Phage Therapy for Antibiotic-Resistant *Pseudomonas aeruginosa*: Overcoming Manufacturing Barriers in the UK

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Background

Phages are viruses that can kill bacteria and are an alternative to antibiotics, crucially they can kill bacteria that are multi drug resistant (MDR)!

There are phages that are known to be extremely active against high numbers of MDR *P. aeruginosa* isolates associated with chronic infection such as Cystic Fibrosis (CF) however, phages cannot be used in clinical trials in the UK due to the lack of GMP production and a regulatory framework for manufacturing.

This project sets to establish a regulatory framework for manufacturing in the UK, in collaboration with the MHRA, and a manufacturing process development that is taking place in collaboration with CPI.

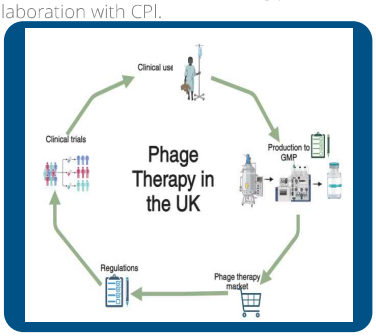


Figure 1: *A flowchart showing the barriers to using phage therapy in the UK, and the importance of this project enabling a process to be scalable and be able to be made to cGMP standards*

Three genetically and morphologically distinct *Pseudomonas* phages were used to determine the impact of diversity on manufacturing processes

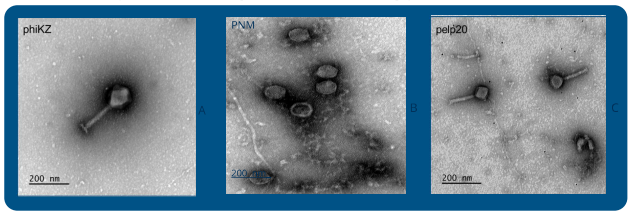


Figure 2: *TEM images of the 3 phages which were evaluated in the context of evaluating manufacturing unit operations A) phiKZ B) PNM C) pelp20*

Aim: Develop key quality attribute packages using previously accepted methods for GMP biotherapeutics that will be transferable for clinical supply processes

Upstream – Shake flask process

Aim: Produce batches of high titre *P. aeruginosa* phages using *P. aeruginosa* strain PAO1 as the host bacteria, using shake flasks.

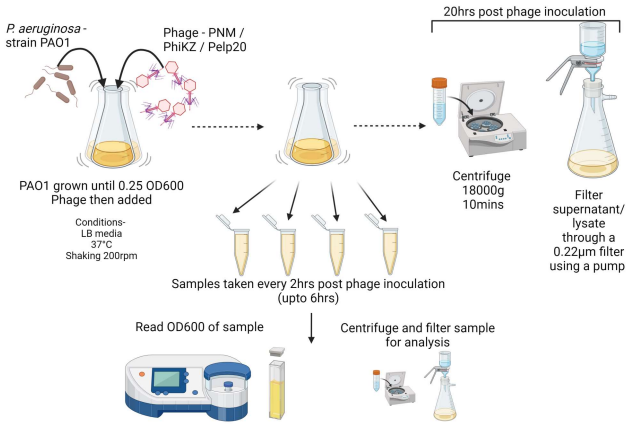
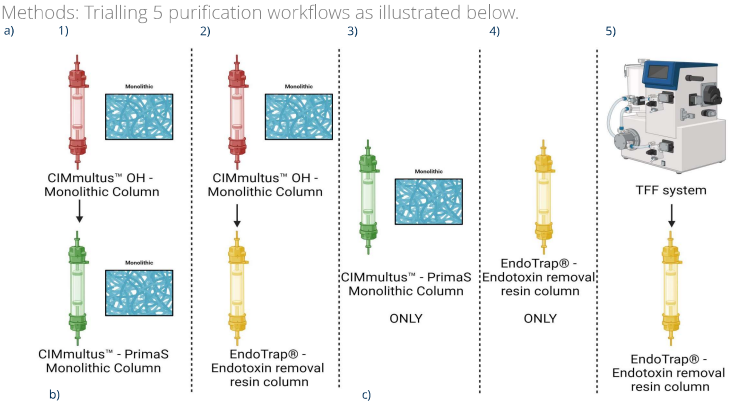


Figure 1: *Schematic of the upstream process workflow*

Downstream purification processes

Aim: Removal of host cell protein, host cell DNA and endotoxin, whilst ensuring phage integrity and purity

Methods: Trialling 5 purification workflows as illustrated below.



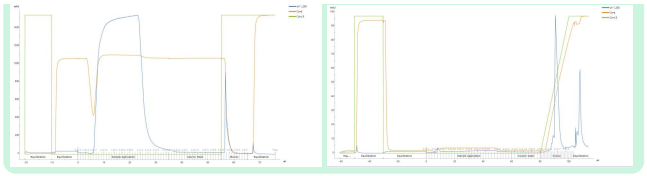


Table 1 *The yield and loss from each fraction collected from the OH method*

Batch 2 - OH	volume (mL)	titre pfu/ml	Total pfu
Input	20	1.5E+11	3E+12
Flowthrough	20	1.5E+10	3E+11
Wash	20	4E+08	8E+09
Elution pooled	10	1E+11	1E+12
Yield			33%

Table 2 *The yield and loss from each fraction collected from the PrimaS method*

Batch2 - PrimaS	volume (mL)	titre pfu/ml	Total pfu
Input	20	1E+10	2E+11
Flowthrough	20	0	0
Wash	20	0	0
Elution	10	2E+11	2E+12
Yield			1000%

Figure 3: a) *A schematic of the downstream process conditions trialled.* ***b)*** *Chromatography trace of the OH capture and elute column running crude phage lysate from PNM batch 2 showing impurities being removed and phages being eluted, one peak showing likely to be one product.* ***c)*** *Chromatography trace of the PrimaS capture and elute polishing column running OH elution from PNM batch 2 , showing less impurities than the OH being removed and phages being eluted, one peak showing likely to be one product.*

Analytical techniques

Screening for residuals
Aim: Analyse/monitor levels of bacterial host contaminates
Host cell protein
- ELISA for exotoxin A
- LC-MS

Host cell DNA
- qPCR
- Endotoxin
- LAL endotoxin testing kit

Phage integrity and stability
Aim: Analyse/monitor integrity and stability of the phages during the manufacturing process
- Dynamic light scattering (DLS)
- qPCR
- HPLC
- Plaque assays

Phage bioactivity
Aim: analyse/monitor numbers of active phage particles (comparing to gold standard plaque assays)
- Octet method
- HPLC
- Plaque assays

Figure 4

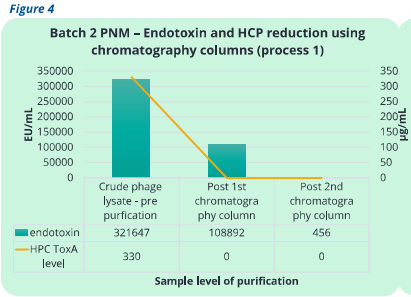


Figure 5

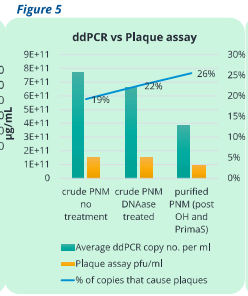


Figure 6

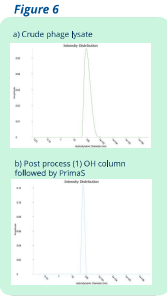


Figure 4: *Reduction in bacterial/host residuals HCP and hcDNA using process 1 OH column followed by PrimaS column reduction 100% and 99% respectively*

Figure 5: *Assessing phage titre using ddPCR comparing to plaque assay*

Figure 6 a) *DLS from a crude PNM phage lysate* ***b)*** *DLS from a purified sample of PNM phage using process 1 OH column followed by PrimaS column, showing a sharper peak at the size of the phage showing less aggregates.*

Conclusion

Demonstration of scalable production methods and robust analytical techniques which could be applied towards the manufacture of phage therapeutics