound infections, including those due to antibiotics resistant strains. The benefit/risk ratio had to be in agreement with the highest standards regulating the evaluation of new therapies in Western countries.

So as to reach this overall final result, several “intermediate” results were to be achieved during the project:

- Bioproduction of both drug products in compliance with GMP;
- Establishment of tests and Quality Controls procedures specific to phage drug products;
- Proof of efficacy and safety (through the phase I-II clinical study and subsequent analysis performed) according to Good Clinical Practices (GCP).

As presented above, the planned objectives were reached, despite the difficulties encountered during the implementation of the clinical trial. Yet, PhagoBurn allowed the Consortium to gain significant and essential knowledge and know-how, and represents a major progress towards the development of phage therapy in the EU and at world level.

In details, the impact of PhagoBurn includes both a scientific part, and a wider socio-economic/societal one.

4.1.1 Scientific impact

In the framework of PhagoBurn, a **first GMP manufacturing process of phage cocktails was developed, validated and authorised** by relevant national agencies. While improvements remained to be achieved notably to increase the production yield and optimise the elimination of bacterial contaminants, this achievement represents **a breakthrough regarding the previous state-of-the-art on phage therapy**.

Furthermore, through the issues encountered in the PhagoBurn clinical study implementation, the Consortium now has a clear understanding of critical elements to be taken into account for a successful phage therapy clinical trial (*see above for details*).

Consequently, the PhagoBurn learning experience represents a **central milestone for the successful development and clinical studying of future phage therapy products**. As such, its scientific impact is significant, as demonstrated by the interest and attention the project generated (*see 4.2 below*).

4.1.2 Socio-economic / societal impact

While it is now clear that the PhagoBurn cocktails will not be commercialised due to stability and shelf life issues (*see above and 4.3 below*), the project led ANSM (the French medicinal agency) to **approve, in principle, the possibility to market on the French territory future phage products, through nominative Temporary Use Authorisations (TUAn, i.e. patient by patient)**.

This authorisation is subject to the following cumulative conditions:

- Production of a phage product meeting GMP standards;

- For cocktails in other areas of infection than burn wounds (i.e. PhagoBurn), submission of specific clinical recommendations to justify the administration mode and the dosage;
- For the treatment against other bacteria than *E. coli* and *P. aeruginosa* (in the frame of other, future cocktails), implementation of preclinical studies, GMP manufacturing and launching of clinical trials, as well as, justification, by area of infection, of the administration mode and dosage.

Also, ANSM authorised the use of bacteriophages in the frame of TUA_n if the following criteria are fulfilled:

- Compromised vital and/or threatened functional prognosis;
- Therapeutic dead-end;
- Single-microbial infection.

Such authorisation thus represents a **new treatment opportunity for patients for whom all other therapeutic strategies have failed**. As such, PhagoBurn should lead to the following advantages:

- To patients: availability of a new, alternative, safe therapeutic option, as a last resort treatment;
- To medical staff: i) availability of a new therapy to fight multidrug resistant bacterial infections for which no other treatment has been efficient, ii) decrease of antibiotics pressure;
- To society as a whole: i) reduction of the high mortality rate of such multidrug resistant bacterial infections, ii) new opportunity to address the current significant issues faced by the antibiotics market.

4.2 DISSEMINATION ACTIVITIES PERFORMED

In PhagoBurn, dissemination and communication activities were performed in WP7, which aimed at:

- Disseminate public relevant information regarding PhagoBurn and its results;
- Optimally prepare exploitation of these results.

All partners were involved in the following activities:

Creation of Monitoring/Management Tools	<ul style="list-style-type: none"> • Dissemination activities table • Planned events table • Documents on Communication Strategy
General public communication	<ul style="list-style-type: none"> • Project Website • YouTube Channel dedicated to PhagoBurn • PhagoBurn Newsletters • Press articles, TV and radio broadcasts • Clinical trial public booklet • Clinical trial launching press release • Phageback – A Swiss National Science Foundation sponsored science meets public program on phagetherapy
Scientific communication	<ul style="list-style-type: none"> • Participation to events • Scientific publications
Institutional communication	<ul style="list-style-type: none"> • Discussions with policy-makers • List of institutional contacts of potential interest

4.2.1 Creation of monitoring / management tools

To ensure the optimal follow-up of all communication and dissemination activities implemented during the project, a set of dedicated tools was created.

Dissemination activities table

Shortly after project launching, a PhagoBurn dissemination activities table was created. Using this table on a day-to-day basis allows closely monitoring all communication/dissemination activities implemented, thus significantly easing the reporting process afterwards. Updates were performed monthly (when relevant) based on information provided by the Consortium.

Planned events table

Similarly, a “planned events” table allowed constant monitoring of events (scientific conferences, meetings...) that partners and subcontractors of PhagoBurn intended to participate in. Once an event had passed, it was added to the “Dissemination activities” table, alongside with the agenda of the event and presentations realised by participating partners. Such table also greatly facilitated updates on the PhagoBurn website. Updates were performed monthly (when relevant) based on information provided by the Consortium.

Documents on Communication Strategy

Two documents were produced and updated by the communication team of PhagoBurn (gathering Percy, Pherecydes Pharma, St Joseph / St Luc Hospital and FEI) and transmitted to all partners and subcontractors of PhagoBurn.

The first document was related to the “Communication Strategy to the General Public”. It aimed at ensuring a coherent and homogenous general public communication on PhagoBurn and notably on the clinical study.

The second document was related to the “Communication Strategy in relation with PhagoBurn Clinical Trial”. It aimed at ensuring a harmonised and coherent communication notably regarding the clinical study launching.

4.2.2 General public communication

PhagoBurn official project website


The PhagoBurn public website – www.phagoburn.eu – was the focal point for all dissemination/communication activities surrounding the project. It was launched in July 2013. Developed through close collaboration between Pherecydes Pharma and FEI, its content was regularly updated by FEI thanks to contributions sent by all project actors.

YouTube channel dedicated to PhagoBurn


A PhagoBurn dedicated YouTube channel was created in November 2014: <https://www.youtube.com/channel/UC74ISFXkcNgab-6WkmU15aQ/feed?filter=2>. Linked to the PhagoBurn website, it aimed at broadening communication on the project through providing a dedicated space for relevant videos.

Created and managed by FEI, the YouTube channel included six videos at the end of the project, provided by actors of PhagoBurn and initial broadcasters (TV channels and producers typically). These videos all evoke the PhagoBurn project, as well as, phage therapy in a broader sense. Thus, they are clearly important to educate the general public to this topic and disseminate relevant information.


← Vidéos mises en ligne Date d'ajout (la plus récente) ▾ Grille ▾




Preview of FutureMag broadcast (ARTE) on phage therapy
62 vues • il y a 9 mois




Des virus qui sauvent des vies - FUTURE - ARTE
66 vues • il y a 10 mois




Cent Regrets M d'Herelle - Premiere Screening
86 vues • il y a 1 an



France 3 Broadcast on Phagoburn
140 vues • il y a 1 an



Jérôme Gabard at 20th Evergreen Phage Meeting
92 vues • il y a 1 an



François Ravat Interview - Cent regrets M. d'Herelle Documenta...
95 vues • il y a 1 an

PhagoBurn Newsletters

Newsletters dedicated to the PhagoBurn project were prepared every six months, up to the launching of the clinical trial, then published on the PhagoBurn website ([dedicated section](#)) and simultaneously sent to the PhagoBurn Diffusion List. Following the launching of the clinical trial and due to high confidentiality requirements, it was decided not to publish further newsletters.

Press articles, TV and radio broadcasts

Communication towards the general public was carried out by all actors involved in PhagoBurn (partners and subcontractors) through diverse media means i.e. written press, TV and radio.

The complete list of actions performed is available on PhagoBurn public website. In short, **77 communication / dissemination activities towards the general public occurred**, including:

- 6 radio broadcasts,
- 8 TV broadcasts,
- 42 press articles,
- 21 articles published following the project official press releases.

Clinical trial public booklet

A public booklet introducing the PhagoBurn clinical trial was finalised in September 2015 in two versions: French and English. The English version is presented hereafter.

About phage therapy

Bacteriophages (phages) are natural viruses which can be found everywhere in our environment, on our skin, in water... They only target bacteria: they are **HARMLESS** to humans, animals, plants, insects...

Phage therapy is based on bacteriophages. Due to their antibacterial action, the therapeutic use of phages started right after their identification (beginning of the 20th century). The discovery of antibiotics in the 1940's and their mass diffusion since then led to phage therapy decline. Yet, countries located beyond the Iron Curtain expanded their usage, whereas the Western world was focusing on antibiotics.

Currently, in response to the constant increase of bacterial resistance to antibiotics, phage therapy encounters a strong revival because of its potent high efficacy combined with an attractive safety profile.

Despite this interest and although phage therapy is currently available in various countries (Georgia, Poland, Russia... according to an historical use but without formal modern standard scientific reporting), very few controlled, well-powered and well-designed clinical trials have tested phage efficacy and safety.

Conducting such trials is thus critical, notably as several governments are currently establishing specific action plans to fight against resistance to antibiotics, and are increasingly considering phage therapy as a potential way to fight these infections.

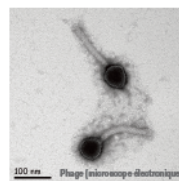
Following the launching of the Phagoburn clinical trial in July 2015, the last regulatory and pharmaceutical production obstacles have now been lifted.



Infections due to bacteria such as *Pseudomonas aeruginosa* (*P. aeruginosa*) and *Escherichia coli* (*E. coli*) are amongst the main causes of death for burn patients.

Fighting such infections in burn trauma is a public health issue

Phagoburn, a European project funded by the European Commission, is focused on a promising alternative or complement to antibiotics:



Phage therapy

Phagoburn aims at assessing the efficacy and safety of two therapeutic phage cocktails to treat either *E. coli* or *P. aeruginosa* burn wound infections.

To this end, a combined Phase I/II multicentre clinical trial is currently conducted

in France, Belgium and Switzerland.

Contribution to the optimisation of regulatory guidelines for phage therapy is also expected.

- 1st patient-in: July 22 2015
- Planned duration: 12 months
- Funding: European FP7 Health Programme
- Website: www.phagoburn.eu
- YouTube: PhagoBurn Project

10 mm

Clinical trial public booklet – Pages 1 (right) and 4 (left)

Phagoburn clinical trial



The synopsis of the trial is available on Clinicaltrials.gov:

clinicaltrials.gov/ct2/show/study/NCT02118010?term=evaluation-of-phage-therapy&rank=1

Hereafter are summarised some of its main components:

- **Objective:** to assess efficacy and tolerance of local phage treatments targeting *E. coli* and *P. aeruginosa* wound infections in burned patients
- **Study Design:** phase I/II efficacy/safety, interventional, randomised, multicentric, open label, controlled against standard-of-care, single-blinded

Phagoburn actors:

- Percy Military Hospital (French Military Health Service) – Principal Investigator
- Pherecydes Pharma (French SME) – Sponsor of the study
- Clean Cells (French SME)
- Queen Astrid Military Hospital – Royal Military Academy, Investigator (B)
- CHU Liège, Investigator (B)
- Charleroi-Loverval Hospital, Investigator (B)
- Lausanne Burn Reference Centre CHU Vaudois, Investigator (CH)
- Saint Joseph-Saint Luc Hospital, Investigator (FR)
- CHU Nantes, Investigator (FR)
- CHU Bordeaux, Investigator (FR)
- CHR Metz Thionville, Investigator (FR)
- HIA Saint-Anne Toulon, Investigator (FR)
- AP-HM, Investigator (FR)
- Statteck (French CRO)
- Laboratoires Brothier (FR), ALGOSTERIL™ band aid provider
- France Europe Innovation (French SME)

- **Primary Outcome endpoint:** time for bacteria reduction in comparison to reference treatment (time frame: 7 days)

- **Secondary Outcome endpoints:** assessment of treatment tolerance (time frame: 21 days), Impact on wound bacterial flora (time frame: 7 days), Evaluation of patients' immune response to the treatment (time frame: 21 days)

The study includes four arms:

- Patients' burn wounds infected by *E. coli* and treated by Silver Sulfadiazine (standard of care);
- Patients' burn wounds infected by *E. coli* and treated with Pherecydes PP0121 cocktail;
- Patients' burn wounds infected by *P. aeruginosa* and treated with Silver Sulfadiazine (standard of care);
- Patients' burn wounds infected by *P. aeruginosa* treated with Pherecydes PP1131 cocktail.

Main inclusion criteria:

- Man or woman, from 18 years old;
- In-hospital patient treated for burn wounds in a burn unit;
- Burn wounds (grafted or not) or graft harvesting area presenting local signs of infection defined by SFETB¹ criteria;
- Burn wounds with microbiologically documented infection and preliminarily treated by povidone-iodine before inclusion;
- Informed consent obtained.

Main exclusion criteria:

- Pregnant or breastfeeding woman;
- Intercurrent condition requiring a treatment which may interfere with analysis results;
- Patient included in an interventional research protocol with therapeutic intervention still ongoing or having participated into anti-infective drug trials in the last month;
- Patient considered as part of a vulnerable population;
- Allergy to Silver Sulfadiazine.

PhagoBurn clinical trial is controlled by an independent Supervisory Committee and by the regulatory agencies:



¹ SFETB: Société Française d'Etude et de Traitement des Brûlures

Clinical trial public booklet – Pages 2 (left) and 3 (right)

This public booklet was disseminated through the publication on the project website, the sending to all actors involved in PhagoBurn for their own communication actions and the sending to the PhagoBurn Diffusion List.

Clinical trial launching official press release

Following the launching of the clinical study in July 2015, an official press release was prepared by Pherecydes Pharma, with the support of the communication agency Andrew Lloyd & Associated (ALA) and FEI. This press release was disseminated by ALA starting on September 9th, 2015. Two versions were prepared: French version and English version.

Phageback

During the 2 ½ year of this SNF-sponsored project, Phageback developed several original tools to present phage therapy to the public. At the core of the concept were practical workshops at the public UNIL Laboratory "L'Éprouvette". In addition, the project developed a website and summarised relevant information on phage therapy on small booklet that were distributed during the held events. Finally, an original exhibition material was designed and used to get in close interaction with various audience. This exhibition appeared to be a great opportunity for scientists, from PhD Student to Senior Researcher to interact on phage therapy with the general population. Thanks to the success we encountered, our workshops have been integrated to the regular offer of the Laboratory "L'Éprouvette". Finally, the exhibition is still presented to other venue during the following years.

4.2.3 Scientific communication

Participation to events

Communication towards the scientific community was carried out by all actors involved in PhagoBurn (partners and subcontractors) through participation – oral presentations or poster presentations – to diverse scientific events (meetings, congresses...). The complete list of scientific presentations is available on PhagoBurn public website. In short, **42 presentations were realised**, including 38 oral presentations and 4 poster presentations.

Publications

Apart from their participation to events, PhagoBurn actors also disseminated information towards the scientific community through publications. Hereafter are the scientific publications realised, either acknowledging PhagoBurn or related to PhagoBurn (published by actors of the project but outside the framework of the FP7 grant):

Publications acknowledging the PhagoBurn grant

- (CHUV, Pherecydes) Oechslin F., Piccardi P., Mancini S., Gabard J., Moreillon P., Entenza J.M., Resch G., Que Y-A. "Synergistic interaction between phage therapy and antibiotics clears *Pseudomonas aeruginosa* infection in endocarditis and reduces virulence" in *J Infect Dis.* 2016 December; jiw632;
- (Percy, Pherecydes, St Luc St Joseph) Jault P., Gabard J., Ravat F. "Le renouveau de la phagothérapie" in *Biofutur* 2016; 35(373):48-51;
- (Percy, Pherecydes) Gabard J., Jault P. "Phage Therapy – Back to the Future" in *AMR Control* 2015; 1:106-109;
- (Percy, Pherecydes, St Luc St Joseph) Ravat F., Jault P., Gabard J. "Bactériophage et phagothérapie : utilisation de virus naturels pour traiter les infections bactériennes" in *Annals of Burn & Fire Disasters* 2015; 23(1):13-20.

Publications mentioning and related to PhagoBurn but not funded through the FP7 grant

- (Queen Astrid) Debarbieux L., **Pirnay J.P.**, **Verbeken G.**, **De Vos D.**, Merabishvili M., Huys I., Patey O., Schoonjans D., Vaneechoutte M., Zizi M. & Rohde C. "A bacteriophage

journey at the European Medicines Agency" in FEMS Microbiology Letter. 2016; 363(2):fnv225;

- (Queen Astrid) **Verbeken G., Pirnay J-P.**, Lavigne R., Ceulemans C., **De Vos D.** & Huys I. "Viruses That Can Cure, When Antibiotics Fail..." in Journal of Microbial & Biochemical Technology. 2016; 8(1):21-24;
- (Queen Astrid) **De Vos D., Verbeken G.**, Dublanchet A., **Jennes S., Pirnay J-P.** "La phagothérapie durable : une question d'évolution" in Biofutur. 2016 February; 35(373):44-47;
- (Queen Astrid) **Verbeken G.**, Huys I., **De Vos D.**, De Coninck A., Roseeuw D., Kets E., Vanderkelen A., Draye J.P., Rose T., **Jennes S.**, Ceulemans C. & **Pirnay J.P.** "Access to bacteriophage therapy: discouraging experiences from the human cell and tissue legal framework" in FEMS Microbiology Letters. 2016 January; 363(2);
- (Queen Astrid) **De Vos D., Pirnay J-P.** "Phage Therapy: Could viruses help resolve the worldwide antibiotic crisis" in AMR Control. 2015; Issue 1:111-114;
- (Queen Astrid, Clean Cells, Pherecydes, CHUV/UNIL) **Pirnay J.P.**, Blasdel B.G., **Bretaudeau L.**, Buckling A., Chanishvili N., Clark J.R., Corte-Real S., Debarbieux L., Dublanchet A., **De Vos D., Gabard J.**, Garcia M., Goderdzishvili M., Górski A., Hardcastle J., Huys I., Kutter E., Lavigne R., Merabishvili M., Olchawa E., Parikka K.J., Patey O., **Pouillot F., Resch G.**, Rohde C., Scheres J., Skurnik M., Vaneechoutte M., Van Parys L., **Verbeken G.**, Zizi M. & Van den Eede G. "Quality & Safety Requirements for Sustainable Phage Therapy Products" in Pharm Res. 2015 July; 32(7):2173-2179;
- (Queen Astrid) Rose T., **Verbeken G., De Vos D.**, Merabishvili M., Vaneechoutte M., Lavigne R., Jennes S., Zizi M. & Pirnay J.P. "Experimental phage therapy of burn wound infection: difficult first steps" in *Int. J. Burn Trauma*. 2014; 4(2):66-73;
- (Queen Astrid) **Verbeken G.**, Huys I., **Pirnay J.P., Jennes S.**, Chanishvili N., Scheres J., Gorski A., **De Vos D.** & Ceulemans C. "Taking Bacteriophage Therapy Seriously: A Moral Argument" in *BioMed Research International*. 2014:621316.

Apart from the above-mentioned publications, two peer-reviewed publications are planned but not yet filed at the end date of PhagoBurn:

- (Pherecydes Pharma) Regulski, K., Champion-Arnaud P. & Gabard J. "Bacteriophage manufacturing: from early 20th century processes to current GMP" in *Bacteriophages, Biology, Technology, Therapy*, edited by Harper D., Abedon S., Burrowes B. & McConville M. *This book chapter will be submitted in April 2017. It is related to PhagoBurn but not funded through the FP7 grant;*
- A peer-reviewed publication presenting the results of the PhagoBurn clinical trial will be prepared and submitted in 2017.

4.2.4 Institutional communication

Finally, the PhagoBurn consortium also participated in some discussions with national (French) and European official institutions. The table hereafter presents these carried-out activities:

Project participants	Title	Date	Place
Pherecydes Percy Clean Cells Reine Astrid	Meeting on phage therapy organised by the European Deputy Michèle Rivasi (The Greens/European Free Alliance) with the objective to progress towards a regulatory proposal	17/09/2013	European Parliament, Brussels, Belgium
Reine Astrid	"The bacteriophage as potential antibacterial" (Oral Presentation)	14/11/2013	Scientific Support for Public Health – EC Joint Research Centre, Brussels, Belgium
Pherecydes	"Evaluation of Phage Therapy for the Treatment of Burn Wound Infection" (Oral Presentation)	04/04/2014	Joint Workshop (EC/JPIAMR/IMI/EFPIA) – Antibiotics & their alternatives

			– fixing & feeding the pipeline, Brussels, Belgium
Percy Reine Astrid	“La phagothérapie : une réponse à l’antibiorésistance”	18/02/2016	French National Assembly Symposium on Phage Therapy

A first list of institutional contact of potential interest was also established in October 2015, including 80 contacts (mainly associations/patients’ associations, political representatives, institutions’ representatives, and health personnel).

4.3 **EXPLOITATION OF RESULTS**

In PhagoBurn, it was planned that the SMEs of the Consortium (Pherecydes Pharma and Clean Cells) would prepare a business plan tackling the future exploitation of project results. While these plans are confidential and cannot be detailed, a short, non-confidential synthesis is provided hereafter.

4.3.1 Context reminder

According to the World Health Organization (WHO), antibiotic resistance (AMR) is currently one of the most serious threats to global health, food security and development, with +/- 10 million deaths a year planned by 2050. Despite actions taken, the number of victims keeps growing, with increasingly pessimistic forecasts. At the same time, antibiotic research strongly decreased between the 1990s and the 2010s, notably as the return on investment is now considered too low by the pharmaceutical industry. Only few new antibiotics have been released on the market in recent years and the ones in development have little chance of becoming actual drugs. In this highly concerning situation, the search for new antibacterial products is critical. This includes antibiotics but also new therapeutic ways, among which **phage therapy** is probably the most advanced and validated approach to date.

In recent years, phage therapy has regained strong interest, due to several factors:

- The enhanced quality control of preparations through the use of new technologies;
- The safety of bacteriophages notably compared to some new-generation antibiotics;
- The high specificity of phages, which respects the microbiota and the beneficial patients’ bacterial environment;
- The ability to obtain evolving treatments in response to the emergence of new antibiotic/phage-resistant bacterial strains;
- The possibility to deliver customized treatments, according to the patient’s microbiological analysis results;
- The different action modes of phages and antibiotics, making them complementary, with the possibility to associate them to enhance the overall treatment.

4.3.2 Exploitation by Pherecydes Pharma

Following the R&D work carried out since its creation in 2006, Pherecydes Pharma currently owns a product portfolio (patent-pending) covering more than 50% of human infections sensitive or resistant to antibiotics.

As explained above, PhagoBurn bacteriophage cocktails will not be pushed further due to stability monitoring and shelf life issues. Nevertheless, the ANSM approval in principle (2016) of the possibility to market on the French territory future phage products, through nominative Temporary Authorisation for Use (ATU), is a direct consequence of PhagoBurn implementation.

Therefore, Pherecydes Pharma will exploit this “indirect” result, alongside with all knowledge, know-how and experience acquired, thanks to PhagoBurn.

4.3.3 Exploitation by Clean Cells

Through PhagoBurn, Clean Cells gained a leading position in Europe regarding phage GMP-manufacturing, through its unique ability to deliver phages as pharmaceutical products for clinical trials implementation.

The SME will exploit this acquired expertise and pioneer position, which constitutes a very strong market differentiating factor.

5. **CONTACT**

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