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A Case Series of Emergency Investigational New Drug Applications for Bacteriophages Treating Recalcitrant Multi-drug Resistant Bacterial Infections: Confirmed Safety and a Signal of Efficacy

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Abstract

The advent and increasing prevalence of antimicrobial resistance commensurate with the absence of novel antibiotics on the horizon raises the spectre of untreatable infections. We must now grapple with infections stemming from extensively multi- and pan-drug resistant bacterial strains. Potential non-antibiotic options to treat Multi-Drug Resistant (MDR) infections include bacteriophages and there has been much fervour in resurrecting research into its clinical use.

Although not subjected to the contemporary rigorous scientific standards for clinical trials, there appears to be an abundance of data purporting safety of bacteriophage therapy regardless of administration route. The US Navy and Adaptive Phage Therapeutics have taken a precision approach to development of bacteriophage therapy. Herein, as opposed to fixed phage cocktails, we exploit the quintessential example of personalized medicine by acquiring the patient's infecting isolate and identifying a phage cocktail proven to lyse the bacteria. As we prepare to execute our FDA regulated clinical phase II bacteriophage therapeutic trials in the ensuing year(s), we have engaged in numerous compassionate use eIND cases to provide potentially life-saving bacteriophage treatment to patients either failing conventional antibiotic therapy due to MDR resistance, or stemming from an inability to secure definitive source control. In all eIND cases, "personalized" bacteriophage cocktails were selected which "targeted" the infecting organism. This case series reports upon 13 emergencies investigational new drug (eIND) cases whereby patients failing antibiotic therapy safely received bacteriophage mixtures (cocktails) without identifying any bacteriophage-mediated adverse effects. Adjudicated microbiologic eradication of the targeted bacterial isolate was achieved in 11 cases, while 6 cases were clinically adjudicated to have achieved therapeutic efficacy defined as clinical resolution. The balance of non-resolved cases was secondary to curtailed therapy (patient expiring), non-infectious mediated organ failure, or relapse of infection from biofilm-mediated infections.

Keywords: Bacteriophages; Antibiotics; Infectious diseases; Multi-drug resistance; Clinical trials

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Introduction

The advent and increasing prevalence of antimicrobial resistance commensurate with the absence of novel antibiotics on the horizon raises the specter of untreatable infections [1]. We must now grapple with infections stemming from extensively multi- and pan-drug resistant bacterial strains. Ultimately, the pervasive fear is regressing to a post-antibiotic era manifesting untreatable bacterial infections. During the past two decades, public health agencies reported on the dramatic increase of drug-resistant pathogens, a worrisome situation leading the World Health Organization to declare a new 'preantibiotic era' in

its 2014 surveillance report (http://www.who.int/antimicrobial-resistance/publications/surveillance report/en/). The annual death toll attributed to MDR organisms is estimated to be over 23,000 in the U.S., 25,000 in Europe and more than half a million people worldwide [2] projected to reach 10 million in 2050 at the current trajectory [3]. The estimated annual cost of treating infections caused by antibiotic-resistant bacteria in Europe is about 1.5 billion euros, while in Canada it is about $200 million, and up to $77.7 billion in the USA [2].

As the development of novel antibiotics stagnates, we must make a concerted effort to identify efficacious non-antibiotic antimicrobial therapies to combat the inexorable increase in bacterial MDR infections. Potential antimicrobials of import include bacteriophage and bacteriophage lysins [4-6]. In fact, bacteriophages have been named by the US National Institute of Allergy and Infectious Diseases as one of the seven weapons we may marshal to fight against antibiotic resistance [6,7]. Phages employed for therapeutic use will need be obligately lytic. Lytic phages do not integrate their genome within the host bacterial genome rather they replicate rapidly using host synthetic machinery. Finally newly synthesized phage particles lyse the bacteria to repeat the cycle again until they kill almost all the bacteria [8,9].

As a class of antimicrobial agent, bacteriophages offer several potential advantages including: (1) absence of safety concerns as delineated in a recent review [1]; (2) bactericidal activity; (3) localized concentration increase at the site of infection; (4) minimal collateral damage to the healthy microbiome; (5) bactericidal efficacy irrespective of antibacterial resistance profiles; (6) potential synergy with antibiotics, (7) potential reversion of bacterial susceptibility to antibiotics; (8) activity against bacterial biofilms; and (9) anticipated cost-effectiveness of pharmaceutical development [7-13].

The advent of multidrug bacterial resistance, commensurate with a paucity of novel antibiotics in the development pipeline, resurrected research into bacteriophage treatment. Presently, phage therapy suffers from insufficient credibility, patient and physician unfamiliarity, limited product availability and an ambiguous navigation of the regulatory environment in which to reach market [14]. However, recent reviews provide valuable insight into the preponderance of historical and contemporaneous clinical use of bacteriophage [1-7,14]. Although not subjected to the contemporary rigorous scientific standards for clinical trials, there appears to be an abundance of data purporting safety of bacteriophage therapy regardless of administration route predominantly borne from the Eastern Bloc nations [14,15]. Given their extensive experience, there appears to be enough historical evidence to motivate contemporary methodologically rigorous clinical trials to evaluate the safety and efficacy of bacteriophage therapy [14].

The US Navy and Adaptive Phage Therapeutics have taken a precision approach to development of bacteriophage therapy. As we prepare to execute our FDA regulated clinical phase II bacteriophage therapeutic trials in the ensuing year(s), we have engaged in numerous compassionate use eIND cases to provide potentially life-saving bacteriophage treatment to patients either failing conventional antibiotic therapy due to MDR resistance, or stemming from an inability to secure definitive source control. In all eIND cases, "personalized" bacteriophage cocktails were selected which "targeted" the infecting organism. In this personalized approach we require acquisition of the patients infecting organism, thence screening against an exhaustive library of characterized phages to identify a personalized "targeted" mixture ("cocktail") of phages which efficaciously killed (*in vitro*) the infecting isolate.

There are two significant challenges to establishing broadly efficacious bacteriophage therapy, which stem from the host specificity of bacteriophages and the naturally occurring genetic diversity of pathogens in circulation. First, any bacteriophage preparation may be inadequate to treat a large fraction of cases. Second, selective pressure from bacteriophage predation commonly results in expansion of variants resistant to the attacking phage. Our resolution to these issues in the clinical application of bacteriophage therapy is a precision approach based on case by case design of personalized (targeted) phage combinations to ensure lytic activity. Furthermore, this strategy can be applied iteratively whenever a round of phage therapy starts to fail due to the emergence of resistance. Herein, we will continually exploit the natural evolution of the bacteria and its associated phage which presumably guarantees the isolation of a phage which targets the mutated bacterial strain. To support this approach, we developed ongoing maintenance and expansion of a library of natural phages which allow open-ended development of precision phage cocktails; *via* a screening strategy we call Host Range Quick Test (HRQT). This testing platform incorporates a colorimetric assay and monitors the growth of bacteria *in vitro* with/without interventions including antibiotics and bacteriophages. Efficacious phage(s) suppress bacterial proliferation and emergence of phage resistance for an enough period reflecting *in vitro* (and in our experience by extension *in vivo*) efficacy. The speed of this strategy, and its ability to identify synergistic phage combinations, and phage-antibiotic synergy, enables practical clinically viable personalized phage therapy, to overcome phage resistant problem during therapeutic application of phages. Future research will strive to exploit the HRQT to additionally identify optimal biofilm degrading phages in isolation and with adjunctive therapies. Additionally, as opposed to the cost and resources required to develop antimicrobials, selecting new phages (e.g., against phage-resistant bacteria) is a relatively rapid and inexpensive endeavor.

An appreciable percentage of the current research effort in the field of antimicrobial phage therapy focusses on the therapeutic efficacy of engineered phages and fixed cocktails. We acknowledge there may be some initial efficacy in this approach, however, we believe that fixed (including engineered) cocktails inevitably exhibit inherent weaknesses stemming from inexorable development of bacterial resistance. Regardless of the engineering executed, the prohibitively high rate at which bacterial populations give rise to phage-resistant strains when under selective pressure will foster treatment failure requiring identification of "next generation phage cocktail" to address the resistant clones. Acknowledging the inevitable development of resistance, we advocate for a strategy in maintaining and

continuous expansion of a phage library and screening the actual pathogen against this library to identify a phage targeting cocktail as the optimal approach to counteract the inevitable resistance development as we will continually exploit the natural evolution of the bacteria and its associated phage which presumably guarantees the isolation of a phage which targets the continuously mutating bacterial strain.

Case Series of Emergency Investigational New Drug Applications

In Table 1, we delineate thirteen eIND cases, in which at least one dose of personalized bacteriophages was successfully administered (all without any safety concerns attributed to the phage therapy). Safety was assessed using clinical and laboratory

Table 1 Synopsis of 10 eIND Bacteriophage treatments delineating demographics, clinical syndrome, bacterial pathogen, route and duration of phage therapy and microbiological and clinical adjudication.

Patient ID	Patient Age/ Gender	Diagnosis (Infectious Clinical Syndrome)	[1]Bacterial Isolate(s)	Route of Phage Administration	[2]Phage Cocktail[#] [[#]Targeted Personalized Phages]	[2]Duration of Phage Therapy	Microbiological Adjudication Microbiological Eradication Phage + Antibiotics (Y, N or Indeterminate)	Clinical Adjudication Clinical Improvement and/or cure (Y, N or Indeterminate)
1	(68/M)	Necrotizing pancreatitis Pancreatic pseudocyst	*A. baumannii*	IV and Percutaneously	2 Phage Treatments Provided[3]	11 weeks	[3]Yes	[3]Yes
2	(2/M)	DiGeorge syndrome Complex congenital heart disease Bacteremia and mediastinal abscess	*P. aeruginosa*	IV	One Phage Treatment Comprising a 2 Phage Cocktail	2 days	[4]Yes (blood) Indeterminate (local)	[4]Indeterminate
3	(77/M)	Traumatic brain injury Post-operative craniectomy site infection	*A. baumannii*	IV	One Phage Treatment Comprising a 2 Phage Cocktail	8 days	[5]Indeterminate (no cultures acquired)	[5]Indeterminate
4	(68/M)	Hypersensitivity pneumonitis Pulmonary fibrosis s/p bilateral lung transplant Post-transplant Pneumonia x 2 episodes	*P. aeruginosa*	IV and Nebulizer	2 Phage Treatments Provided Episode 1 (3 phage cocktail) Episode 2 (2 phage cocktail) *used in suppressive fashion after resolution of infection *A non Navy phage was initially administered prior to the start of the Navy-APT phages	2 weeks (episode 1) 4 weeks (episode 2)	[6,13]Yes (Negative blood and BAL cultures)	[6]Yes

5	(25/F)	Cystic fibrosis s/p bilateral lung transplant Post-transplant pneumonia and sepsis	*Burkholderia cenocepacia*	IV	One Phage Treatment Comprising "1" Phage	2 doses	[7]No (Only two doses administered prior to expiring)	[7]Indeterminate
6	(60/M)	Left Ventricular Assist Device Infection and septicemia	*P. aeruginosa*	IV	One Phage Treatment Comprising "3" Phages	6 weeks	[8]Yes (Negative Blood Cultures but not Durable (presumed stemming from a secondary strain emanating from biofilm)	[8]Indeterminate
7	(41/M)	Post-surgical L knee wound infection	1 *K. pneumoniae* 2 *A. baumannii*	IV	*K. pneumoniae* One Phage Treatment Comprising 1 Phage *A. Baumanii* Two Treatments each Comprosing 1 Phage	2 weeks	[9]Yes (Negative Blood Cultures)	[9]Yes
8	(M/28)	Cystic Fibrosis s/p Bilateral Lung Transplant	*Burkholderia dolosa*	IV	One phage Treatment Comprising "1" Phage	5 weeks	[10]Yes (Negative Blood and BAL Cultures)	[10]Indeterminate
9	(18/F)	Bacteremia/ urosepsis s/p kidney transplant	ESBL *E. coli*	IV	One Phage Treatment Comprising "2" Phages	23 days	[11]Yes (Negative Blood and Urine Cultures)	[11]Yes
10	(47/M)	Ventriculitis Meningitis	*A. baumannii*	IV	One Phage Treatment Comprising "1" Phage	8 days	[12]Yes (Negative CSF Culture)	[12]Indeterminate
11	(23/M)	Cystic Fibrosis Lung Transplant and Sternal Wound Infection	*Burkholderia gladioli*	IV	1 Phage Treatment Compromising "1" Phage	19 weeks	[15]Yes (Negative Culture)	[15]Yes
12	(36/M)	Recurrent Urinary Tract Infection	ESBL *E.coli*	IV and Intravesicular	One Phage Treatment Compromising "2" Phages	20 days	[16]Yes	Indeterminate (Recurrence After 2 Months)

13	(10/F)	Cystic Fibrosis Lung Infection	*A.xylososidans*	Nebulizer and IV	One Phage Treatment Compromising "1 phage"	3 weeks	[17]Yes Sterile Culture (Active)	[17]Yes

[1]All bacterial isolates were MDR, and clinical syndromes reported had all failed optimal antibiotic therapy with clinician directed antibiotics continued during phage therapy.

[2]Clinical resolution attributed to bacteriophage treatment often required multiple cocktails (mixtures) to accommodate either emerging bacterial resistance to phage and/or novel strains (biofilm-mediated).

[3]For methods and discussion regarding microbiological and clinical course see Schooley et al, 2017 Antimicrobial Agents and Chemotherapy.

[4]The phage therapy was interrupted and truncated given cardiac issues. The fragmented and short period of administration undermined clinical interpretation. Surgical team refused local instillation into the abscess. The patient did manifest negative blood cultures upon each phage introduction (Duplessis, 2017, JBIDS).

[5]Cerebral infection in a comatose patient without microbiologic specimens acquired during or post phage administration given reluctance to pursue given poor clinical condition. Family refused local therapeutic instillation (nor attempts at intracerebral specimen acquisition). Technical limitations constrained the desired optimal phage concentrations. Family withdrew care with patient expiring shortly thereafter. For methods, and discussion regarding microbiological and clinical course see Lavergne et al, 2018; Open Forum Infectious Disease.

[6]Pulmonary concentration of phage was several log10 higher than the given dose and indicative of bacteriophage replication in the lung. Variability in antibiotic sensitivity patterns of Pseudomonas aeruginosa (PsA) was noted with phage treatment. The patient clinically responded to phage and antibiotic therapy with resolution of two distinct episodes of PA pneumonia and improved respiratory status. Phage was administered as suppressive therapy without any breakthrough PsA infection.

[7]The patient received only two doses of intravenous bacteriophage therapy before she passed due to progressive respiratory failure. Preliminary autopsy results reportedly identified phage in her lung tissue.

[8]The patient was administered a three-phage cocktail intravenously for six weeks at variable titers, a pioneering case for the use of outpatient intravenous phage without sequelae. The patient cleared blood cultures, but upon treatment cessation, relapse occurred with the same strain (confirmed by genetic sequencing) presumably emanating from the LVAD biofilm.

[9]Sustained multiple traumas after a motor vehicle accident, developing post-operative wound infections on his left knee caused by multidrug resistant *Klebsiella pneumoniae* and *Acinetobacter baumannii*. A second muscle flap surgery was performed in a final attempt to save the patient's leg from amputation. Following surgery, the patient received two one-week courses of intravenous bacteriophage therapy with titers of 5.0 × 107 and 5.3 × 107 PFU/mL for the *K. pneumoniae* and *A. baumannii* phage, respectively achieving microbiological and clinical resolution (muscle flap healed completely).

[10]The patient's bacterial titer dropped significantly, and the physician reported minor clinical improvements after 34 days of treatment. However, the patient suffered from an unrelated splenic pseudoaneurysm, and underwent a splenectomy and distal pancreatectomy, cessation of phage therapy, and subsequent dissemination of infection, leading to death.

[11]Phage therapy consisting of two different phage products with titers ranging from 1.0 × 109 to 1.0 × 1010 PFU/mL. The patient ended the course of antibiotics 15 days after start of phage therapy. She became afebrile exhibiting marked clinical improvement after one week of treatment without antibiotics and remained culture negative 11 days after a total 23-day course.

[12]Significant head traumas after a motor vehicle accident complicated by an abscess and ventriculitis caused by a multidrug resistant strain of *Acinetobacter baumannii*. After 8 days of treatment, the patient's CSF was culture negative for the MDR *A. baumannii*, however, he was culture positive for a strain of *Klebsiella pneumoniae* and *Staphylococcus aureus* leading to brain death.

[13]In Aslam, 2019, three case reports utilizing adjunctive phage therapy are described including the initial case involving a pulmonary transplant secondary to hypersensitivity pneumonitis.

[14]Note, in Case 1 and 4, non-Naval-APT fixed phage cocktails supplied by alternative entities were initiated in treatment prior to commencing the Naval-APT personalized phage cocktials.

[15]The patient received 19 weeks of phage therapy consisting of 1 phage. After 11 days of phage therapy significant clinical improvement was observed, after 35 days patient was culture negative therapy continued as the sternal wound healed.

[16]After 13 doses of phage therapy the patient became culture negative, the therapy was stopped, and patient remained culture negative for two months. After two months the patient was readmitted to the hospital for similar symptoms. At this time the patient was no longer interested in receiving phage therapy and the new bacterial isolate was not tested further for phage sensitivity.

[17]Patient was initially treated with the experimental antibiotic cefiderocol and after 17 days her phage therapy was initiated. On the fifth day of a receiving both phage and antibiotic, the antibiotic regimen was stopped and phage therapy continued for approximately 2 weeks.

parameters, including clinician-maintained adverse event logs, vital sign monitoring, and serial complete blood count and comprehensive metabolic collection. Administration of phage was conducted at each institution with individual emergency Investigational New Drug applications (eIND) from the U.S. Food and Drug Administration (FDA), Institutional Review Board notification and approval, and patient informed consent. This table doesn't capture the host of cases in which phages were solicited, but conditions changed (patient improved upon salvage therapy or expired prior to phage delivery). In synopsis, the tabulation provides the patient demographics, clinical syndrome, bacterial pathogen, route of administration, duration of phage therapy, and clinical outcome including adjudicated clinical response and microbiological results. In all cases, it may be assumed that the endotoxin content in all phage preparations were well below the maximum (5U/kg/hr) allowable levels as disseminated by the FDA. All infections were secondary to MDR bacterial isolates, and in all clinical syndromes the treating physician (eIND sponsor) deemed the patient was failing optimal standard of care (SOC) antibiotic therapy and unlikely to respond (survive the infection) without herculean interventions (i.e., the addition of innovated antimicrobial approaches including phage therapy).

Saliently, the inclusion criteria for accepting the eIND request required that the patient's treating physician (again in all cases serving as the eIND sponsor) in consultation with the medical consultants of APT all reached the conclusion (after an exhaustive review of the patient's medical history and current response to treatment) that the patient (a) was experiencing a severe infection due to an MDR bacteria; (b) was not responding to at least one antibiotic course of treatment; (c) experienced optimization of their host immunity and source control (as dictated by the treating staff, which could have declared suboptimal source control constrained by surgical restrictions); (d) would experience a poor prognosis without aggressive attempts to eradicate the infecting isolate in the throes of presumptive failure with any subsequent antibiotic trials and (e) was expected to survive for a sufficient time to have received a complete course of the novel phage therapy (somewhat arbitrarily perceived to be at least 5 days based on review of the observational data in the literature). There were no pre-ordained restrictions (exclusion criteria) in considering an eIND case based on age, clinical syndrome, or infecting pathogen. The eIND was declined if there could be no timely identification of a phage cocktail targeting the infecting isolate. In general, phage cocktails could be identified for non-mycobacterial, non-fastidious (Burkholderial) infections. There were cases in which a phage cocktail couldn't be identified in enough time targeting these two infecting pathogens. Aside from the, there was one case presenting with a PsA isolate for which we couldn't identify a successful lytic phage due to the carriage of a lysogenic phage. Fortunately, this scenario is quite rare and can be overcome by selecting a lytic mutant of lysogenic phage. In all cases, the personalized phage cocktails were administered intravenously (IV). Preclinical and observational data suggests that phages achieve widespread penetration throughout all organ systems. Intuitively, for specific clinical syndromes (pneumonia, abscesses) we attempted to concomitantly administer phage therapy locally (*via* nebulized phage delivery in the former and percutaneous instillation in the latter case). Formal clinical trials will need to assess the potential synergy in treatment efficacy employing such an approach, or if the additional topical/local phage delivery is extraneous to IV phage delivery. Without clear guidance nor insight regarding the pharmacokinetics, and pharmacodynamics of phage administration, and acknowledging the rapid clearance from the blood stream [16], in all cases we recommended a frequency of IV administration at 6 to 8 hour intervals accepting what was accommodated by the treating staff. At the treating physician discretion, phage administration occurred at more frequent intervals (for example, 2 hours in case-4, never engendering safety concerns). Similar recommendations were rendered for the two cases receiving local phage instillation. In most cases, multiple phages targeting the organism, and exhibiting proven additive or synergic *in vitro* killing (lytic) activity (*via* the HRQT) could be identified. As tabulated, a few cases required identification of a second cocktail (owing to developing bacterial resistance to phage, or presumptive novel bacterial strains which originated from biofilms). Finally, in two cases (cases 1, 4), non Navy fixed phage cocktails were initially administered, prior to the request for the Navy-APT personalized phage cocktails. We do observe that successful implementation of adjunctive phage therapy may often require extended therapeutic courses as intimated below, wherein abridged therapeutic courses may be unsatisfactory [17-19,20]. Additionally, given the excellent adjuvanticity of phage, prolonged phage therapy may provoke both cell mediated and humoral responses against both the bacteria and phage due to presentation of phage bacterial complexes to the immune system. Finally, an additional benefit and clinical use of phage therapy may be to administer them in a prophylaxis fashion in an attempt to repopulate a salubrious microbiome, while promoting a conversion of antibiotic resistant bacteria to a more sensitive population [20].

In all cases, the sponsoring physician continued optimal antibiotic courses (according to local SOC guidelines) concomitant to phage therapy. Adjudication was executed by the patients treating physician (serving as eIND sponsor in all cases) and the medical consultant from APT assisting with the compassionate use eIND.

Definitions for Microbiological eradication and Clinical Improvement

Microbiological eradication implies durable elimination of all targeted bacterial infection (negative blood culture and negative local infection as appropriate to the given clinical syndrome [for example, negative cultures from a bronchoalveolar lavage upon treatment of ventilator associated pneumonia]) out to 28 days post treatment. Clinical improvement implies resolution of the infectious clinical syndrome which in all successful delineated cases was adjudicated to be ascribed at least partially to the introduction of bacteriophage therapy, and highly unlikely to have been achieved if continuing SOC therapy without the introduction of adjuvant phage therapy. In all indeterminate cases, the phage therapy was abridged secondary to patient expiring, or adverse

outcomes occurred secondary to non-infectious mediated organ failure, or relapse of infection from biofilm-mediated infections. In none of these cases, did the adjudication bodies identify adverse effects attributed to the phage therapy.

Our results from this case series of eINDs corroborate the safety of phage as recently reported in a comprehensive review [1]. We still await results from methodologically rigorous clinical trials assessing the clinical efficacy of phage therapy as an adjunct to antibiotics, source control, and host immunity optimization. However, these cases provide a signal of efficacy as recently reported in contemporary literature including successful topical phage therapy for (a) infected (*S. aureus*) diabetic ulcers [21]; (b) infected venous stasis ulcers (polymicrobial) exploiting the PhagoBioderm topical biopolymer licensed in the Republic of Georgia [22]; (c) otitis externa (*Pseudomonas aeruginosa*) [23]; and (d) chronic prostatitis (*Enterococcus faecalis*) and recently published results suggesting phage mediated efficacy in eradicating an infected aortic graft with *Pseudomonas aeruginosa* employing a single local instillation of phage [24]; and (e) IV phage administration to clear a PsA bacteremia [25].

Preparations for Assessing Clinical Efficacy of Personalized Phage Cocktails in Phase II Clinical Trials

Although we've garnered tremendous insight into optimizing the process of personalized phage delivery (acquiring the infecting isolates, identifying personalized "targeted" phage cocktails, processing a phage cocktail in a clinically viable and expedient formulation), there are too few cases to draw major conclusions regarding clinical therapeutic efficacy. However, we have observed a signal suggesting efficacy in this case series of eIND cases reflecting some of the most refractory cases. We have expedited our process, reducing our processing and manufacturing times significantly and have developed novel proprietary methodology to minimize the endotoxin content of our preparations eliminating this prior constraint to maximizing phage concentrations, all of which are necessary steps toward the successful implementation of phage therapy on a larger scale than individual eIND cases.

We are now pursuing phase II clinical bacteriophage trials to commence this year, specifically assessing the safety and efficacy of adjunctive bacteriophages in treating recalcitrant MDR urinary tract infections and infected ulcers (diabetic, venous stasis, decubitus). Integral to our trial designs herein, we will endeavor to (1) improve understanding of phage pharmacokinetics, and pharmacodynamics including patients experiencing renal and/or hepatic insufficiency, (2) optimize clinical phage administration clarifying the optimal administration frequency, route of administration, dosing, and duration of therapy, (3) optimize the timing and sequence of phage administration relative to antibiotics (pre-clinical data suggests optimization with sequential introduction of phage followed by antibiotics) [26,27], (4) clarify the breadth, and depth of the host immune response (adaptive and innate) to phage and its influence upon treatment efficacy, (5) clarify non-IV administration efficacy [potential additive, competitive interference or extraneous treatment efficacy (i.e., nebulized phage therapy for pulmonary infections; intra-vesicular administration for GU tract infections)],(6) optimize our HRQT procedures for *in vitro* assessments of phages which not only target the bacterial isolate but harbor biofilm degrading activity (only a percentage of phages harbor this activity), and (phage coupled to compounds forstering biofilm degradative activity, (7) optimize topical phage formulations (encapsulation methods) for a wide spectrum of cutaneous infections,(8) garner insight into factors promoting bacterial resistance to phage during treatment, and (9) optimize phage administration in a prophylaxis fashion to achieve a healthy microbiome while resensitizing antibiotic resistant bacteria.

Commensurate with executing the clinical trials, we will continue optimizing (streamlining) our personalized phage development process: (1) minimizing the time from isolate acquisition to patient treatment with a personalized phage cocktail; (2) optimizing our proprietary HRQT to seamlessly execute phage-antibiotic synergy testing and identify phage mediated biofilm activity (to optimize treatment of prosthetic joint infections, cystic fibrosis cases, and all clinical infections associated with biofilms). Ultimately, our vision will be to perch dedicated local phage banks at major hospital centers accommodating expedient phage therapy.

Conclusion

This case series reports upon thirteen eIND cases whereby patients received at least one dose of bacteriophage mixtures (cocktails). Most saliently, there were no safety concerns identified with phage administration in these thirteen cases. We noted clinically adjudicated microbiologic eradication of the targeted organism in 11 cases. We acknowledge in all cases. Therefore, antibiotics were continued therefore; we may not attribute success entirely to introduction of phage. However, in all cases the treating physician believed the patient wouldn't clear the infection with antibiotics alone. In the 2 indeterminate microbiological assessments, specimens were not acquired as the patient expired. In 6 cases, we identified clinically adjudicated evidence of phage mediated therapeutic efficacy. In indeterminate cases, the reasons cited for failure included (1) sub-optimal phage administration duration, (cases 2, 3, 5 and 10); (2) non-infectious complications including cardiac decompensation (case 2); perceived brain death and withdrawal of care (case 3); post-surgical complications (case 8); relapse confirmed by genetic sequencing of the same bacterial strain presumably emanating from the LVAD biofilm (case-6) and relapse *vs.* appearance of a novel strain from the bladder (case 12). We cannot conclude whether extended phage therapy would have unequivocally cleared all infectious nidi and cured the clinical syndrome. We do hypothesize that optimal outcomes require a minimal threshold course of phage (coupled to antibiotics) coupled to source control and optimization of the patients' immunity. As intimated earlier, we posit that phage therapy may provide clinical efficacy in recalcitrant MDR infections but as always will necessarily be one of the integral components of a four-pronged approach to treatment.

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