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SUPPLEMENTARY INFORMATION

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Engineered bacteriophages for treatment of a patient with a disseminated drug-resistant *Mycobacterium abscessus*

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Supplementary Information

Supplementary Background and Results

Prior reports of phage therapy: To our knowledge, mycobacteriophages have not been previously used to treat mycobacterial infections in humans, although phages have protected guinea pigs[22] and mice[23] from experimental tuberculosis and mice[24] from experimental *M. ulcerans* infection[9-11]. The therapeutic use of bacteriophages has garnered considerable attention for the past 100 years but remains controversial, with widespread usage in the former Soviet Union[8] but few randomized double-blind studies. Among the many challenges for broad phage therapeutic use are the variability in phage susceptibilities of clinical isolates and the need to counter phage resistance. A phase 1/2 randomized double-blind study of phage therapy for *Pseudomonas aeruginosa* burn infections was confounded by poor stability of the phage preparation[22], and a phase II randomized trial of phage control of *Escherichia coli* diarrhea showed little efficacy[23]. Although treatment effectiveness remains ill-defined, there is substantial evidence that a primary safety concern is the presence of lipopolysaccharide (LPS) toxins in phages prepared by growth in gram-negative bacteria[8]. Personalized treatments with phage cocktails known to infect the clinical isolate are promising, and this approach has been used with promising outcomes[12,24]. Mycobacteria do not contain LPS, and thus LPS toxicity is not anticipated to be a major concern with phages grown on *M. smegmatis*.

Additional clinical information: The patient had chronic infections with *Pseudomonas aeruginosa* and *Stenotrophomonas maltophila* that continued post-transplant and were isolated from sputum samples on sixteen and eight occasions respectively. These were treated with short courses of oral antibiotics (Extended Data Fig. 1) if they were associated with increased

clinical symptoms such as cough or sputum production. Although lung function improved post-transplantation it appeared to plateau at approximately just below 60% predicted capacity six months after transplantation (see Fig. 1A), although this is not atypical for this type of transplantation. The initial *M. abscessus* isolate from the patient was resistant to all antibiotics tested: clarithromycin, amikacin, tobramycin, ciprofloxacin, moxifloxacin, cefoxitin, cotrimoxazole, doxycycline and linezolid.

From initial discharge on a palliative care pathway, the patient had two skin lesions and the sternal wound infection. The patient developed further skin lesions (approx. 2-3/week) on limbs until phage therapy was commenced, after which only one possible new lesion developed (this was a deep-seated lesion in buttock area which may have already been present but came to skin surface). Repeat blood cultures were negative on a number of occasions.

***M. abscessus* GD01**: Genomic DNA isolated from a liquid culture showed a close similarity to the previously sequenced *M. abscessus* subsp. *massiliense* FLAC047 (GenBank accession # CP021122), from which it differs by 44 insertions/deletions (including 13.3, 15.3 and 36.5 kbp insertions) and over 400 single nucleotide polymorphisms (SNPs). However, genomic reads assembled into two distinct complete contigs, suggesting there are two closely-related strains (GD01-A and GD01-B), 4,901,034 and 4,889,373 bp long, respectively. They differ by 18 SNPs and five insertion/deletions, the largest of which is 11.6 kbp absent from GD01-B. No integrated prophages or extrachromosomal plasmids were detected. Phage isolation and subsequent analyses performed prior to genomic sequencing indicated the differences between the two strains are not obviously related to phage susceptibility profiles.

Host range mutants of phage BPs: The genomes of HRM1 and HRM10, derived from wild-type BPs and a lytic mutant BPs*33*ΔHTH, respectively – were sequenced and each contain a

single base change in the portal gene *3* (C2083T and A2695G) conferring R66W and N270D amino acid substitutions, respectively. Halo – a phage closely related to BPs – also yielded a host range mutant (HRM20) which contains the same nucleotide substitution as in BPs HRM1.

Isolation of additional phages: During a search for additional phages, we tested over 100 environmental samples on strain GD01. Only one (Isca) was isolated (Supplementary Table 2), which groups genomically in Subcluster A3. However, although Isca propagated on *M. abscessus* GD01 efficiently infects *M. smegmatis*, it does not efficiently infect *M. abscessus* GD01 when grown on *M. smegmatis*, perhaps reflecting an uncharacterized restriction system in GD01.This prompted a search among other Subcluster A3 phages, and phages BabyRay, Heldan, Fred313, Puppy, and TNguyen7, were identified as potential candidates (Supplementary Table 2). In addition, we prepared several large pools of phages from the Pittsburgh collection, including many that have not been sequenced, and used these for enrichment with *M. abscessus* GD01 in liquid culture, followed by plaque isolation. Using this approach, we identified two phages – Gabriela and Itos – both of which were shown to genomically map in Cluster L2 (Supplementary Table 2).

Consents and other approvals

The approvals process was initiated with a conversation with the United Kingdom Medicines and Healthcare Regulatory Agency (MHRA), reporting the urgent clinical status of the patient on a palliative care pathway for which other treatments had failed, and that other lung transplant patients with disseminated *Mycobacterium abscessus* infections had high mortality. A Notification of Intention to Import was completed and forwarded to a pharmaceutical importer/distributor who was licensed to import unlicensed pharmaceuticals. Information pertinent to the Transmissible Spongiform Encephalopathies (TSE) status, manufacturing

details, and Certificate of Analysis were provided to the importer in compliance with MHRA regulations. The phage cocktail was thus treated as an unlicensed product from a non-GMP research facility, under the Notification of intention to Import under Part 2 and 4 of schedule 4 of The Human Medicines Regulations 2012 (SI 2012/1916).

Because two of the phages in the cocktail had been engineered, the United Kingdom Health and Safety Executive (HSE) was contacted to establish the Genetically Modified Organism (GMO) status, and a letter of explanation was provided with descriptions of each of the bacteriophages and detailed descriptions of how the genetic modifications were engineered. Pursuant to HSE advice, the Genetically Modified Safety Committee and the Biological Safety Officer (BSO) for the University College London, University College London Hospitals NHS Trust and Royal Free Hospital NHS Trust were contacted. Their genetic modification risk assessment was completed and communicated to the BSO and HSE. Following discussions between the BSO and HSE it was concluded that the phage cocktail was not considered to be GMO because only native phage DNA sequences were present, the engineering involved precise removal of DNA, and no exogenous reinsertion of DNA sequences had occurred. The phages were considered to be unlikely to cause disease or harm in humans, plants, or animals, and did not warrant classification as Genetically Modified Microorganisms (GMM).

Risks associated with the patient, the product, the environment, to health care workers, and risks to future bacteriophage research were all considered, as follows. Quality Assurance information was reviewed by both the pharmaceutical importer/distributor and the Quality Assurance team at the Great Ormond Street Hospital (GOSH), a National Health Service Foundation Trust. Following bacteriophage purification, the cocktail was tested for sterility prior to importing, and again at GOSH in the in-house certified cell therapy labs using BactAlert tests. Possible risks to the patient were considered including anaphylaxis and immune responses to

bacterial lysis, and these were discussed with the GOSH Drugs and Therapeutic Committee, the Ethics Committee, and the clinical team. Mitigating actions were implemented including an initial test of topical test doses, an initial low intravenous dose prior to administration of the full dose, a therapeutic plan for treatment of anaphylaxis or immune reactions, and the immediate availability of a bed in the intensive care unit if needed. To address potential risks associated with the product, the Quality Assurance team at GOSH required Certificates of Analyses for materials used in the preparation of the phage cocktail and a Quality Risk Assessment was completed and approved by the GOSH Qualified Person and Chief Pharmacist. Risks to the environment were considered minimal based on the cocktail non-GMO status. To minimize risks to health care workers, accredited staff at the certified cell therapy lab at Great Ormond Streeat Hospital used sterile conditions to dilute the cocktail and distribute to unit dose syringes. Protocols for all of the above were prepared, agreed on, and signed by K. F., K.C.G., and H. S. All risks including GMO status and product and patient risks and impact on future phage research were discussed with the GOSH Ethics Committee, and a commitment was made to publish irrespective of the outcomes.

Patient and parental consent were obtained for experimental treatment, and consent was provided for presentation of patient images.

Data availability

Information of all phages tested here including genome sequence data and GenBank accession numbers are all available at https://phagesdb.org. Genome sequence and raw reads of *M. abscessus* GD01_A and GD01_B sequences are available in GenBank with Accession #'s CP035923 and CP03592, respectively.

Supplementary References

22. Jault, P.*, et al*. *Lancet Infect Dis* (2018).

23. Sarker, S.A. & Brussow, H. *Ann N Y Acad Sci* **1372**, 42-52 (2016).

24. Chan, B.K.*, et al. Evol Med Public Health* **2018**, 60-66 (2018).

Table S1. Recovery of *M. abscessus* from patient samples

Date	Isolation site	Culture Positive	Kirchner[1]
Before phage treatment			
19/3/18	Sputum	-	
22/3/18	Pleural fluid	-	
23/3/18	Pleural fluid	-	
6/4/18	Pleural fluid	-	
8/4/18	Sputum	+	
12/4/18	Sputum	-	
13/4/18	Chest wound	+	
13/4/18	Arm	+	
13/4/18	Sputum	+	
22/4/18	Sputum	-	
15/5/18	Sputum	-	
After start of phage treatment			
17/7/18	Sputum	-	
29/8/18	Arm	+	
3/10/18	Chest wound	+	
3/10/18	Arm	+	
13/10/18	Chest wound	-	
17/10/18	Chest wound	+	14 Days
17/10/18	Arm	+	14 Days
17/10/18	Wrist	-	
26/11/18	Arm	+	34 Days
26/11/18	Chest wound	-	
11/12/18	Chest wound	+	15 Days
28/12/18	Arm	-	

[1]Days to positive, in cases for which this is known

Table S2. Plating efficiencies of phages on *M. smegmatis* and *M. abscessus* GD01

Phage[1]	**Cluster**	***M. smegmatis* mc²155**[2]	***M. abscessus* GD01**[2]	**EOP**
[3]Bxb1	A1	1×10^{9}	1×10^{9}	$<1 \times 10^{-9}$
[3]D29	A2	1×10^{6}	0	$<1 \times 10^{-6}$
[3]Elmo HRM$^{mc2\ 155}$	A3	1×10^{10}	1×10^{5}	1×10^{-5}
[3]Fernando	A3	1×10^{9}	1×10^{3}	1×10^{-6}
[3]Isca	A3	1×10^{8}	1×10^{8}	1
[3]Peaches	A4	3×10^{8}	0	$<3 \times 10^{-8}$
[3]DaVinci	A6	1×10^{10}	0	$<1 \times 10^{-10}$
[3]Et2Brutus	A11	1×10^{8}	0	$<1 \times 10^{-8}$
[3]EagleEye	A16	2×10^{8}	0	$<2 \times 10^{-8}$
[3]Muddy	AB	1×10^{10}	1×10^{10}	1
[3]Hedgerow	B2	2×10^{10}	0	$<1 \times 10^{-10}$
[3]Dandelion	C1	1×10^{10}	0	$<1 \times 10^{-10}$
[3]Adjutor	D1	3×10^{8}	0	$<3 \times 10^{-8}$
[3]Courthouse	E6	1×10^{8}	0	$<1 \times 10^{-8}$
[3]Tweety	F1	1×10^{10}	0	$<1 \times 10^{-8}$
[3]BPs	G1	1×10^{10}	1×10^{7}	1×10^{-3}
[3]BPs HRM10	G1	1×10^{10}	1×10^{10}	1
[3]Halo HRM20	G1	1×10^{10}	1×10^{10}	1
[3]Halo	G1	2×10^{10}	2×10^{7}	1×10^{-3}
[3]Island3	I1	1×10^{9}	0	$<1 \times 10^{-9}$
[3]Che9c	I2	1×10^{10}	0	$<1 \times 10^{-10}$
[3]Adephagia	K1	2×10^{9}	1×10^{7}	5×10^{-3}
[3]AdephagiaΔ*42*Δ*43*	K1	1×10^{10}	2×10^{5}	2×10^{-5}
[3]TM4	K2	2×10^{9}	1×10^{6}	5×10^{-4}
[3]TM4Δ	K2	1.3×10^{10}	2×10^{5}	1.5×10^{-5}
[3]ZoeJ	K2	1×10^{9}	1×10^{5}	1×10^{-4}
[3]ZoeJΔ*45*	K2	2×10^{10}	2×10^{10}	1
[3]ShedlockHolmes	K3	3×10^{10}	1×10^{8}	3×10^{-3}
[3]Fionnbharth	K4	1×10^{11}	1×10^{8}	1×10^{-3}
[3]Fionnbharth Δ*47*	K4	1×10^{11}	1×10^{8}	1×10^{-3}
[3]Wintermute	K4	2×10^{10}	1×10^{6}	5×10^{-5}
[3]Larva	K5	1×10^{9}	0	$<1 \times 10^{-9}$
[3]PegLeg	M1	2×10^{10}	0	$<2 \times 10^{-10}$
[3]Nanosmite	M3	1×10^{9}	0	$<1 \times 10^{-10}$
[3]Charlie	N	1×10^{10}	0	$<1 \times 10^{-10}$
[3]Giles	Q	1×10^{10}	0	$<1 \times 10^{-10}$
[3]Jeon	W	1×10^{9}	0	$<1 \times 10^{-9}$
[3]Gaia	X	2×10^{9}	0	$<2 \times 10^{-9}$
[3]Rem711	Z	1×10^{10}	0	$<2 \times 10^{-10}$
[3]Cuke	Singleton	1×10^{5}	0	$<1 \times 10^{-5}$
[3]DS6A	Singleton	1×10^{9}	0	$<1 \times 10^{-9}$
[3]Kumao	Singleton	3×10^{9}	0	$<3 \times 10^{-9}$
[3]Moomoo	Singleton	3×10^{6}	0	$<3 \times 10^{-6}$
[3]Sparky	Singleton	2×10^{6}	0	$<2 \times 10^{-6}$
[4]BabyRay	A3	1×10^{9}	1×10^{9}	1
[4]Fred313	A3	1×10^{7}	1×10^{7}	1
[4]Fred313cpm1	A3	1×10^{10}	1×10^{10}	1
[4]HelDan	A3	2×10^{7}	1×10^{5}	5×10^{-3}
[4]Puppy	A3	1×10^{8}	1×10^{8}	1
[4]TNguyen7	A3	1×10^{9}	1×10^{8}	10^{-1}
[4]Cambiare	G2	1×10^{10}	0	$<1 \times 10^{-10}$
[4]Flagstaff	G2	2×10^{10}	0	$<2 \times 10^{-10}$
[4]Pinnie	G3	2×10^{8}	0	$<2 \times 10^{-10}$
[4]Validus	K1	3×10^{10}	1×10^{5}	3×10^{-6}
[4]Keshu	K3	2×10^{10}	2×10^{10}	1
[4]KeshuΔ*47*	K3	1×10^{10}	1×10^{10}	1
[4]SamScheppers	K4	2×10^{10}	0	$<2 \times 10^{-10}$
[4]Amgine	K6	3×10^{10}	0	$<3 \times 10^{-10}$

[4]Krueger	K6	2×10^{10}	0	$<2 \times 10^{-10}$
[4]Gabriela	L2	1×10^{10}	1×10^{9}	1×10^{-1}
[4]Itos	L2	3×10^{10}	1×10^{10}	3×10^{-1}

[1]Phage lysates were grown on *M. smegmatis* mc^2155, except Isca and DS6A, grown on GD01 and M. tb respectively.
[2]Plaque forming units ml^{-1}
[3]Phages tested in an initial screen from which phages for a therapeutic cocktail were selected
[4]Additional phages tested after the initial therapeutic phages were selected

Table S3. Plating efficiencies of phages on *M. abscessus* strains GD01 and GD02.

Phage	Cluster	mc^2155	GD01	EOP	GD02	EOP
Bxb1	A1	1×10^{9}	0	$<1 \times 10^{-9}$	0	$<1 \times 10^{-9}$
D29	A2	1×10^{6}	0	$<1 \times 10^{-6}$	0	$<1 \times 10^{-6}$
Elmo HRM^{mc2155}	A3	1×10^{10}	1×10^{5}	1×10^{-5}	1×10^{10}	1
Fernando	A3	1×10^{9}	1×10^{3}	1×10^{-6}	0	1×10^{-6}
Peaches	A4	3×10^{8}	0	0	0	$<3 \times 10^{-8}$
DaVinci	A6	1×10^{10}	0	$<1 \times 10^{-10}$	0	$<1 \times 10^{-10}$
Alma	A9	2×10^{9}	0	0	0	$<2 \times 10^{-9}$
Et2Brutus	A11	1×10^{8}	0	$<1 \times 10^{-8}$	0	$<1 \times 10^{-8}$
EagleEye	A16	2×10^{8}	0	$<2 \times 10^{-8}$	0	$<2 \times 10^{-8}$
Hedgerow	B2	2×10^{10}	0	$<1 \times 10^{-10}$	0	$<1 \times 10^{-10}$
Dandelion	C1	1×10^{10}	0	$<1 \times 10^{-10}$	0	$< 1x 10^{-10}$
Adjutor	D1	3×10^{8}	0	$<3 \times 10^{-8}$	0	$<3 \times 10^{-8}$
Courthouse	E6	1×10^{8}	0	$<1 \times 10^{-8}$	0	$<1 \times 10^{-8}$
Tweety	F1	1×10^{10}	0	$<1 \times 10^{-8}$	0	$<1 \times 10^{-8}$
DeadP	F1	1×10^{11}	0	$<1 \times 10^{-11}$	0	$<1 \times 10^{-11}$
BPs	G1	1×10^{10}	1×10^{7}	1×10^{-3}	0	$<1 \times 10^{-10}$
BPs*33*ΔHTH	G1	3×10^{10}	1×10^{7}	1×10^{-3}	0	$<3x10^{-10}$
BPs*33*ΔHTH, $HRM^{GD01}10$	G1	1×10^{10}	1×10^{10}	1	0	$<1 \times 10^{-10}$
Halo HRM20	G1	1×10^{10}	1×10^{10}	1	0	$<1 \times 10^{-10}$
Halo	G1	2×10^{10}	2×10^{7}	1×10^{-03}	0	$<2 \times 10^{-10}$
Cambiare	G2	1×10^{10}	0	$<1 \times 10^{-10}$	0	$<1 \times 10^{-10}$
Island3	I1	1×10^{9}	0	$<1 \times 10^{-9}$	0	$<1 \times 10^{-9}$
Che9c	I2	1×10^{10}	0	$<1 \times 10^{-10}$	0	$<1 \times 10^{-10}$
Adephagia	K1	2×10^{9}	1×10^{7}	5×10^{-3}	0	$<2 \times 10^{-9}$
Adephagia*Δ42Δ43*	K1	1×10^{10}	2×10^{5}	2×10^{-5}	0	$<1 \times 10^{-10}$
TM4	K2	2×10^{9}	1×10^{6}	5×10^{-4}	1×10^{2}	5×10^{-8}
TM4Δ	K2	1.30×10^{10}	2×10^{5}	1.54×10^{-5}	1×10^{2}	1.5×10^{-8}
ZoeJ	K2	1×10^{9}	1×10^{5}	1×10^{-4}	0	$<1 \times 10^{-9}$
Keshu	K3	2×10^{10}	2×10^{10}	1	0	$<1 \times 10^{-10}$
ShedlockHolmes	K3	3×10^{10}	1×10^{8}	3.33×10^{-3}	0	$<3 \times 10^{-10}$
Fionnbharth	K4	1×10^{9}	0	$<1 \times 10^{-9}$	0	$<1 \times 10^{-9}$
Fionnbharth Δ47	K4	2×10^{10}	0	$<1 \times 10^{-10}$	0	$<1 \times 10^{-10}$
Wintermute	K4	2×10^{10}	1×10^{6}	5×10^{-5}	0	$<2 \times 10^{-10}$
Larva	K5	1×10^{9}	0	$<1 \times 10^{-9}$	0	$<1 \times 10^{-9}$
PegLeg	M1	2×10^{10}	0	$<2 \times 10^{-10}$	0	$<2 \times 10^{-10}$
Nanosmite	M3	$1x10^{9}$	0	$<1 \times 10^{-10}$	0	$<1 \times 10^{-10}$
Charlie	N	$1x10^{10}$	0	$<1 \times 10^{-10}$	0	$<1 \times 10^{-10}$
Giles	Q	$1x10^{10}$	0	$<1 \times 10^{-10}$	0	$<1 \times 10^{-10}$
Jeon	W	$1x10^{9}$	0	$<1 \times 10^{-9}$	0	$<1 \times 10^{-9}$
Gaia	X	2×10^{9}	0	$<2 \times 10^{-9}$	0	$<2 \times 10^{-9}$
Cuke	Singleton	1×10^{5}	0	$<1 \times 10^{-5}$	0	$<1 \times 10^{-5}$
DS6A	Singleton	1×10^{9}	0	$<1 \times 10^{-9}$	0	$<1 \times 10^{-9}$
Kumao	Singleton	3×10^{9}	0	$<3 \times 10^{-9}$	0	$<3 \times 10^{-9}$
Moomoo	Singleton	3×10^{6}	0	$<3 \times 10^{-6}$	0	$<3 \times 10^{-6}$
Muddy	Singleton	1×10^{10}	1×10^{10}	1	1×10^{7}	1×10^{-3}
Sparky	Singleton	2×10^{6}	0	$<2 \times 10^{-6}$	0	$<2 \times 10^{-6}$

Table S4. Cytokine responses to phage treatment

Date collected[1]	IFNγ[2]	TNFα[2]	IL-2[2]	IL-4[2]	IL-6[2]	IL-10[2]
6/21/18	<5	<5	<5	<5	<5	<5
6/23/18	<5	<5	<5	<5	<5	<5
6/24/18	<5	<5	<5	<5	6.90	<5
6/25/18	<5	<5	<5	<5	<5	<5
6/26/18	<5	<5	<5	<5	<5	<5
6/27/18	<5	<5	<5	<5	<5	<5
7/4/18	5.74	<5	<5	<5	17.21	10.44
7/9/18	<5	<5	<5	<5	<5	<5
7/18/18	<5	8.31	<5	<5	114.05	7.74
7/25/18	<5	<5	<5	<5	<5	<5
8/1/18	<5	<5	<5	<5	<5	<5
8/8/18	<5	<5	<5	<5	<5	<5
8/14/18	<5	<5	<5	<5	7.86	<5
8/29/18	<5	<5	<5	<5	<5	<5
9/19/18	<5	<5	<5	<5	7.11	<5
10/3/18	<5	<5	<5	<5	<5	<5
10/16/18	<5	<5	<5	<5	<5	<5

[1]Phage treatment was started on 6/19/18.
[2]All values are reported as pg/ml.

Table S5: Primer and probe sequences for qPCR and dPCR assays

Oligo name	Target phage	Sequence[1]
Muddy-F	Muddy	5’ – ACCGAATATGCTGCGTACAA - 3’
Muddy-R	Muddy	5’ – GGGAAGGCAGACTCAGATAAAG – 3’
Muddy-Probe	Muddy	5’- FAM-TGATACCTCGCACAGATGACGCTT – 3’-BHQ1
ZoeJ-F	Zoe-J	5’ – CCTTCTGGCGTGTCACTTTA – 3’
ZoeJ-R	Zoe-J	5’ – CTGAACCATCGGAGGCTATTC – 3’
ZoeJ-Probe	Zoe-J	5’-FAM – TAAACAATAGGCCCGACCTGCGTC – 3’ BHQ1
BPs-F	BPs	5’ – CGGGTTTCACGGACCTTATT – 3’
BPs-R	BPs	5’ – CGTGGTCAACCCTGGATAAA – 3’
BPs-probe	BPs	5’ FAM – CTAACTACCGTCCTCTGCCTGGGA – 3’ BHQ1

[1]FMA: Fluorescein amidite; BHQ1: Black Hole Quencher 1