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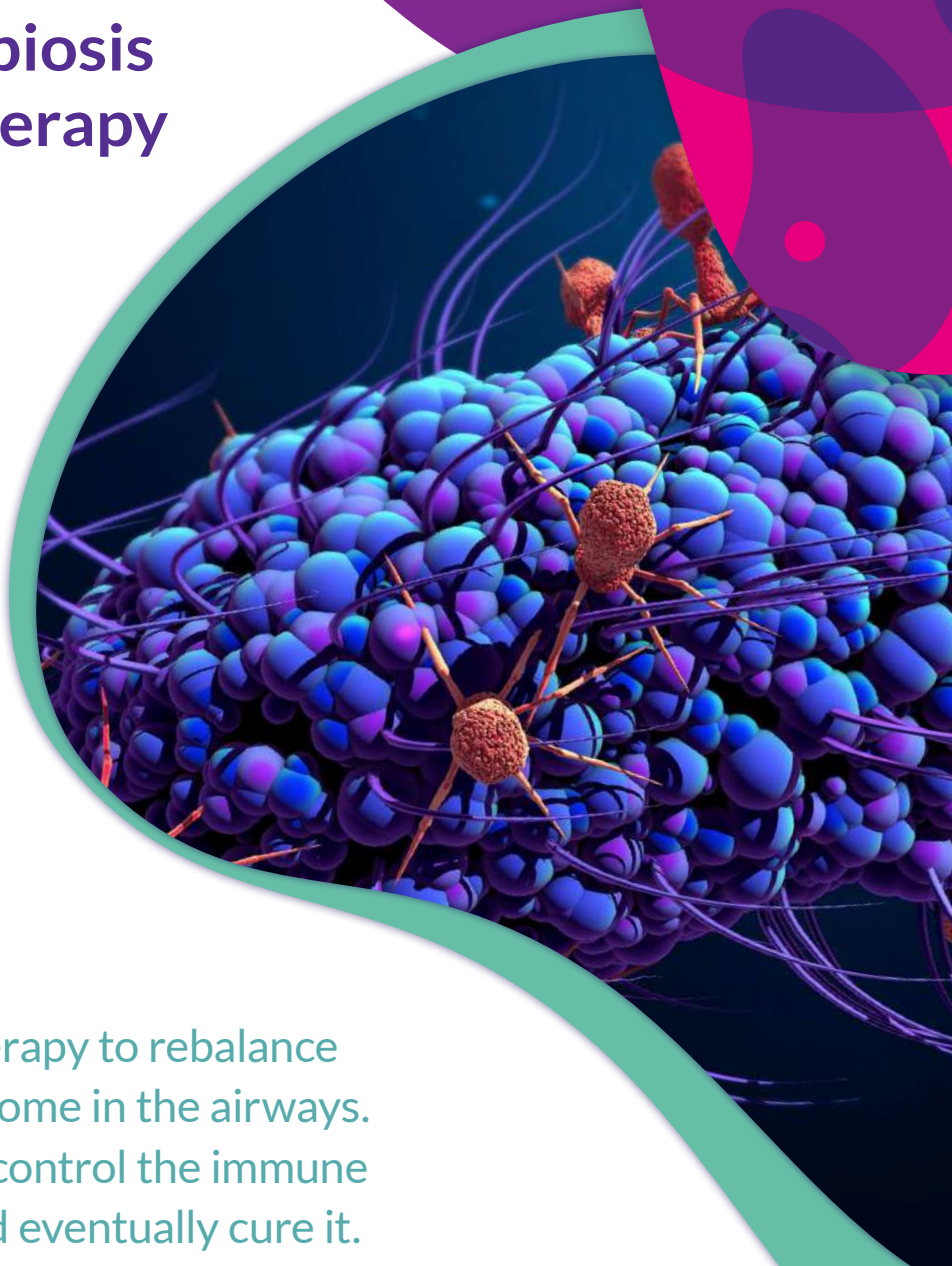
CURE

Constructing a **Eubiosis Reinstatement Therapy** for Asthma



CURE proposes a phage therapy to rebalance the structure of the microbiome in the airways. If proved, this therapy may control the immune dysregulation of asthma and eventually cure it.

This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 767015

Where are we now in the CURE project?

CURE is now at half way of the project and interesting new findings are cumulating. The annual meeting took place in Syros, Greece on 15–16 September 2019, where all the partners presented the progress in their work and many more outcomes have arisen since then!

Significant amount of samples have been gathered for biological analyses

The National and Kapodistrian University of Athens (N.K.U.A) and the Medical University of Lodz (MUL), clinical partners of CURE, have followed about 60 patients with asthma as well as 30 healthy individuals in two phases: during the first month, when they tool samples at 8 different time points and, during a whole year, adding another 5 regular samplings. The aim of these two phases is to study if and how the microbiome[1] changes in time and if it correlates with symptoms and/or the immune response.

In addition to regular samples, 32 asthma exacerbations were identified, and samples obtained. These are important because it is expected that major changes of the microbiome may happen during such events, usually caused by a viral infection.

Do phages play a role in regulating the inflammation of asthma?

The Swiss Institute of Allergy and Asthma Research (SIAF) and the Biomedical Research Foundation, Academy of Athens (BRFAA) looked further into tissue integrity[2] and the effect of phages[3] on innate immunity. Using epithelial cells cultures exposed to Staphylococcus phages, they did not find any effect of the phages on the integrity of the tissue or on the molecules that hold the tissue together ('tight junctions'). Nevertheless, phages were able to change the numbers, activation state and affect differently subgroups of 'innate lymphoid cells' (ILCs), suggesting a potential contribution of phages in immune tolerance and inflammation regulation.

Changes in the microbiome are associated with the disease state of asthma

A key question on evaluating the possibility of influencing the microbiome on the airways is linked with how it changes in time. **The University of Manchester (UMAN)** has sequenced and evaluated samples from different groups of people. In all these groups, the microbiome was more different between patients than within a patient at different times (i.e. the microbiome is 'personalised'). Dominant families of microbes remained relatively stable within each patient; a core microbiome representing 10-15% of species, with a stable proportion among bacteria, fungi, archea and viruses was observed and was often shared between patients. They also observed that asthma patients have more dispersed microbiomes than healthy individuals. This observation supports the idea that while health is organized around a particular state (i.e. it's stable), disease may have many different states. Finally, they have observed, that baseline (steady state) microbiome characteristics can be used to cluster together patients, who had or not an exacerbation. These data further our understanding about the dynamics of the microbial communities in the airway and the opportunities for intervention.

1 Microbiome: The microorganisms in a particular environment (including the body or a part of the body).
2 Epithelial or tissue integrity: The ability of body tissues to regenerate and/or repair to maintain normal physiological process.
3 Phage or bacteriophage: Type or virus that infect bacteria.

Establishing a well characterised bacteriophage cocktail as a potential treatment for asthma

The Georgi Eliava Institute of Bacteriophagy, Microbiology and Virology (ELIAVA) and the ELIAVA Bio Preparations LTD (ELIBIO) are working intensely to generate a well characterised collection of bacteriophages. These bacteriophages are aimed at targeting bacteria considered important in the upper respiratory microbiome in regard to asthma (Moraxella, Acinetobacter, Streptococcus, Haemophilus and Staphylococcus). A large number of strains from these bacteria have been collected from various sources and used for experiments. At the moment, over 30 new phages have been discovered and characterized.

However, there are so many bacteriophages that we will never be able to isolate them all[4]. For this reason, we need to build computer predictions ('networks') of how different phages interact with different bacteria. We developed and tested an algorithm able to describe the phage-bacteria network[5] and saw that there are phages who infect many bacteria and others that are more selective.

We have also started to set up a culture system that will represent the human airway and in which phages and bacteria will interact over human epithelial cells. A prototype of the device to house this culture system has been developed.

Building a prediction model on the impact of bacteriophage interventions in asthma

Finally, **the University of Manchester** is exploring several approaches for developing mathematical models of how the microbiome changes over time. They adapted some existing programs that could model how a small number of species may interact between themselves, to describe more complex bacterial and viral communities. Currently they are also tweaking a tool designed for large datasets, such as the one that CURE is developing.

"New phages have been identified, we have confirmed and are starting to understand the tolerance of the human immune system towards phages and we have visualised for the first time how the communities of microbes change in time and in relation to asthma activity. The group remains passionately interested and intrigued by the CURE concept. It appears that we will continue having great and satisfactory time in the next two years." Prof. Nikos Papadopoulos, Coordinator of CURE.

4 There are an estimated 10^{31} phages on the planet and only 2000-3000 phages have been isolated and studied by now in bacteriophage research.
5 Bacterial-phage network or interaction: Phage means 'bacteria eater'. Phages interact and infect specific bacteria without infecting other types of bacteria. Therefore, there is a constant interaction between both.

"The microbiome has been shown to influence the progression of asthma and viruses can be key triggers"

David L Robertson is a research professor and Head of CVR Bioinformatics at the MRC-University of Glasgow Centre for Virus Research (CVR) and supervises with Nikos Papadopoulos, Mark Muldoon and Tucker Gilman a multidisciplinary team of researchers at the University of Manchester. He also leads a research group within the CVR composed of bioinformaticians, computational biologists and computer scientists focussed on virus research, particularly, on virus-host interactions and change in viral genomes. Prof. Robertson's interests in research are centred on viral evolution and systems virology with focus on virus-host specificity, change in the virome and the trade-offs between molecular evolution and disease susceptibility.

The University of Manchester will conduct the largest and most complete metagenomics analysis of the respiratory track undertaken to date. What does it mean for the clinical and research community to have such knowledge?

One of the most interesting components of the project is the longitudinal nature of the samples. In the CURE project, we are studying through days, weeks and months the change in the microbiome among asthma patients and healthy individuals (controls). This will result in a large dataset on the ecology and dynamics change of the microbiome in the respiratory system.

The premise of the CURE project is that the composition of the microbiome can contribute to an individual's health and disease status. Based on our data, the respiratory metagenome of healthy individuals seems to be more stable than from asthma patients. Therefore, when looking at a disease state, characterising the dynamics and the composition of the respiratory microbiome, its interaction with the immune system as well as the related problems is key to understanding the relationship between asthma and the microbiome. A central hypothesis of the project is if it will be possible to influence the microbiome so as to control inflammation in the respiratory tract in asthma. Unlike antibiotics, bacteriophage therapy can be used to target individual bacteria with high specificity.

For now, the first set of analysis on the geographical and temporal variability of the respiratory metagenome in healthy and asthmatic individuals is completed. What do the results show?

One of the most interesting findings is the change in bacteriophages present in asthmatic patients. Based on the results from PREDICTA, an EU-funded research project, the presence of bacteriophages appears to be reduced in the airways of asthma patients. Bacteriophages are important regulators of the microbiome, thus, their absence suggests that some bacteria in the system are not present or are present but their numbers unregulated.

Our data shows a clear variability of the respiratory metagenome between asthmatic and healthy individuals. Definitely, there is an association between the changes in the microbiome and the disease state of asthma. For example, adenoviruses seem to be more prevalent in asthma patients. While some literature suggests that there might be some causation, this could just as equally be correlate of disease state. It's important to discriminate when we see change in the microbiome associated with disease, what are causative factors from what are correlated factors, for example, treatments or the action of immune response.

The changes in the respiratory microbiome of asthma patients are unpredictable, for example, a person's age can influence the composition of the system. These factors make it difficult to predict the impact of bacteriophage-based interventions in the respiratory metagenome and, even harder, to define a generic prediction that fit all patients.

We are currently studying the longitudinal patterns to identify the composition and dynamics of the system. Working with ecologists and mathematicians at the University of Manchester we can make predictions, but for now we need more data and that is something that is ongoing in the project.

Do you think that metagenomics patterns may end in predicting the risk of acute asthma exacerbations?

The microbiome has been shown to influence the progression of asthma and viruses can be key triggers. By identifying the typical characteristics of the species interactions using ecological measures in the asthmatic respiratory tract, we should hopefully be able to predict the microbiome that correlates with the disease state of asthma. Asthma exacerbations are often associated with rhinoviruses, the viruses causing common colds, and microbiome composition can be used to predict health status, so yes, an individual being prone to asthma exacerbation, should be predictable.

At what stage is your research as the UK partner in this project linked to CURE? What are the next steps and objectives?

We are in the process of finalising the sequencing of the data from asthma patients and healthy individuals involved in the CURE clinical cohort. Half of the samples are already sequenced. We are characterising and classifying the data in order to build a picture of the microbial ecology in the respiratory system. This involves looking at the composition of the data, the species present and inferring interactions between them. The technical challenges of this kind of data shouldn't be underestimated. In the respiratory system you have particular problems such as the sampling strategy and the presence of eukaryote viruses which when abundant can interfere with detection thresholds. With the existing data and the application of machine learning tools we will be able to define mathematical models to be able to predict future datasets.

What are your expectations about CURE? And what are your main concerns?

We are studying the nature of the species in the respiratory microbiome in healthy individuals and how it can contribute to a disease state. The real challenge in future work is to use this kind of information with a therapeutic purpose. It seems clear that the microbiome is essential to understand asthma as a disease. A key question is whether it is possible to recreate the healthy microbiome in the respiratory system. This has been done with some diseases of the gut, specifically, faecal transplants have been used to transfer the microbiome from a healthy to a diseased individual with positive outcomes. Whether the respiratory microbiome can be 'fixed' in this way is an open question and one we feel worth exploring. Our longitudinal study will be an interesting contribution to this area of research.

European Regulatory Framework for Bacteriophage therapy: Opportunities and limitations

Antimicrobial resistance has become a global threat [1] and alternatives to antibiotics are urgently needed. In this context, bacteriophage therapy has become a potential alternative in the treatment of multi-drug resistant bacterial infections.

Historical clinical data on the effective use of phages in patients exists, mostly from Eastern Europe, particularly Poland, and former Soviet Republics, concretely, Russia and Georgia. However, these data have not been validated under European Regulatory standards and no evidence exists under these standards, proving the safety and efficacy of this therapy. For this reason, the use of phage therapy on humans is not yet authorised in Europe.

Under what Regulatory Framework does phage therapy operate?

Natural bacteriophages used as therapeutics are considered by the European Medicines Agency (EMA) medicinal products [2], placing them under the Directive 2001/83/EC on the Community code relating to medicinal products for human use [3], under the category of biologicals.

Under this regulatory framework, bacteriophage therapy can be authorised for its use on humans after marketing authorisation approval (Article 6). A marketing authorisation can only be obtained after the quality, safety and effectiveness of the therapy is proven, through the application of Good Manufacturing Procedures (GMP) and the review of preclinical data, followed by Phase I, II and III clinical trials (Article 8).

Limitations for bacteriophage therapy under the Medicinal Products Directive

On March 2011, European authorities formally stated that the existing Regulatory Framework is adequate for the use of bacteriophage therapy on humans [4]. However, a large part of the scientific community disagrees and calls for an urgent adaptation of the current legislation [2].

A legislation for evolving bacteriophages is needed

Phage preparations become less effective with time and updates are needed. Bacteriophages and bacteria co-evolve in a dynamic system and, as with antibiotics, bacteria can develop resistance to phages during the course of the treatment to survive the phage intervention [5].

However, after the marketing approval of a phage preparation, any modification on its composition as an addition, removal or replacement of a phage strain, aiming at improving its effectiveness, could only be possible through the application of a new marketing authorisation [6], following Article 8 of the Directive 2001/83/EC.

The current licensing pathway for medicinal products is not suitable for evolving bacteriophages. A revision of the current legislation is crucial to allow rapid updates and licensing of bacteriophage preparations as it is the case with the influenza vaccine [7].

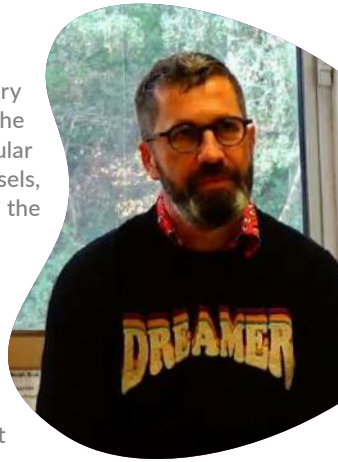
'One size fits all' or 'personalised' approach?

The provisions described in the Directive 2001/83/EC *apply to industrially produced medicinal products for human use intended to be placed on the market in Member States* (article 2).

The current legislation imposes many requirements and procedures for manufacturing and obtaining marketing authorisation for bacteriophage therapy. In this context, these requirements would not limit industry actors to produce and pursue uniform market placement of bacteriophage-based products, but the lack of strong intellectual property protection and bacteriophage-bacteria resistance issues discourage pharmaceutical companies to invest in bacteriophage therapy. Paradoxically, non-profit hospitals are willing to develop personalised bacteriophage preparations to treat patients but the provisions imposed by the Directive are too costly and time-consuming for them [8].

However, non-profit hospitals in a few European countries, such as Belgium, have sporadically applied bacteriophages to treat individual patients under very exceptional circumstances. This was only possible by exclusion from the provisions of the Directive 2001/83/EC, under two exemptions defined in the legislation: the magistral formula exemption (Article 3) and the "special needs" exemption (Article 5).

Phage therapy in Belgium



In Belgium the authorities developed a more suitable regulatory environment for phage therapy. Dr Jean-Paul Pirnay, member of the Ethics Advisory Board of CURE and head of the laboratory for molecular and cellular technology at the Queen Astrid Military Hospital in Brussels, Belgium, took part in the process of conforming phage therapy to the Belgian law.

The Queen Astrid Military Hospital is a non-profit hospital working independently from industry actors. In 2007, it was the first Belgian hospital to reinitiate a focus on phage therapy. Since then, phage therapy was applied to more than 60 patients, first under the umbrella of article 37 from the Declaration of Helsinki and later under the magistral preparation framework. According to Dr Pirnay, "we almost always had success and we could not see any adverse effects".

Article 37 from the Declaration of Helsinki [9] states that *in the treatment of an individual patient, where proven interventions do not exist or other known interventions have been ineffective, the physician, after seeking expert advice, with informed consent from the patient or a legally authorised representative, may use an unproven intervention if in the physician's judgement it*

offers hope of saving life, re-establishing health or alleviating suffering. Dr Pirnay pointed out that "this umbrella only applies in very desperate cases. It is more like a guideline and not a law".

In July 2016, Belgian authorities acknowledged that phage therapy had no specific Regulation in Europe [8]. "We decided that we needed to have something stronger to give more protection to doctors to use phages. That is how, together with authorities we put the phages into an existing framework: the Magistral Preparations", Dr Pirnay said.

In January 2018, a Belgian Magistral Phage Framework was created, allowing phages to be processed as active pharmaceutical ingredients of magistral formulations. Magistral preparations operate in accordance with Article 3 of the Directive 2001/83/EC defined as *any medicinal product prepared in a pharmacy in accordance with a medical prescription for an individual patient*. They are not subjected to constraints such as marketing authorisation and are a practical way for a medical doctor to personalise patient treatments to specific needs and to make medication available that do not exist commercially [8].

Active pharmaceutical ingredients (APIs) of magistral preparations (in this case the phages) must conform to the provisions of a monograph, an official document that defines the quality standards for the elaboration of medicines. Ideally, this monograph has to be integrated in the European Pharmacopeia, of the Belgian Pharmacopeia or of any official Pharmacopeia [10].

In Europe a phage monograph does not exist and therefore experts of the Queen Astrid Military Hospital together with the Belgian Authorities took the initiative to elaborate a generic monograph on how to safely prepare and test phages for use on patients. On 10 January 2018, version 1.0 of the monograph was deemed appropriate by the Belgian competent authorities for medicines [8]. "The next step would be to have the Belgian monograph integrated into the European Pharmacopeia. If we succeed in this, then it will be easier for phage therapy in other European countries and for commercial stakeholders to bring phage APIs to the European market and to produce (outsourced by the pharmacist) magistral phage preparations".

Watch here the full interview with Dr Jean-Paul Pirnay explaining more in detail the use of bacteriophage therapy in the Queen Astrid Military Hospital and how the Belgian law integrated bacteriophage therapy.

An adaptation of the current European legislation is needed for bacteriophage therapy

It is clear that the Medicinal Products Directive did not consider medicinal products with the characteristics that phages have and, thus, cannot be considered suitable for phage therapy. The current provisions that apply to industrial processes of bacteriophage preparations contrast with the idea and practical aspects of bacteriophage therapy itself, based on a sustainable and tailor-made patient approach [2].

European Regulators should define a Regulatory Framework differentiating the industrial and personalised approaches and allowing rapid updates of phage preparations and licensing procedures. Overcoming the current limitations will strengthen a further development of phage therapy in Europe.

It is time to implement a broader phage therapy regulatory framework. Supranational medicine agencies, such as the European Medicines Agency (EMA) should build on the initiatives developed by some national regulatory authorities [11]. Policymakers need to open the door for a broad and fast (possibly interim) solution with reduced stringency as compared to the current pharmaceutical requirements [12]. Phage banks containing large amounts of characterized (e.g. host range, genomic passport,...) and safe therapeutic phages need to be set up. Medical Doctors need to be made aware of the existence and content of these banks.

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CURE at the European Research and Innovation Days

On September 25th 2019, the coordinator of CURE, Nikos Papadopoulos, was invited to present the CURE concept during the European Research and Innovation Days in Brussels.

The European Research and Innovation Days is an annual policy event of the European Commission. It brings together all relevant stakeholders to debate and shape the future European research and innovation strategic priorities to address new societal challenges.

The CURE Coordinator was invited to speak during the session "EIC Pathfinder showcases: medical & neuro-technologies (I) and energy & environment (II)" to present CURE as a Future and Emerging Technology in Europe and share our vision of phage therapy application in the future in humans to treat bacterial infection.

The Biomedical Research Foundation Academy of Athens (BRFAA), nominated for the Innovation Radar Prize 2019

The Biomedical Research Foundation Academy of Athens (BRFAA), partner in CURE, was selected in July 2019 as one of the 36 finalists for the prestigious European Commission's "Innovation Radar Prize 2019" under the "Innovative Science" category, linked to their research in CURE.

The Innovation Radar is an initiative from the European Commission to identify high quality innovations from EU-funded research projects grouped under 4 categories: Tech for Society, Innovative Science, Industrial & Enabling Tech and Women-led innovations. A public poll was open to vote for the best 3 EU-funded innovators of each category.

The 12 finalists (3 in each category), in terms of votes cast, were selected to the finals. A jury of experts reviewed the candidates and announced the winner at the Research and Innovations Days in Brussels (24, 25 and 26 September 2019). We are proud that BRFAA was initially nominated for the prize and reached a good number of votes.

Congratulations to the 3 finalists. These were Oslo University Hospital, Space Structures and Max-Planck-Gesellschaft Zur Förderung Der Wissenschaften.

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