Review



# Phage Therapy: An Alternative Approach to Combat Multi-drug Resistant Infections in Cystic Fibrosis

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*Review*

# Phage Therapy: An Alternative Approach to Combat Multi-Drug Resistant Infections in Cystic Fibrosis

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**Abstract:** Patients with cystic fibrosis (CF) are prone to develop life-threatening lung infections with a variety of pathogens difficult to eradicate, which still remain an important issue, despite the therapy for CF has considerably improved in recent years. Moreover, prolonged exposure to antibiotics in combination favours the development and spread of multi-resistant bacteria, thus the development of alternative strategies is crucial to counter antimicrobial resistance. In this context, phage therapy, i.e. the use of phages, viruses that specifically infect bacteria, has become a promising strategy. In this review we aim to address the current status of phage therapy in the management of multidrug-resistant infections, from compassionate use cases to ongoing clinical trials, as well as the challenges this approach presents in the particular context of CF patients.

**Keywords:** cystic fibrosis; bacterial infection; antimicrobial-resistance; alternative strategies; phage therapy

## 1. Cystic Fibrosis and Related Bacterial Infections

Cystic fibrosis (CF) is an autosomal recessive disease caused by mutations in the *ctrl* gene leading a misfunction in the CF transmembrane conductance regulator (CFTR), a cAMP-regulated chloride channel expressed in epithelial cells of different tissues [1]. The loss of ions regulation involves the accumulation of a thick mucus over the lungs, causing an insufficient mucociliary clearance as well as and alteration in phagocytosis. This condition give rise to a suitable environment for the colonization of different bacteria, which often establish persistent infections [2].

Furthermore, as the stagnation of mucus inside the lungs lead to a chronic inflammation and a general decrease of innate defenses, these conditions tend to worsen over time, leading to the onset of respiratory failure [1,3].

The severity of the disease may depend on a combination of genetic and environmental factors [4]. More than 2000 CFTR gene mutations have been reported so far, associated with more or less serious forms of CF [5,6]. In addition, different genes, called modifiers, have been identified able to interact positively or negatively with the CFTR [7,8]. Moreover, environmental factors as type of treatments, the adherence to the therapy and the life-style adopted can significantly influence the evolution of the disease. Anyway, although the severity of the disease may vary considerably from person to person, the progressive impairment of lung tissue caused by persistent infection and inflammation, remain the main cause of morbidity pwCF. In any case, in recent years the therapy for CF has considerably been improved, especially following the introduction of the so-called CTFR modulators, drugs that can restore the function of the CFTR protein of about 40-50% [9].

CTFR modulators are drugs that can improve or restore the expression, the function or the stability of a malfunctioning CFTR. Based on the effects they exert on CFTR mutations, they are classified into five groups: potentiators, correctors, stabilizers, read-through agents, amplifiers [10].

The potentiators are molecules that increase the probability of channel opening in CTFRs with mutations leading to impaired channel gating; these mutations are present in about 5% of pwCF [11].

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Correctors can rescue folding or the proccessing of CFTRs carrying mutations that cause a mistrafficking of the protein to the plasma membrane. This is the case with the F508del mutation, the most prevalent in pwCF [11]. Stabilizer increase the stability CFTR proteins with mutations that significantly reduce the half-life. The read-through agents are compounds that can induce a ribosomal the over-reading of premature termination codon, allowing the introduction of an amino acid instead, thus permitting the translation to continue. Therefore, these compounds can rescue mutations that can introduce a termination codon such as nonsense variants, frameshift or splicing variants. Finally, amplifiers increase expression of the mRNA coding for the CTFR, thus the production of the protein. These compounds are useful for mutations leading to reduced synthesis or maturation of the protein [11].

Nevertheless, although these innovative therapy is improving the lives of several patients with CF (pwCF), these type of drugs are not yet available to treat all known genetic variants, and opportunistic infections still remain an important issue [12].

## 2. An Overview on Main Pathogens of Patients with CF

Among the pathogens that generally infects pwCF Staphylococcus aureus, Pseudomonas aeruginosa, Hemophilus influenzae, Mycobacterium abscessus (Mab) and Burkholderia cepacia complex (Bcc) are the main bacteria [13,14]. These pathogens are difficult to treat since their intrinsic and acquired resistance to antibiotic or different strategies to avoid the host's immune system.

For example, *M. abscessus*, but also the Gram-negatives *P. aeruginosa* and *B. cenocepacia*, possess thick cell walls that impair the crossing of many antibiotics, as well as efflux pumps able to export several molecules toxic for the microorganism [15].

Moreover, the natural antimicrobial resistance is combined to their attitude to establish biofilms [2], communities of the same or different species impenetrable to most drugs [15]. These structures are composed by a matrix of autogenic extracellular polymers that surround the bacteria cells and characterized by a specific elasticity resistant to mechanical stress. This matrix is causative of the improvement of the antibiotic resistance of the bacterial community of even thousand times [16].

*M. abscessus* (*Mab*) is a nontuberculous mycobacterium and an emerging pathogen responsible of severe infections particularly in people suffering from congenital lung diseases, including pwCF. this opportunistic pathogen is acquiring always more importance since its incidence in some industrialized Countries is even higher compared to *M. tuberculosis* [17]. *Mab* may establish chronic infections in immunocompromised patients and people with respiratory diseases and may be causative of complications after pulmonary transplant [18–20]. *Mab* can be found in a smooth variant rich in glycopeptidolipids and associated to biofilm or in a more virulent rough variant poor in glycopeptidolipids and related to persistent and chronic infections [21–23]. As previously stated, *Mab* is intrinsically resistance to many antibiotics due to his thick cell wall and efflux pumps [15,17]. The treatments against this opportunistic pathogen are based mostly on repurposed drugs from therapeutic regimens against *M. tuberculosis* and *M. avium* [24]. Moreover, currently a standard drug regimen to treat *Mab* infections is not established, although consensus recommendations have been pubblished by the US Cystic Fibrosis Foundation and the European Cystic Fibrosis Society [25]. So, the high difficulty to fight and eradicate *Mab* infections is pushing the researchers to develop novel anti-*Mab* strategies and specific drugs.

*P. aeruginosa* is today one of the most studied microorganisms and one the main pathogens of pwCF [14]. Its plastic genome, featured by numerous virulence factors both intrinsic and acquired, confers to this bacterium a high adaptability to the environment, such as the thick mucus of pwCF [26]. Commonly, this plasticity is also causative of *P. aeruginosa* multi-drug resistance, such as resistance to aminoglycosides, cephalosporins, fluoroquinolones and carbapenems [27]. Finally, it may also establish biofilms in chronic infections, exacerbating the clinical pictures of patients [28].

*S. aureus* is a Gram-positive bacterium and an opportunistic superbug belonging to ESKAPEs [29]. Its genomic plasticity was causative of the acquisition of different virulence factors and drug resistances, so that multi-drug resistant *S. aureus* and hypervirulence strains are nowadays a challenging concern for human health [30]. For example, the widespread methicillin-resistant *S.*

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*aureus* (MRSA) may cause infections in skin and soft tissues as well as endocarditis, bacteremia, and osteomyelitis [14,31].

*H. influenzae* is a Gram-negative pathobiont classified in six capsulated serotypes and non-capsulated strains [32]. Overall, the serotype B capsulated strain is the most infective but an effective vaccine is available (Hib). Recently, pathogenic non-typeable serotypes are emerging and they already became a global concern mostly in America and Europe [32].

*Bcc* is composed by around 26 species of which *B. cenocepacia* and *B. multivorans* are the most common [33]. Among all the species of this complex, most of them may cause severe or deathly infections in pwCF, that are hard to eradicate with antibiotics, giving low priority for lung transplantation to infected pwCF [34].

Since antibiotics are not effective to eradicate the infections of the above-described bacteria alternative or complementary therapies are required.

For this reason, along with the classical antibiotic therapies, novel strategies are under development to fight multi-drug resistant strains as well as chronic infections in pwCF [35]. Among these, anti-virulence compounds like quorum sensing inhibitors and iron chelators, the use of nitric oxide or bacteriophages have been successfully exploited to treat some infections of *M. abscessus*, *S. aureus* and *P. aeruginosa* in pwCF [15,36–39].

The use of bacteriophages in therapy is not something new. It has been largely exploited in East Europe for decades and more recently it caught the attention of West Europe [40] so that new treatments are under development against persistent infections, in particular in pwCF and fragile individuals [13].

## 3. Phage Therapy Overall

Since the 1940s, the use of modern antibiotics as anti-microbial therapeutics in the Western world has largely replaced the administration of phages. However, recently the use of phage as supportive therapy to systemic antibiotics for the treatment of complex infections, known as "phage therapy", has gained interest considering the increasing burden of antimicrobial resistance (AMR), multi-resistant bacteria (MDR) and the stalled development of new antibiotics. This is confirmed by the numerous published cases series and case reports of the use of bacteriophages for the treatment of bacterial infections [41,42].

Bacteriophages have been considered for therapeutic use for almost a century, since they were first identified as viruses that attack bacterial targets, and, due to thesuccesses reported by the end of the Fisrt World War, phage therapy became widespread in Eastern Europe and the Soviet Union [43]. Although the first antibiotics, penicillin and streptomycin, became widely available in the early 40s and were adopted in the Western world, phage therapy continued to be used for many years in Eastern Europe. In fact, active therapy programs still exist in few countries [43].

Phages are a class of viruses that infect bacteria in order to complete their own life cycle. After having introduced the genetic material into their host, phages exploit bacterial metabolic functions to either enter a lysogenic state, also called temperate phages, or follow a strictly lytic life cycle. During the lysogenic cycle, phages integrate their genome into the bacterial chromosome, called prophage, so it is replicated together with DNA of the bacterial host. During this phase, they are mostly dormant, but can can anyway modify gene expressions, and they can be activated by many different signals to the lytic state. On the contrary, lytic phages cannot enter in a lysogenic state, but after having infected the host organism, the latter is rapidly killed by the lysis and the releasing of subsequent generation of biologically infectious viruses [41].

Phages are widely distributed in the natural environment and have considerable value for clinical use. Therapeutic phages are usually lytic and recognise and bind to specific bacteria mostly through specific receptors of the external membrane of the host. They offer many different advantages compared to antibiotic treatments, such as shorter time of treatment and a high specificity, although their larger size may restrict the penetration into bacterial replication sites. Moreover, they have the unique ability to replicate at the sites of infection, and hava usually a low

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toxicity, so they can be used in combination as a phage cocktail to better fight the target organisms [36,44].

The characteristics of an ideal phage for phage therapy are typically described as obligately lytic hence virulent, with a broad and also species-specific host range. Notably, phage host range is primarily determined by recognition of bacterial cell surface features by the phage tail fibre, and the modification of these structures should be able to increase phage host range for therapeutic applications [45].

In the choice of phages for clinical use, it is important to consider host specificity. This condition prevents the lysis of bacteria which should not be targeted with the subsequent effect that can arise. However, it is also important to be aware of the potential development of resistance mechanisms in bacteria due to continuous phage applications, which can lead to the co-evolution. To enhance the effectiveness of phages and address phage resistance, several approaches have been adopted, for instance, phage cocktails or combinations of phage and antimicrobial drugs. The use of phages offers several benefits like their self-replicating capacity which allows them to replicate easily, moreover they are also much cheaper and faster to prepare in contrast with antibiotics. Further, they are pretty specific, and no phage replication occurs in human tissues, supporting the observation of remarkable safety in reported cases of treatments [46,47].

The different specificity of these phages depends on their classification. Phages can be roughly divided into monovalent (with a narrow host range) and polyvalent (with a broad host range). Nevertheless, researchers often describe phages differently, and there is still much inconsistency in the use of these terms. Significant investigation has been carried out into the use of both narrow- and broad-host range phages in the treatment of infections and diseases caused by MDR bacteria. The effectiveness of phage therapy is therefore determined by the host range of these phages [48].

It is noteworthy that phage therapy can 're-sensitise' previously antibiotic-resistant bacteria to antibiotics in vitro, a concept known as “phage steering”. However, it has not yet been demonstrated that phage steering can properly work against an infection. Therefore, the phage steering is a therapeutic strategy that utilises phages to kill phage-sensitive bacteria while directing the bacterial that survived to acquire an antibiotic-sensitivity. Actually this therapy seems like able to improve bacterial clearance through antibiotic re-sensitisation in association with phage resistance.

The whole idea of phage steering is to get phage resistance and subsequent susceptibility to antibiotics. It the study of Ashworth *et al.*, the phage resistance developed in the *in-vivo* model, but also in the lungs even in the absence of phage treatment. The lung was the only tissue to exhibit near-total resistance to the incoming phages, while other tissues showed varying levels of phage resistance in the absence of phage treatment. The final point of this approach is that phage steering involves the use of bacteriophages to target and eliminate most phage-sensitive bacteria, while also promoting the development of an antibiotic-sensitive phenotype in the remaining population [49].

It is often believed that phages that replicate using the lytic life cycle are the only ones that have therapeutic value, as the only result of infection is the lysis of the host bacterial cell and release of progeny phages. On the other hand, temperate phages are generally considered somewhat unfavourable because a high proportion of the infected bacterial cells persist to become lysogens and carry the virus as a prophage. It has been observed that temperate phages can be engineered to suppress the lysogenic state by removing part or all of the repressor gene. That is important since also if the better choice is still the use of lytic phages, for some pathogens there is not enough availability of this kind of phages, hence the interest will move toward the available temperate phages which can be improved by the lysogenic state silencing by removing part or all of the repressor gene [50]. For instance, bacteriophages for *Burkholderia*spp are mainly temperate phages [51], limiting their use against these important pathogens of pwCF. However, temperate phages can represent a useful platform for engineering a phage dysplaying a higher virulence and broader host range, as was done in the case of the phage Milagro [52]

However, lysogenic phage can still produce lysis and be virulent, and not all lysogenic phages are able to form stable lysogens, so it is being debated if they may have a therapeutic utility. Moreover, the formation of the lysogens can also depend on external factors, such as the host and the

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environment [53]. In this context, Lauman and Dennis developed a set of metrics to evaluate eight specific phages of *Burkholderia* spp, to determine the tendency to form stable lysogens and their antibacterial activity, in order to assess their potential therapeutic role. This approach should be useful to also exploit temperate phages in a polyphage cocktail [54]. The various recent studies moving in this direction are indeed beginning to lay important foundations for the isolation and development of new phages for use in phage therapy [52,54–56].

Tipically, bacteria lysogenized with a phage are resistant to the infection by phage of the same type, a phenomenon called superinfection exclusion. However, in particular stress conditions, superinfective phages, with the ability the successfully infect lysogenized-bacteria, can emerge [57]. This observation led to the idea of engineering phages, with a view to obtaining superinfectious variants. For example, Prokopczuk and co-workers realized an engeneered superinfective version of the *P. aeruginosa* filamentous phage Pf4, which was succesfully tested in a burn wound infection model, confirming the potential of this approach [58].

Bacteria and phages have a co-evolutionary relationship, where bacteria develop mechanisms to resist phage infection and phages adapt to overcome those bacterial defences. The emergence of phage-resistant bacteria presents a challenge for phage therapy. However, phages have the potential to be pre-adapted to their target bacteria, both in vitro and in vivo. Phage pre-adaptation involves a process of training phages in prior in vitro antagonistic evolution [46].Phage therapy, which has been a long-standing practice in the beginning of 20$^{th}$ century, has undergone a strong revival in the last years, after being largely abandoned in Western countries for many years. A growing number of clinical trials are underway to test the role of various phage products in the fight against multi-drug infections. It is intriguing to note that clinical trials of recombinant and synthetic phage are now being initiated, but are subject to greater scrutiny in terms of safety. Although there is a lack of efficacy data from clinical trials, several countries, have implemented a 'parallel track' for the approval of phage therapy for individual cases of phage therapy on a supportive care basis when antibiotic options have failed [59].

Exploiting the synthetic biology, which combines the principles of biology and engineering to design, existing biological agents to perform tasks that do not occur naturally. There is a clear and remarkable potential in therapeutic phage bioengineering, with innovative approaches being tested on a continuous scale [60]. Improving the knowledge phage-host and phage-human interactions, phage dynamics and genome function is fundamental to enable the generation of a new strategy to combat bacterial infections and to face the challenges related to phage therapy [61].

## 4. Phage Therapy in Patients with Cystic Fibrosis

Although the introduction of CFTR modulators has improved lung function in many pwCF, these innovative drugs cannot be used to treat all mutations, and benefits against inflammation and infection still remain limited. Bacterial infections therefore remain a major challenge. Moreover, infections with pathogens such as *B. cenocepacia* or *M. abscessus* are particularly difficult to eradicate and often preclude lung transplantation [62].

The increasing spread of antibiotic resistant bacteria has revived the use bacteriophages to treat infections. Indeed, phage therapy may have several advantages with respect to conventional antimicrobials, for instance a high specificity for the bacterial host, which limits off-target effects as well as the killing of commensal bacteria, a lower toxicity, as well as a lower cost for the manufacturing [63–65].

Phages have been recently used in several cases as compassionate therapy to treat drug-resistant bacterial infections [66–68], including infections in pwCF caused by *Pseudomonas aeruginosa* [37,69], *Staphylococcus aureus* [37], *Achromobacter* [70–73], *Burkholderia multivorans* [74] and *Burkholderia dolosa* [75] as well as *Mycobacterium abscessus* [64,76,77]. These cases demonstrated the potential of this approach for the treatment of lung infections resistant to all conventional therapies, as well as the safety of phage therapy, also supported by the a positive outcome in some of them.

For instance, interesting information emerged from the compassionate use of phages in 20 pwCF with *M. abscessus* infection [64]. In this study, phage treatment was well tolerated, and no phage

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resistance occurred, even in cases where a single phage was administered. Moreover, half of the patients showed a favourable response and, in some cases, complete resolution of the infection. However, for some patients the clinical benefit was rather low and the reason for the variability in response is still unclear [64].

It is anyway worth noting that *M. abscessus* lineages are characterised by relatively high genetic stability, which could limit the emergence of phage-resistant strains [77].

By contrast, *P. aeruginosa* shows high genetic plasticity and hypermutator genotypes [78], favouring the emergence of phage-resistance [79]. This has been reported, for instance, in a recent case report describing the individualised use of phage therapy in two pwCF with *P. aeruginosa* infection [80]. Indeed, both patients displayed symptomatic improvement after the treatment, but at the end of therapy had a regrowth of *P. aeruginosa*, conceivably due to the emergence of phage resistance [80]. This does not exclude that cases of *P. aeruginosa* infection can be successfully treated by phage therapy, as described in a case report in which a pwCF with chronic *P. aeruginosa* infection was successfully treated with intravenous administration of a cocktail of 4 bacteriophages, enabling the patient to undergo lung transplantation [81].

Beside the treatment of pwCF with mycobacterial or pseudomonal infections, cases of compassionate treatment with phage in patients with infection with *Burkholderia* spp [74,75], and *Achromobacter* spp infections [70,72,82] have also been reported. Even in this type of infection, however, the effects of phage therapy, as well as the outcome of patients, proved to be very heterogeneous .

It must always be taken into account that these cases of compassionate use of phages are characterised by a high heterogeneity, regarding the pathogens involved, the severity of the disease and the overall condition of the patients, and often lacks standardisation and a control group, so conclusions must still be taken with caution [83].

However, the potential of phage therapy for the treatment of lung infection ina particular context such as in pwFC has yet to be fully explored, and several questions such as the administration route, length of the treatment, the concomitant administration of mucolytic agents, the formulation have yet to be solved.

For example, the altered mucociliary clearance, and the production of dense, thick mucus, favours the establishment of microbes and affects immune responses to infection and inflammation. Moreover, in this type of environment, bacteria tend to form biofilms.

It is clear that this peculiar environment can have a strong impact on the efficacy of drugs, especially when administered by inhalation. For example, it has been seen that high mucin and DNA levels increase the viscosity of CF dysfunctional mucus, limiting the diffusion of antibiotics [84,85]. The same problem could conceivably occur with phage administration.

For instance, a study on the compassionate use of phages, in a series of patients with mycobacterial lung infections, showed that intravenous administration was more effective in treating disseminated infections, particularly in the presence of structural damage to the lungs or dysfunctional mucus. In contrast, administration by nebulisation limited neutralisation of the phages by the immune system of the patients [36].

As mentioned above, CF pathogens tend to form a biofilm which, due to its complex composition, can hinder the diffusion of antibiotics, making treatment even more difficult. However, some bacteriophages produce depolymerases, capable of modifying the polysaccharides in the biofilm, thus improving their penetration into the matrix and their effectiveness [86]. Together with the aforementioned phage steering, this evidence supports the potential of combining phage therapy with conventional antibiotics for the treatment of multidrug-resistant infections, as confirmed by several *in vitro* and *in vivo* studies [87–91].

A further problem could arise from the fact that some pathogens, such as *M. abscessus*, are intracellular, implying the need for phages to reach bacteria residing in human cells. Indeed, the absence of specific receptors on the surface of mammalian cells would preclude phage internalisation, suggesting that phage therapy could only be effective with extracellular bacteria [13]. Furthermore, *M. abscessus* usually resides in macrophages, so there is also the possibility of phage clearance from

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the intracellular environment [92]. However, different recent studies reported the possibility of internalization of phages by phagocytic cells [93,94], as well as the possibility of phages to penetrate into mammalian cells and to kill intracellular *M. abscessus* [13].

The restricted host range of phages could be another limitation for phage therapy, particularly in the context of cystic fibrosis, which is characterised by multiple infections. To circumvent this problem, several approaches are used, such as the use of phage mixtures, phage libraries, extensive screening and genetic engineering techniques [95].

Currently, phage therapy for the treatment of infections in pwCFs has been used mainly in compassionate use cases, which, despite the lack of rigour and consistency of treatment and monitoring, have nevertheless provided a number of useful insights for the design of clinical trials. A multicentre clinical study was recently concluded. This study was conducted to evaluate the safety, tolerability, pharmacokinetics and pharmacodynamics of a multiphage cocktail in pwCF with chronic *P. aeruginosa* lung infections, demonstrating the tolerability and efficacy of the formulation used [96]. In addition, several clinical trials of phage therapy in CF infections by *P. aeruginosa* (NCT01818206, NCT04684641, NCT05453578, NCT05010577, and NCT04596319) or by non-tuberculous mycobacteria (NCT06262282) are currently ongoing (Table 1) [97].

**Table 1.** Currently ongoing or completed clinical trials of phage therapy in pwCF [80].

| ClinicalTrials.gov ID | Official title | Pathogen | Type of phage(s) | Current Status | Last update posted |
|---|---|---|---|---|---|
| NCT04684641 | CYstic Fibrosis bacterioPHage Study at Yale (CYPHY): A Single-site, Randomized, Double-blind, Placebo-controlled Study of Bacteriophage Therapy YPT-01 for Pseudomonas Aeruginosa Infections in Adults With Cystic Fibrosis | *P. aeruginosa* | single phage | Completed | 2023-11-18 |
| NCT01818206 | Bacteriophages Effects on Pseudomonas Aeruginosa Presents in Sputum of Cystic Fibrosis (CF) Patients | *P. aeruginosa* | cocktail of 10 phages | Completed | 2013-09-05 |
| NCT05453578 | A Phase 1b/2, Multi-Centered, Randomized, Double-Blind, Placebo-Controlled Trial of the Safety and Microbiological Activity of a Single Dose of Bacteriophage Therapy in Cystic Fibrosis Subjects Colonized With Pseudomonas Aeruginosa | *P. aeruginosa* | cocktail of 4 phages | Recruiting | 2024-06-03 |
| NCT05010577 | A Phase 1b/2a, Randomized, Double-Blind, Placebo-Controlled, Multicenter Study to Evaluate Nebulized Bacteriophage Treatment in Outpatient Adult Cystic Fibrosis (CF) Subjects With Chronic Pseudomonas Aeruginosa (PsA) Pulmonary Infection | *P. Aeruginosa* | single phage | Active, not recruiting | 2023-10-18 |
| NCT06262282 | A Prospective Standardized Assessment of People With Cystic Fibrosis and Non-tuberculosis Mycobacteria Pulmonary Disease Undergoing Treatment With Mycobacteriophage (POSTSTAMP) | Non-tuberculous mycobacteria (NTM) | | Enrolling by invitation | 2024-02-16 |
| NCT04596319 | A Phase 1b/2a, Multi-Center, Double-Blind, Randomized, Placebo-Controlled, Single and Multiple Ascending Dose Study to Evaluate the Safety and Tolerability of AP-PA02 Multi-Phage Therapeutic Candidate for Inhalation in Subjects With Cystic Fibrosis and Chronic Pulmonary Pseudomonas Aeruginosa (Pa) Infection | *P. Aeruginosa* | multi-phage cocktail | Completed | 2024-01-31 |

## 5. Conclusion

Although progress in research has dramatically improved the life of patients with cystic fibrosis, infections by multidrug resistant bacteria continue to represent a major challenge. Among the alternative therapeutic strategies to overcome the lack of new effective antibiotics, phage therapy is

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standing out in particular. However, the peculiarities found in cystic fibrosis infections pose several difficulties to be overcome, leaving some questions still unanswered, such as the best route of administration, phage formulation and concomitant use of antibiotics or mucolytic agents, and the duration of the treatment period. However, the recent use of phage therapy in compassionate cases has provided encouraging indications of its efficacy, and several clinical trials currently underway will help implement this important approach in the cure of pwCF.

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