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

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Summary

Background Wound infections are the main cause of sepsis in patients with burns and increase burn-related morbidity and mortality. Bacteriophages, natural bacterial viruses, are being considered as an alternative therapy to treat infections caused by multidrug-resistant bacteria. We aimed to compare the efficacy and tolerability of a cocktail of lytic anti-*Pseudomonas aeruginosa* bacteriophages with standard of care for patients with burns.

Methods In this randomised phase 1/2 trial, patients with a confirmed burn wound infection were recruited from nine burn centres in hospitals in France and Belgium. Patients were eligible if they were aged 18 years or older and had a burn wound clinically infected with *P aeruginosa*. Eligible participants were randomly assigned (1:1) by use of an interactive web response system to a cocktail of 12 natural lytic anti-*P aeruginosa* bacteriophages (PP1131; 1×10^6 plaque-forming units [PFU] per mL) or standard of care (1% sulfadiazine silver emulsion cream), both given as a daily topical treatment for 7 days, with 14 days of follow-up. Masking of treatment from clinicians was not possible because of the appearance of the two treatments (standard of care a thick cream, PP1131 a clear liquid applied via a dressing), but assignments were masked from microbiologists who analysed the samples and patients (treatment applied while patients were under general anaesthetic). The primary endpoint was median time to sustained reduction in bacterial burden by at least two quadrants via a four-quadrant method, assessed by use of daily swabs in all participants with a microbiologically documented infection at day 0 who were given at least one sulfadiazine silver or phage dressing (modified intention-to-treat population). Safety was assessed in all participants who received at least one dressing according to protocol. Ancillary studies were done in the per-protocol population (all PP1131 participants who completed 7 days of treatment) to assess the reasons for success or failure of phage therapy. This trial is registered with the European Clinical Trials database, number 2014-000714-65, and ClinicalTrials.gov, number NCT02116010, and is now closed.

Findings Between July 22, 2015, and Jan 2, 2017, across two recruitment periods spanning 13 months, 27 patients were recruited and randomly assigned to receive phage therapy (n=13) or standard of care (n=14). One patient in the standard of care group was not exposed to treatment, giving a safety population of 26 patients (PP1131 n=13, standard of care n=13), and one patient in the PP1131 group did not have an infection at day 0, giving an efficacy population of 25 patients (PP1131 n=12, standard of care n=13). The trial was stopped on Jan 2, 2017, because of the insufficient efficacy of PP1131. The primary endpoint was reached in a median of 144 h (95% CI 48–not reached) in the PP1131 group versus a median of 47 h (23–122) in the standard of care group (hazard ratio 0·29, 95% CI 0·10–0·79; p=0·018). In the PP1131 group, six (50%) of 12 analysable participants had a maximal bacterial burden versus two (15%) of 13 in the standard of care group. PP1131 titre decreased after manufacturing and participants were given a lower concentration of phages than expected (1×10^2 PFU/mL per daily dose). In the PP1131 group, three (23%) of 13 analysable participants had adverse events versus seven (54%) of 13 in the standard of care group. One participant in each group died after follow-up and the deaths were determined to not be related to treatment. The ancillary study showed that the bacteria isolated from patients with failed PP1131 treatment were resistant to low phage doses.

Interpretation At very low concentrations, PP1131 decreased bacterial burden in burn wounds at a slower pace than standard of care. Further studies using increased phage concentrations and phagograms in a larger sample of participants are warranted.

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See Comment page 2

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Research in context

Evidence before this study

Human phage therapy is empirical and only used routinely in Georgia, Poland, and Russia. Although medical standards exist, to our knowledge no clinical studies or recommendations according to current evidence-based medical standards have been developed by these countries but, if there are any, they are only available in Russian. We searched PubMed for publications between Jan 1, 1990, and July 26, 2018, with no langauge restrictions, using the MeSH search term "Phage therapy" and found only one randomised trial to assess an oral cocktail targeting children with acute diarrhoea in Bangladesh, which was supported by Nestlé Research and published in 2016. However, several successful case reports have been published previously. A design for a randomised controlled trial was published in 2017 to assess the use of bacteriophages for treating urinary tract infections. But, to our knowledge, no randomised controlled trial has been published to assess the efficacy and tolerability of a cocktail of bacteriophages produced under good manufacturing practice conditions—a prerequisite for approval by national regulators.

Added value of this study

To our knowledge, this is the first time a cocktail of natural lytic bacteriophages has been produced according to good manufacturing practices and approved by three national health regulators. Although the effect was slower than in the control group, we found that our phage cocktail reduced bacterial burden in the infected wounds of patients with burns, one of the most difficult populations to treat, and something that, to our knowledge, has never been achieved in a human trial before. Monitoring the shelf-life of the phage cocktail and its active components suggested a personalised medicinal approach to reduce potential causes of phage therapy failure.

Implication of all the available evidence

Development of phage therapy is a promising alternative to combat bacterial resistance to antibiotics. Close cooperation with national regulators is needed for good manufacturing practices and the future design of studies. Further efficacy and tolerability assessments of phage therapy are needed with more participants. In France, when this trial was ending, the national health regulator authorised the first treatment of patients with extremely drug resistant and difficult to treat infections by use of phage therapy. Since then, six cases with various bacterial infections have been successfully treated.

Introduction

Lytic bacteriophages replicate by infecting and multiplying within a specific bacterium until the bacterium is destroyed, and the released virions promote this infecting cycle. An estimated 1×10^{32} phages exist in nature. Phages were discovered by Frederik Twort in 1915 and phage therapy was first described by Felix d'Herelle in 1917 as a complex process.[1] 10 years after these discoveries antibiotics emerged and their ease of use resulted in phage therapy being essentially abandoned. With the emergence of antibiotic resistance, phage therapy now seems to be a promising alternative to antibiotics, with some successful case reports[2–4] supported by a large fundamental knowledge base.[5,6] As natural biological regulators, bacteriophages fit within the WHO One Health strategy for animals, humans, and the environment.[7] However, to our knowledge, no randomised controlled trial has ever investigated phage therapy in humans.

Initiated in 2010, this study (PhagoBurn) is the first randomised controlled trial to investigate phage therapy. The trial was designed with the cooperation and scrutinisation of three drug safety agencies (National Agency for the Safety of Medicines and Health Products [ANSM], France; Federal Agency for Medicines and Health Products [AFMPS], Belgium; and Swissmedic, Switzerland) to ensure it complied with good manufacturing and clinical practices. The trial's objective was to target infected burn wounds because these infections are the main cause of sepsis and death in patients with burns.[8] Two features of phage therapy that were considered in the trial design were that treatment with phages could reduce the risks associated with iterative antibiotic use on burn wounds and resistant strains of bacteria, and that, according to recommendations by the French Society for Burn Injuries,[9] infected burns should be treated topically because of poor diffusion of systemic antibiotics in burn wounds; therefore, a topically administered phage therapy should remain active until it reaches its target.

This study aimed to evaluate the efficacy and tolerability of a pre-assembled cocktail of 12 natural lytic anti-*Pseudomonas aeruginosa* bacteriophages compared with standard of care in patients with infected burn wounds.

Methods

Study design and participants

In this multicentre, double-blind, randomised phase 1/2 trial, participants were recruited from Hôpital d'instruction des armées Percy in Paris, France, and five other specialised burn centres in hospitals in Lyon, Nantes, Metz, Toulon, and Marseille in France, and the Queen Astrid Military Hospital in Brussels, Belgium, and two other burn centres in hospitals in Liège and Loverval in Belgium. The study was coordinated and overseen in France by the French Ministry of Defence, and in Belgium it was overseen by The Royal Military Academy. Ethical approval of the study protocol was given by each country's regulatory agency and by the

appropriate institutional review boards. An independent data and safety monitoring board was appointed by the principal investigator (PJ) to review safety during the trial.

Patients were recruited over two periods between which amendments were made to the protocol and inclusion criteria in light of safety reviews, such that overall recruitment lasted 13 months. Patients were eligible for inclusion if they were aged 18 years or older and being treated in hospital burn centres with a clinically infected burn wound. A clinically infected wound was classified as a *P aeruginosa* infection, confirmed by swab culture, with or without general signs (ie, clinical or biological signs of sepsis), and meeting one or more of the following consensual clinical infection criteria of the French Burn Society:[10] inflammation, pus, delayed healing or wound reopening, and spontaneous debridement. Initially, only *P aeruginosa* had to be isolated from the wound; however, the data and safety monitoring board added two new eligibility criteria for the second period of the study: first, the patient's Sequential Organ Failure Assessment score must not increase by more than 2 points in the 48 h before inclusion, and second, wounds can be infected with *P aeruginosa* alone or marginally colonised by other species. Full inclusion criteria and amendments and additions made for the second recruitment period are in the appendix (p 2). Exclusion criteria included pregnancy or breastfeeding for women, comorbid conditions that might interfere with analytical results, and any adverse reaction to the standard of care treatment (full exclusion criteria, with second recruitment period amendments, are in the appendix, p 2). Written informed consent was provided by the participants or their next of kin.

Randomisation and masking

After confirmation of infection, participants were randomly assigned (1:1) to receive PP1131 or standard of care by use of an interactive web response system with an electronic case report form. Randomisation was stratified according to study site and ongoing active antibiotic treatments at baseline, if any. Because of differences in treatment appearances, standard of care being a white emulsion and PP1131 a clear solution, treatment was not masked from clinicians. However, because burn dressings were changed while participants were under general anaesthesia, treatment allocation was masked from them. Treatment allocation was masked from microbiologists who did the microbiological evaluations for the primary endpoint and investigators.

Procedures

The cocktail of 12 natural lytic anti-*P aeruginosa* bacteriophages, PP1131, was designed and qualified (ie, tested in agreement with quality control tests of the regulator) by Pherecydes Pharma (Romainville, France). All phages were natural lytic phages that were collected from hospital sewage water and subsequently purified. Each phage included in the cocktail belonged to either the Podoviridae or Myoviridae family and was isolated and characterised by phenotyping and genotyping. From a regulatory aspect, each phage of the cocktail was considered as a drug and the cocktail as a drug product. The cocktail was produced by Clean Cells (Boufféré, France) following good manufacturing practices. A complete description of each manufacturing step and external validations by health authorities were reviewed by ANSM in an Investigational Medicinal Product Dossier and a clearance certificate was issued on June 24, 2015.

A single batch of PP1131 was produced, formulated in phosphate-buffered saline, and comprised 12 different phages. The global phage titre in a vial was set to 1×10^9 plaque-forming units (PFU) per mL, and the concentration of each phage was a twelfth of that concentration. Before the trial, preliminary studies of shelf-life stability were done by use of two representative single phage preparations. The upper endotoxin threshold was defined at 5 EU/kg per h according to European Pharmacopeia regulations. To prepare the topical PP1131 treatment, a vial of PP1131 was diluted 1000-fold in isotonic saline solution immediately before application and to reach an expected phage titre of 1×10^6 PFU/mL. An alginate template (Algosteril, Les Laboratoires Brothier, Paris, France) was soaked with the diluted solution (20 mL for 200 cm² Algosteril), and directly applied onto the wounds.

See Online for appendix

Sulfadiazine silver was selected as the standard of care comparator because it is the recommended first-line treatment for burn infections and used worldwide.[10] It targets aerobic Gram-positive and Gram-negative bacteria and fungi. A thick layer, as determined by the clinic staff, of 1% sulfadiazine silver emulsion cream was applied directly onto the wounds.[11]

When patients were being screened for eligibility and clinic staff were waiting on microbiological confirmation (infection status known within 2–3 days), if a burn wound was suspected to be clinically infected patients were given povidone-iodine cream dressings, which acted as an antibacterial drug. Because silver might inhibit phage activity, no dressings containing silver were used before confirmation of infection and randomisation.

At day 0, participants were given either sulfadiazine silver or PP1131. All treatments were applied daily for 7 days. Observations were made for 21 days, including the initial 7 days of treatment and 14 days of follow-up. Treatment was applied to all infected open-wound sites, except grafts and donor sites before the first post-operative dressing, usually the first 3 days after surgery (some patients had surgery for their wounds, but those with partial thickness wounds were treated with non-surgical methods to avoid unecessary scars). Adjunctive antibiotic therapy during the 7-day treatment period was allowed at the discretion of the treating physician according to French Burn Society recommendations.[9]

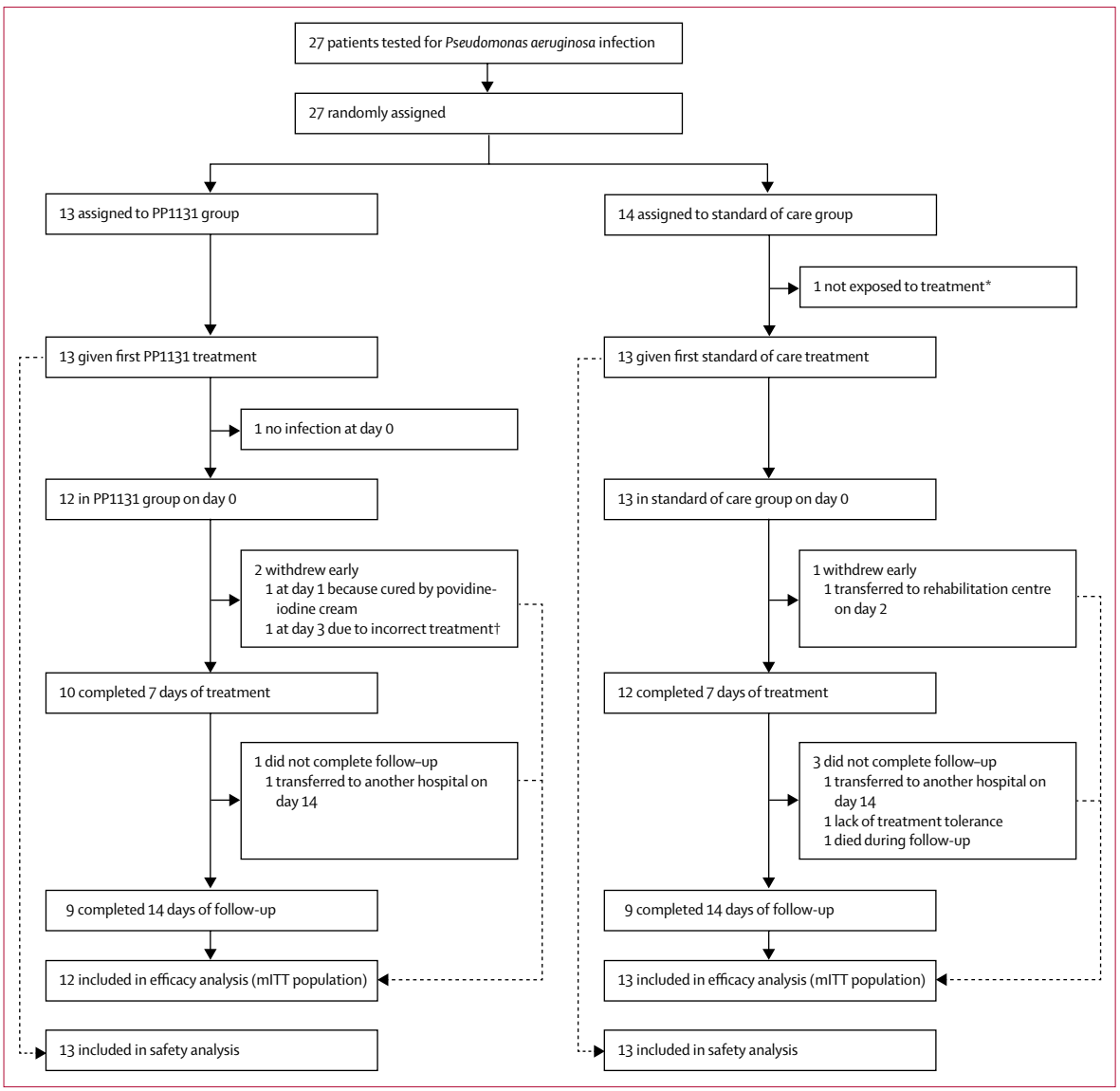

Figure 1: **Trial profile**
The safety population contains participants who were given at least one dressing. mITT population includes all patients from the safety population with a microbiologically documented infection at day 0. mITT=modified intention to treat. PP1131=cocktail of 12 natural lytic anti-*Pseudomonas aeruginosa* bacteriophages. *Patient randomised on the day the trial was suspended (Jan 2, 2016). †Patient was exposed to treatment for a different clinical study.

Data collection

Sampling of each infected wound was done once a day during the 7 days of treatment at dressing opening before any additional care by use of two identical ESwabs (Copan Diagnostics, Murrieta, CA, USA). The entire area of each wound was thoroughly sampled with a Z gesture until swab saturation according to European Society for Clinical Microbiology and Infectious Diseases recommendations.[12] One swab was seeded on selective medium (cetrimide agar) within 2 h at the onsite microbiological diagnostic laboratory by a microbiologist and analysed by use of the semiquantitative four-quadrants method (details of this method are in the appendix [p 4]). The other swab was immediately supplemented with 20% glycerol onsite and stored at –80°C before being sent to the sponsor (Pherecydes Pharma) for evaluation of bacterial burden by colony counting on selective media (quantitative method and ancillary analysis; appendix p 5).

Ancillary analyses, planned in advance with the methods determined post-hoc, focused on the phage susceptibility of *P aeruginosa* strains from participants given PP1131 who completed at least 7 days of treatment. Strains were isolated on selective cetrimide agar plates from the second swab taken from each participant at day 0. This second swab was collected exclusively from the most infected wounds. At least five separate colonies

were randomly selected for analysis from the culture of each participant. In a few cases in which two colony morphotypes were observed, at least five separate colonies of each morphotype were randomly selected. By use of a standard spot-test assay, susceptibility patterns in response to a freshly prepared PP1131 cocktail were visually determined for each colony after 24 h of incubation at 37°C.

Pharmacovigilance was carried out by an independent pharmacist (SD Pharma Consulting, Clermont-Ferrand, France) supported by a substitution pharmacist (ASPE Conseil, Toulouse, France) under the supervision of the principal investigator (PJ).

Outcomes

The primary efficacy endpoint was the time taken for a sustained reduction in bacterial burden of two quadrants or more assessed by semiquantitative culture results, adjusted to newly introduced antibiotic treatment (active on targeted *P aeruginosa* strain). If a participant presented with multiple wounds infected with *P aeruginosa*, daily bacterial burden was defined as that of the wound with the highest bacterial burden for that day, to account for putative cross-contamination between wounds.

Key secondary endpoints included the following: the primary endpoint without adjustment for antibiotic treatment, and evolution of bacterial burden for all infected wounds; evolution of French Burn Society clinical criteria; timing and indication of adjunctive systemic antibiotic treatment that has activity on the pathogenic strain; incidence and time of first diagnosis of any novel bacterial infections during the 7 days of treatment; and reasons for participant withdrawal from the study (eg, tolerability).

Safety assessments included frequency, duration, and severity of adverse events, clinical laboratory tests (haematology, chemical, and urine analyses), vital sign measurements, physical examinations, and clinical assessment of burn wounds. Adverse events were described using the system organ classes and preferred terms of the MedDRA dictionary (version 18.0).[13]

The trial was also designed to evaluate an anti-*Escherichia coli* fixed cocktail (PP0121) under similar conditions, but that part of the study was stopped after only one patient was recruited over a period of 7 months and hence is not reported here.

Statistical analysis

Considering the paucity of evidence on the efficacy of phage therapy, we set the desired original sample size at 125 participants. With an estimated 20% dropout rate, we calculated that this sample size should detect a twice as fast reduction in bacterial burden between groups, with a two-sided 5% significance level and a power of 90%.

We reported continuous normally and non-normally distributed variables as mean (SD) or median (IQR) and categorical variables as numbers and percentages.

	PP1131 (n=12)	Standard of care (n=13)
Age, years	61 (35–75)	37 (32–62)
Sex		
Male	8 (67%)	7 (54%)
Female	4 (33%)	6 (46%)
Burn severity		
TBSA (%)	19 (12–39)	35 (13–39)
Third degree burn TBSA (%)	6 (3–11)	23 (7–30)
Inhalation injury	3 (25%)	4 (31%)
At randomisation, time since injury, days	29 (21–43)	23 (23–36)
On active antibiotics on day 0	2 (17%)	2 (15%)

Data are median (IQR) or n (%). mITT population comprised patients with a burn wound infected with *Pseudomonas aeruginosa* who were given at least one application of PP1131 or standard of care. mITT=modified intention to treat. PP1131=cocktail of 12 natural lytic anti-*Pseudomonas aeruginosa* bacteriophages. TBSA=total body surface area.

Table 1: **Baseline characteristics of mITT population**

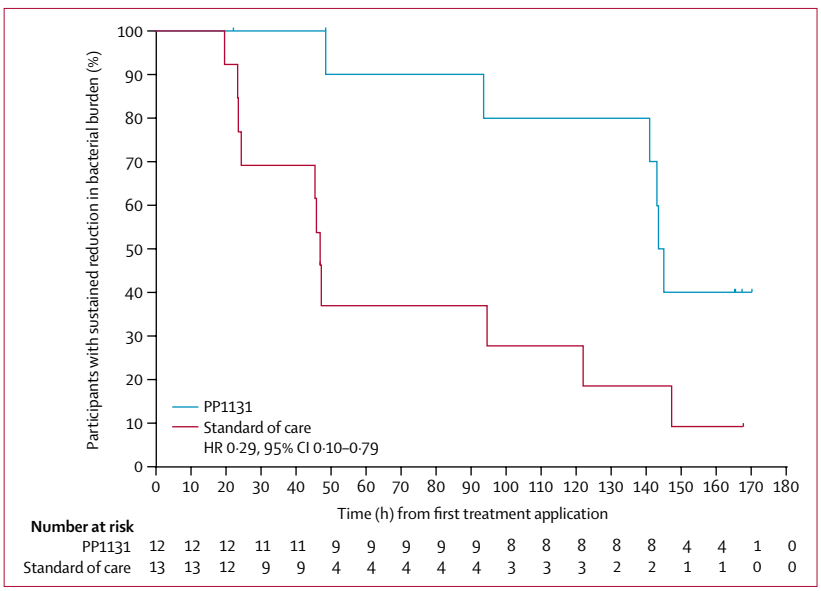

Figure 2: **Time to observe reduction in bacterial burden in the most infected wound**
Kaplan-Meier analysis of median time to sustained semi-quantitative reduction of two or more quadrants of highest daily bacterial burden compared with day 0. HR=hazard ratio. PP1131=cocktail of 12 natural lytic anti-*Pseudomonas aeruginosa* bacteriophages.

The safety was assessed in participants who received at least one dressing according to the protocol. The primary endpoint was assessed in participants with a microbiologically documented infection at day 0 who were given at least one sulfadiazine silver or phage dressing (ie, modified intention-to-treat [mITT] population). Modification of the ITT population was needed to assess efficacy because some patients might have been cured by initial treatment with povidone-iodine dressing before randomisation. The ancillary analysis was done in the per-protocol population, which comprised participants in the PP1131 group mITT

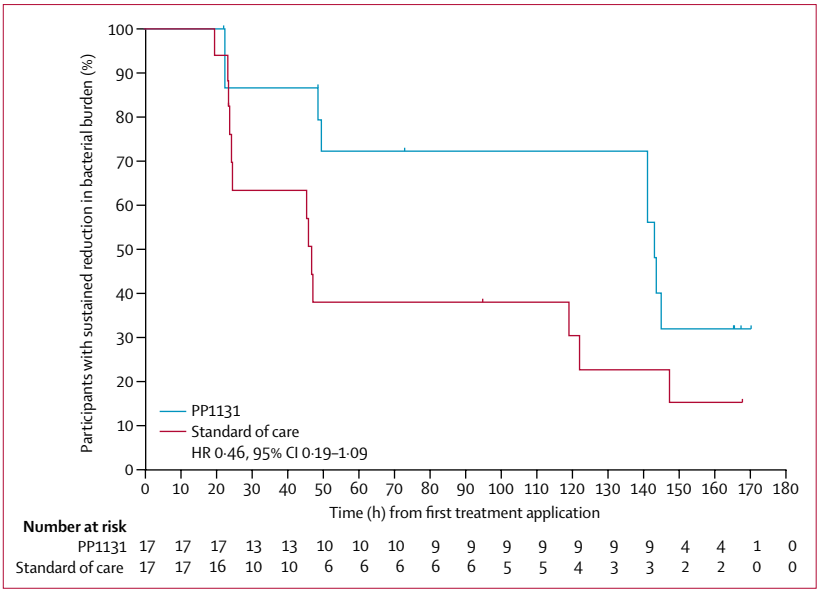

Figure 3: **Time to observe reduction in bacterial burden for all areas**
Kaplan-Meier analysis of median time to sustained semi-quantitative reduction of two or more quadrants of daily bacterial burden across all wounds compared with day 0. HR=hazard ratio. PP1131=cocktail of 12 natural lytic anti-*Pseudomonas aeruginosa* bacteriophages.

population without any major deviation—ie, completed at least 7 days of treatment as per protocol.

We compared the primary endpoint between groups using a Cox regression model adjusted for the potentially confounding time-dependent covariate of systemic antibiotic treatment introduced between day 0 and day 7, because antibiotics could be given at any time during the trial, and for the stratification variable of ongoing systemic antibiotic use at day 0. We did a time-to-event analysis stratified by antibiotic intake at day 0 using the log-rank test and Kaplan-Meier method. We compared binary endpoint (eg, yes or no) proportions between treatment groups using χ^2 or Fisher's exact tests. All statistical tests were two-sided at a 5% significance level. We used SAS version 9.4 for all statistical analyses, tables, graphs, and subject data listings.

The study is registered with the European Medicine Agency, number EudraCT 2014-000714-65, and ClinicalTrials.gov, number NCT02116010.

Role of the funding source

The funder of the study had no role in study design, data collection, data analysis, data interpretation, or writing the clinical report. The study sponsor, Pherecydes Pharma, had no role in the core clinical study. JG and CF participated in the study design and performed, analysed, and interpreted ancillary quantitative microbiological data, including evaluation of *P aeruginosa* strain susceptibility to phages at day 0. The corresponding author had full access to all data in the study and had final responsibility for the decision to submit for publication.

Results

Between July 22, 2015, and Jan 2, 2017, 27 patients with infected burn wounds were recruited, 16 (59%) during the first recruitment period (July 22, 2015, to Jan 2, 2016) and 11 (41%) during the second (May 31, 2016, to Jan 2, 2017). Patients were randomly assigned to the PP1131 group (n=13) or the standard of care group (n=14). In the standard of care group, one patient was not exposed to treatment because they were randomly assigned to the group on the day the trial was suspended (Jan 2, 2016) and one patient in the PP1131 group had no infection at day 0; the mITT population therefore consisted of 12 in the PP1131 group and 13 in the standard of care group (figure 1). Baseline characteristics of patients in the mITT population are shown in table 1. Four participants, two in each group, had ongoing active antibiotic treatment for *P aeruginosa* at day 0. Participants in the PP1131 group were less extensively burned and older than those in the standard of care group. Distribution of participants included in analyses among study centres is given in the appendix (p 6).

The bacterial burden of the most infected burn wounds ranged from one to four quadrants according to the semiquantitative method. Notably, six (50%) of 12 participants in the PP1131 group and two (15%) of 13 in the standard of care group had a very high bacterial burden (ie, four quadrants) in their most infected wound at day 0 (appendix p 6), and similar proportions of participants in each group were observed when all non-contiguous infected burn wounds were tested (appendix p 8). Overall, *P aeruginosa* strains isolated from participants in the standard of care group had broader antibiotic susceptibility at day 0 than those from participants in the PP1131 group (appendix p 9).

In the mITT population, the median time to sustained semiquantitative reduction of two or more quadrants of highest daily bacterial burden compared with day 0 was significantly longer in the PP1131 group (median 144 h, 95% CI 48–not reached) compared with the standard of care group (47 h, 23–122; hazard ratio [HR] 0·29, 95% CI 0·10–0·79; p=0·018 log-rank test stratified by ongoing treatment with antibiotics; figure 2). At day 7 (168 h), the primary endpoint was achieved in a lower proportion of patients in the PP1131 group (six [50%]) than in the standard of care group (11 [85%]), although this result was not significant (p=0·097).

In the Cox regression analysis, adjusted for ongoing active antibiotic use at inclusion (stratification variable) and active antibiotics introduced while on study treatment (potentially confounding factor), being in the PP1131 group was the only predictor associated with a reduction in the time to bacterial burden (HR 0·24, 95% CI 0·08–0·75; p=0·014; appendix p 11).

Considering all infected areas without adjustment for antibiotic treatment, no significant difference was seen between groups in the median time to achieve at least a reduction of two quadrants of bacterial burden compared

with day 0 (143 h [95% CI 48–not reached] in PP1131 group *vs* 47 h [24–122] for standard of care group; HR 0·46, 95% CI 0·19–1·09; p=0·07; figure 3). A higher proportion of participants had a successful treatment outcome in the standard of care group (13 [76%] of 17) than in the PP1131 group (nine [53%] of 17; p=0·15) by the end of follow-up.

For the secondary endpoint of clinical French Burn Society criteria evolution, no difference was seen between the PP1131 and standard of care groups in changes from baseline. Only conversion of superficial wound into deep wound and presence of pus seemed to be less frequent in the PP1131 group than in the standard of care group at day 14 (data not shown). For open wounds, two (29%) of seven participants in the PP1131 group still had open wounds versus seven (88%) of eight participants in the standard of care group (p=0·025); and for presence of pus, three (43%) of seven participants in the PP1131 group still had pus versus eight (100%) of eight participants in the standard of care group (p=0·016). However, because the study was not powered to detect differences in these secondary outcomes, no definitive conclusions can be drawn.

As decided by the attending physician, antibiotic treatment active against *P aeruginosa* was introduced after day 0 for two (17%) of 12 participants in the PP1131 group and for five (38%) of 13 in the standard of care group (HR 0·46, 95% CI 0·09–2·39; p=0·38).

92 adverse events were reported in ten (38%) of 26 participants in the safety population (table 2), with 40 adverse events reported in three (23%) of 13 participants in the PP1131 group and 52 events in seven (54%) of 13 in the standard of care group. Three (23%) participants in the PP1131 group and six (46%) in the standard of care group had infectious adverse events. These differences were not substantial. Median duration of exposure to dressing was not substantially different between participants in the PP1131 and standard of care groups (148·5 h [SD 46·5] *vs* 157·0 h [SD 33·1]). One patient died in each treatment group after day 21. Both were older than 70 years and their deaths were not related to study treatment (appendix p 13).

Ancillary studies testing PP1131 stability found that each individual phage component of PP1131 was very stable at concentrations of 1×10^9 PFU/mL or higher for over 24 months after manufacturing. However, once assembled, the global titre of PP1131 decreased from 1×10^9 PFU/mL to 1×10^4–1×10^5 PFU/mL (first titration at 6 months), then remained stable. Therefore, because the PP1131 cocktail had to be diluted to reduce the concentration of endotoxin for application to wounds, patients were given a daily dose of approximately 10–100 PFU/mL instead of the expected dose of 1×10^6 PFU/mL. A reconstruction stability study showed that the global phage titre decreased by three logs (ie, 1000-fold) within 15 days following manufacture.

	PP1131 (n=13)	Standard of care (n=13)
All	3 (23%)	7 (54%)
Blood and lymphatic system disorders		
All	0	1 (8%)
Pancytopenia	0	1 (8%)
General disorders and administration-site conditions		
All	1 (8%)	2 (15%)
Hyperthermia	1 (8%)	1 (8%)
Impaired healing	0	1 (8%)
Infections		
All	3 (23%)	6 (46%)
Bacteraemia	1 (8%)	1 (8%)
Bronchitis	0	1 (8%)
Ear infection	1 (8%)	0
Fascial infection	0	1 (8%)
Pneumonia	0	1 (8%)
Pseudomonas sepsis	1 (8%)	0
Pseudomonas infection	0	1 (8%)
Septic shock	0	3 (23%)
Skin-graft infection	0	2 (15%)
Superinfection	1 (8%)	0
Urinary tract infection	1 (8%)	0
Procedural complications		
All	0	1 (8%)
Post-procedural haemorrhage	0	1 (8%)
Investigations		
All	1 (8%)	0
Oxygen saturation decreased	1 (8%)	0
Renal and urinary disorders		
All	1 (8%)	0
Haematuria	1 (8%)	0
Haemorrhage urinary tract	1 (8%)	0
Respiratory, thoracic, and mediastinal disorders		
All	1 (8%)	1 (8%)
Hypoxia	1 (8%)	0
Lung disorder	0	1 (8%)
Skin and subcutaneous tissue disorders		
All	0	1 (8%)
Urticarial	0	1 (8%)
Vascular disorders		
All	0	1 (8%)
Haemorrhagic shock	0	1 (8%)

Data are n (%). PP1131=cocktail of 12 natural lytic anti-*Pseudomonas aeruginosa* bacteriophages.

Table 2: **Adverse events in the safety population**

The susceptibility of the bacterial strain was tested in ten participants in the PP1131 group who completed 7 days of treatment (ie, the per-protocol population). A freshly prepared PP1131 cocktail was tested on *P aeruginosa* colonies isolated at day 0 from the most infected wounds. Samples taken from two participants had to be excluded from analysis because no colony regrowth was seen from

A

	Number of colonies	Resistant	Intermediatly susceptible	Susceptible
Participants who reached primary endpoint by day 7				
1	5	0	0	5
2	5	0	0	5
3	6	0	2	4
4	6	0	0	6
5	6	0	1	5
Total	**28**	**0**	**3**	**25**
Participants who did not reach primary endpoint by day 7				
1	13	1	12	0
2	5	5	0	0
3	11	0	11	0
4	11	1	4	6
5	5	0	0	5
Total	**45**	**7**	**27**	**11**

B

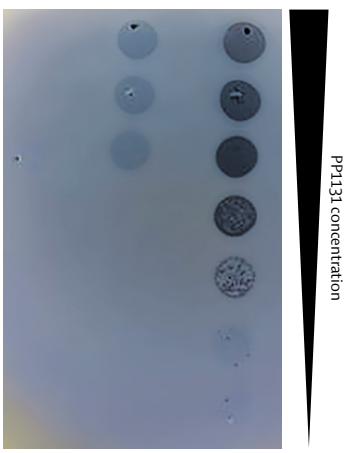

Figure 4: **PP1131 susceptibility of *Pseudomonas aeruginosa* strains recovered from the day 0 swabs of participants in the per-protocol population**

(A) Susceptibility of the different recovered clones. The five participants did and five participants did not reach the primary endpoint by day 7. (B) Three different patterns observed in spot tests. Three different susceptibility patterns to PP1131 are shown: resistance, intermediate susceptibility, and susceptibility. PP1131=cocktail of 12 natural lytic anti-*Pseudomonas aeruginosa* bacteriophages.

their swabs from day 0 after storage (–80°C). Overall, 73 colonies were tested for phage susceptibility. Figure 4 shows three different susceptibility patterns to PP1131: resistance, intermediate susceptibility, and susceptibility. Among 28 colonies isolated from five (50%) participants in the per-protocol population who reached the primary endpoint by day 7, 25 (89%) colonies were susceptible, three (11%) were intermediately susceptible, and none were resistant. Among 45 colonies tested from five (50%) participants who did not reach the primary endpoint by day 7, 11 (24%) were susceptible, 27 (60%) were intermediately susceptible, and seven (16%) were resistant. Phage susceptibility at day 0 was significantly associated with reaching the primary endpoint by day 7 ($p<0·0001$).

Discussion

We found that daily bacterial burden in the most infected wound was successfully reduced by two quadrants or more in half of participants at the end of phage treatment. However, the median time to achieve this endpoint was significantly longer for those in the PP1131 group than for those in the standard of care group. This result was independent of systemic use of antibiotics active against the infecting strain, either ongoing at day 0 or introduced later during study treatment. To our knowledge, this is the first time that a clinically relevant reduction in bacterial burden has been observed in a clinical trial after 7 days of topical phage application.

To our knowledge, PhagoBurn is the first clinical trial of phage therapy that follows good clinical practices. The study focused on severe burn wound infections because infection of wounds is a major cause of morbidity and mortality after burn trauma and is often caused by multidrug-resistant bacteria.[14,15] The phage treatment was designed to be applied topically, avoiding a systemic injection, which was considered too risky by agencies in 2013 when we first proposed our study design. A systemic phage injection was potentially more prone to adverse events associated with endotoxin residues than a topical treatment would be, and so to meet with good manufacturing practices we designed a topical treatment.

Although the study was launched in June, 2013, pharmaceutical manufacturing of the bacteriophage mixture was challenging, with the establishment of a sterile process in line with specifications for good manufacturing practices requiring 25 months instead of the predicted 14 months. Recruitment was suspended between Jan 2, and May 31, 2016, when data on the instability of the PP1131 cocktail emerged via an independent investigation. These data confirmed that, although the concentration of phages had decreased from 1×10^9 PFU/mL to 1×10^6 PFU/mL, the global phage titre remained stable over time. After this instability was discovered, recruitment was restarted with amendments made to the protocol to include complementary data on shelf-life stability, and modified inclusion criteria were approved by the data and safety monitoring board, ethical committees, and regulators. However, because of the instability of the phage mixture, all patients had been treated with a reduced dose, regardless of being included at the beginning or end of the study. On Jan 2, 2017, the sponsor stopped recruitment because of concerns regarding patient exposure to insufficient phage concentrations. The decision to terminate the study early was confirmed on March 20, 2017, when the second data and safety monitoring board meeting recommended stopping the trial because of insufficient efficacy.

Epidemiological estimates available at the time of study design via hospital records overestimated the numbers of *P aeruginosa* monoinfections (defined as an inclusion criteria) because hospital records did not differentiate monomicrobial from polymicrobial infections. Additionally, bioproduction of the treatment took twice as long as expected, and so reduced the time dedicated to the clinical study. Together these factors contributed to a lower than expected number of patients meeting inclusion criteria. Furthermore, inclusions were discontinued because of PP1131 activity loss; hence, only 27 patients were randomised, with a final mITT population of 25 participants. This major deviation from the original statistical analysis plan reduced the statistical power of this study.

The semiquantitative method used to assess the primary endpoint is a validated standard practice in microbiology labs. Although this method does not count colonies, it is still an objective technique that yields a reproducible result, provided that the swabbing and seeding techniques are done properly and uniformly. To achieve the highest level of reproducibility, investigators

and microbiologists in all centres were trained to do the tests the same way at trial initiation. Stratification of randomisation by centre also reduced the risk of between-centre variation influencing the results.

Our study had several limitations and encountered many unexpected difficulties. First, the treatment groups were unbalanced despite randomisation. Participants in the PP1131 group were less extensively burned and older than those in the standard of care group. Age and scale of burn wounds are both key burn severity risk factors.[16] Bacterial burden was also higher in the PP1131 group than in the standard of care group. Such unexpected differences could be explained by the small number of participants recruited.

Second, success in achieving the primary endpoint was linked to initial (ie, day 0) *P aeruginosa* susceptibility to the phage cocktail, highlighting the importance of doing preliminary phage-susceptibility testing (ie, so-called phagograms like in our ancillary studies) in future trials before applying a phage treatment. Here, avoiding a preliminary phagogram for each patient had the advantage of simplifying the protocol and enabling treatment of infected patients without further delay.

Third, stability issues resulted in administration of a 1000-fold to 10 000-fold lower dose of active phages to patients in the PP1131 group. This low phage dose, in combination with the high bacterial burden in the PP1131 group, resulted in a low (1:10 000) phage to bacteria multiplicity of infection at the wound site. On the basis of a literature search and preclinical data, we originally decided to use a phage to bacteria multiplicity of infection of 10:1 in PhagoBurn—ie, 1×10^6 PFU/mL for an estimated 1×10^5 colony-forming units (CFU) per mL of bacteria in the burn wounds. But after the titre loss, we had an estimated 10 PFU/mL of phages versus 1×10^5 CFU/mL of bacteria. With a multiplicity of infection of 10, fast phage replication should occur within hours to overcome the infection. At a low multiplicity of infection, phage propagation is slowed and a slow decrease in bacterial burden is observed. Additionally, the daily cleaning of the wounds, washing away some of the multiplying phages, would have affected the speed of activity.

Additionally, sulfadiazine silver cream was applied directly onto wounds without any interface, whereas PP1131 was applied via an alginate template. Although preclinical in-vitro testing of phage solutions at 1×10^9 PFU/mL showed that the template had no effect on phage release, the possibility of phage trapping at lower concentrations could not be excluded.

Few adverse events were reported in PP1131 group, and they were not substantially different from those reported in the standard of care group. One could argue that the absence of major adverse events is associated with the low doses of active phages given to participants. However, despite a low concentration of functional phages, the combined concentrations of active, inactive, and fragmented phages (eg, heads, tails, fibres) was close to 1×10^6 PFU/mL. Therefore, phage components in PP1131 did not provoke safety issues, which agrees with the documented favourable safety profiles of bacteriophages.[4,5,17]

The most serious septic complications, including septic shock and infectious complications, appeared twice as frequently in the standard of care group than in the PP1131 group. This result was clinically surprising because we expected that a worsening of sepsis would occur in the group comprising older individuals for whom the rate of bacterial reduction was slowed with PP1131 and who had the highest initial bacterial burden. The skin barrier is destroyed in serious burn wounds, increasing the systemic absorption of topical products. The seemingly few serious septic adverse events seen in the PP1131 group led us to hypothesise a clinical effect via the systemic passage of phages with a possible immune stimulation.[18,19] Non-specific immunomodulatory activity occurs with phage therapy,[20] including anti-inflammatory effects (lipopolysaccharide inhibition, and action on interleukin 1 and Toll-like receptor 4), and phagocytosis activation.[21] If confirmed, immune response modulation could be observable by use of a low bacteriophage concentration, an effect that was already described by D'Hérelle and Smith in 1922.[1]

Phage bioproduction for phage therapy and clinical trials, regardless of preparation type (magistral or ready to use), must meet good manufacturing practice standards and be approved by national health authorities.[22] After substantial improvements in the development process, which focused on reducing the concentration of residual endotoxins (by more than a 1000-fold) and other bacterial debris, phage therapy moved from being an exclusively topical treatment in 2015 to an intravenous treatment in 2017; hence, in future studies exploring phage therapy the use of intravenous formulations should be considered.[3] Additionally, decreasing the number of phage types in a product and improving their stability are key points that should be explored in future trials. Development of improved formulations is warranted.

Phage therapy can be given via two strategies: for severe infections caused by multidrug-resistant or extensively drug-resistant bacteria, when time is the limiting factor, a ready-to-use product could be applied without a phagogram, whereas for acute or chronic infections for which treatments can wait for a few days, a phagogram followed by treatment involving a magistral preparation of selected active phages that are compliant with good manufacturing practices seems more appropriate. In both cases, a sufficient shelf-life is needed, whether the product is a single phage or a combination. When the best association of phages targeting the bacterial strain is identified, the mixture should be assembled at the point of care just before use. This approach relies on the availability of phage libraries that are compliant with good manufacturing practices, a compliant production chain, and standardised distribution for clinical use. If phagogram duration can be shortened (currently 2 days

via a manual system, and would need to be shortened to about 0·5 days), a selected phage strategy would also be applicable to infections that evolve quickly.

To benefit from the whole potential of phage therapy, regulatory framework must evolve from fixed treatments adapted to antibiotics (as in 2013) to personalised precision medicine.[6,23,24] Similarly, although the use of a pre-assembled cocktail was mandatory for PhagoBurn, stability issues associated with such a complex product led to changing opinions among both industry experts and active regulating agencies (ANSM, AFMPS, European Medicines Agency, and US Food and Drug Administration). Nowadays, following a preliminary diagnostic test (ie, phagogram), the optimal phage choice to target a patient's bacterial strain can be provided to hospital pharmacists for mixing and dilution before administration. The risk of manipulation errors is decreased by providing clear instructions for use. Additionally, this strategy makes it possible to consider polymicrobial infection management. Between 2017 and 2018, four patients with antibiotic-resistant (multidrug-resistant or extremely drug-resistant) bone, joint, or prosthetic infection, and endocarditis were experimentally treated in Lyon's Centre Hospitalier Universitaire with phages produced by Pherecydes Pharma. ANSM initiated this option by setting up a specialised scientific committee (Comité Specialisé Scientifique Temporaire) that centralises, analyses, and follows up on requests for care from clinicians.

Designing a clinical trial for this mode of personalised treatment is challenging. Standardisation of manufacturing techniques and cohort treatment monitoring in close collaboration with drug safety agencies is needed for a successful study.

PhagoBurn is a foundational trial. From being an unauthorised therapy in 2013, phage therapy has progressed to successful personalised treatment in 2017 under the watch of the French national health authorities. In this study, for the first time, three regulatory agencies approved the use of a phage cocktail produced according to good manufacturing practices in a multicentre, randomised controlled study in a population of patients who had severe clinical conditions. Although progress was slower than in the control group, a clinically relevant reduction in bacterial burden was observed in the phage group, with numerically fewer serious adverse events seen in those treated with the phage cocktail than the standard of care, indicating a favourable potential of phage therapy.

Contributors

The study was conceived and designed by PJ, JG, FR, SJ, Y-AQ, GR, TL, RLF, AFR, and JPP. The data were acquired by JVS, TL, RLF, AFR, FR, HC, CS, and SJ; analysed by PJ, TL, Y-AQ, GR, FR, JPP, SJ, CF, and JG; and interpreted by PJ, JG, TL, FR, GR, SJ, AFR, CF, and RLF. PJ and JG initiated the study and PJ coordinated and oversaw the study. Cocktails were manufactured by Clean Cells (Boufferé, France) and coordinated by IA and LB. Statitec (Toulouse, France) did the trial monitoring and statistical analyses with the support of PJ and TL. PJ was the study coordinator. The manuscript was drafted by PJ, JG, GR, Y-AQ, and TL, and revised for intellectual content and approved for publication by PJ, TL, GR, Y-AQ, FR, SJ, JPP, AFR, RLF, HC, CS, CF, JVS, and JG.

Declaration of interests

We declare no competing interests.

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