CURE NEWSLETTER N. 3
Issue Summer 2021

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 a special moment, where interesting and relevant findings are arising. The project partners met online last 22nd of October for the annual meeting, to present their progress and discuss next steps. Due to the COVID-19 outbreak, the project has been granted a 6 month extension; March 2022 is the new date for the project's completion.

Follow-up completed

The National and Kapodistrian University of Athens (N.K.U.A) and the **Medical University of Lodz (MUL)** followed asthmatic and healthy individuals over a one-year period to characterize microbiome dysbiosis, a condition defined by an imbalance of an individual's microbiome. Researchers traced microbiome changes in the upper respiratory tract over time, alongside disease activity and immunological patterns, to investigate possible relationships.

During the visits, exposures, such as viral infections or allergens were registered, and respiratory samples were obtained for metagenomic analyses, host immune responses and phage isolation. Serum samples were taken for immunology assessment and plasma samples for total and specific IgE analyses.

The follow-up was finalised in February-March 2020 in both clinical centres. However, due to the COVID-19 pandemic, certain visits had to be cancelled and were performed after restrictive measures were lifted. Despite these accommodations, all the challenges related to COVID-19 were overcome.

The presence of bacteriophages contributes to a reduction of inflammation

The Swiss Institute of Allergy and Asthma Research (SIAF) and the **Biomedical Research Foundation, Academy of Athens (BRFAA)** are responsible for evaluating the immunological reactions and mechanisms of our body associated with microbial composition and effects of bacteriophage[1] introduction in the airways.

Based on the above-mentioned samples collected by the clinical partners, BRFAA performed a detailed immune cell profiling using multiparametric flow cytometry[2]. The immunological data was then shared with the University of Manchester to develop predictive mathematical models to understand the associations between asthma and microbiome composition in the lungs.

In addition, BRFAA also studied the effect of bacteriophages on blood cells' ability to fight infection and adapt to intruders, also referred to as peripheral blood mononuclear cells (PBMC) viability. To test the effect, several phage

[1] A bacteriophage is a type of virus that infects bacteria; in fact, it literally means "bacteria eater".
[2] Powerful analytical preparative tool. It enables the rapid measurement of multiple physical and chemical characteristics of individual cells.

preparations were introduced in cell culture models with a clinical isolate of *Staphylococcus aureus* from the nasopharynx[3] of a 4-year-old male (resistant to penicillin and erythromycin). Data show that the presence of bacteriophages (from the commercial PYO cocktail by ELIAVA Institute) improved PBMC viability[4] and reduced inflammation, as an immunological response against bacteria.

In parallel, SIAF examined the effects of bacteriophages on epithelial barrier integrity[5] and on human innate lymphoid cells[6]. It is of interest that bacteriophages, although they are viruses, do not attack or destroy the epithelium.

Taken together, these findings suggest that bacteriophages support the immune system without compromising human tissues; these are promising findings for a developing asthma treatment.

There is strong evidence of a relationship between microbial dysbiosis and asthma

The **University of Manchester (UMAN)** is studying the microbial composition of the respiratory track and how it evolves over time, using metagenomics[7]. Overall, there is good evidence of a correlation between microbiome dysbiosis and asthma. Considering the large variability of the metagenome, many factors influence its composition, such as geography and age. The question that is now being explored is how asthma influences the change of the metagenome over time.

Cooperation among partners is key for the success of the project

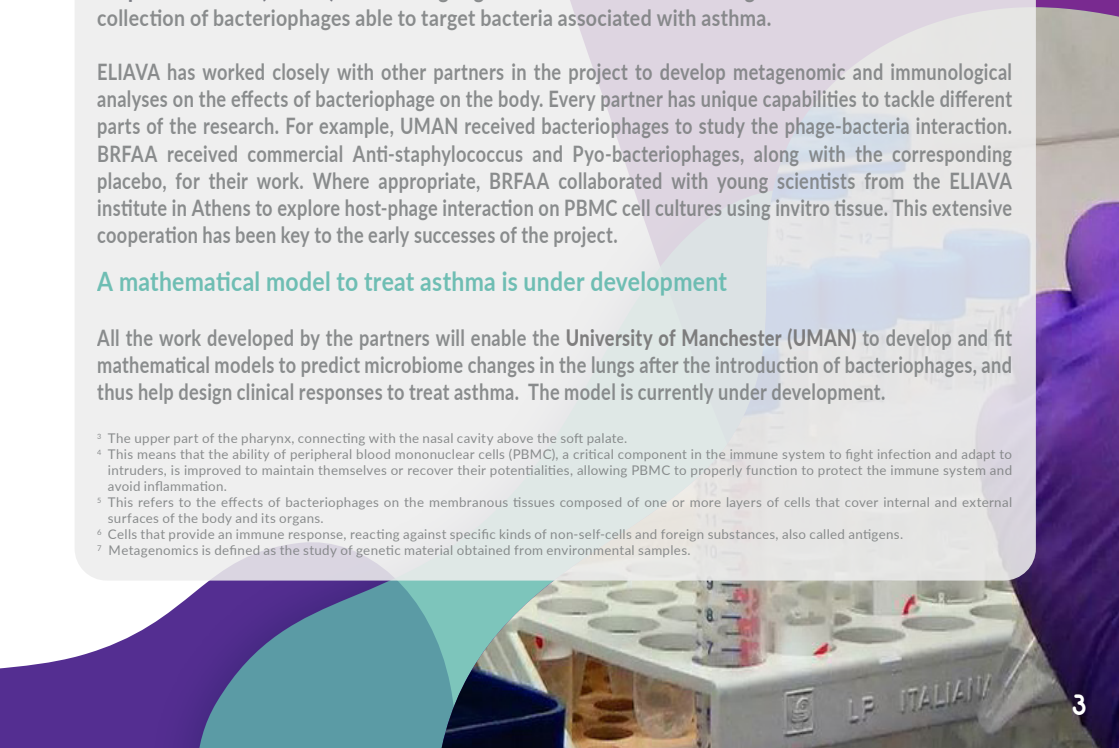
The Georgi Eliava Institute of Bacteriophagy, Microbiology and Virology (ELIAVA) and the **ELIAVA Bio Preparations LTD (ELIBIO)** are working together in order to isolate and generate a well-characterised collection of bacteriophages able to target bacteria associated with asthma.

ELIAVA has worked closely with other partners in the project to develop metagenomic and immunological analyses on the effects of bacteriophage on the body. Every partner has unique capabilities to tackle different parts of the research. For example, UMAN received bacteriophages to study the phage-bacteria interaction. BRFAA received commercial Anti-staphylococcus and Pyo-bacteriophages, along with the corresponding placebo, for their work. Where appropriate, BRFAA collaborated with young scientists from the ELIAVA institute in Athens to explore host-phage interaction on PBMC cell cultures using invitro tissue. This extensive cooperation has been key to the early successes of the project.

A mathematical model to treat asthma is under development

All the work developed by the partners will enable the **University of Manchester (UMAN)** to develop and fit mathematical models to predict microbiome changes in the lungs after the introduction of bacteriophages, and thus help design clinical responses to treat asthma. The model is currently under development.

[3] The upper part of the pharynx, connecting with the nasal cavity above the soft palate.
[4] This means that the ability of peripheral blood mononuclear cells (PBMC), a critical component in the immune system to fight infection and adapt to intruders, is improved to maintain themselves or recover their potentialities, allowing PBMC to properly function to protect the immune system and avoid inflammation.
[5] This refers to the effects of bacteriophages on the membranous tissues composed of one or more layers of cells that cover internal and external surfaces of the body and its organs.
[6] Cells that provide an immune response, reacting against specific kinds of non-self-cells and foreign substances, also called antigens.
[7] Metagenomics is defined as the study of genetic material obtained from environmental samples.



"Recognition and description of specific metagenomic profiles in conjunction with disease characteristics will contribute to precision medicine in patients with respiratory allergy associated diseases"

Dr. Paraskevi Xepapadaki is a Paediatrician and Associate Professor in Paediatrics-Allergology within the 2nd Paediatric Clinic, Allergy Department at the University of Athens.

Can you please introduce yourself and describe your background?

I am Pediatrician and Associate Professor in Pediatrics- Allergology in the Allergy Department, 2nd Pediatric Clinic, University of Athens, also partner in CURE. My main research focus involves the effects of viral infection on asthma and lung function testing in children, while in clinical daily practice, I am supervising and actively participating in the evaluation of children with allergy related diseases (food, skin, respiratory and drug allergy, immunotherapy and biologicals). In addition, I am responsible for the Respiratory Allergy outpatient clinic within the Allergy Department and since June 2014 I am also responsible for the Pulmonology outpatient clinic. Last, I served as a past member of the Pediatric Board of the European Academy of Allergy and Clinical Immunology (EAACI), and from 2018 I am a member of the Pediatric Asthma Section of the World Allergy Organization (WAO).

The National and Kapodistrian University of Athens (NKUA) and the Medical University of Lodz (MUL) are together responsible for the clinical cohort of the CURE project. What does the clinal cohort do, what is your role, and how is it linked to the rest of the project?

The clinical cohort is a group of asthma patients and healthy individuals observed over time and plays a central role in the development of the CURE project. The main objective of the cohort is to prospectively monitor variations in respiratory symptoms. In parallel, it serves to collect biological samples from the participants, supporting the progress of our work and of other partners in the project for phage isolation, host response and metagenomic longitudinal pattern analyses. Prospective data of disease activity and exposures are now merged into a common database with trajectories from the same subjects obtained from the metagenome metadata and host responses.

As part of my role in the project, I designed the study protocol, I coordinate the research teams involved in the clinical study and I am responsible for recruiting children with asthma and controls in my centre but also for following up with the recruitment in other clinical centres. Moreover, I monitor the progress and adherence to the protocol, ensuring the overall success and scientific value of the study.

What can you tell us about your research so far?

We were able to complete the intense one-month, and one-year follow-up of the clinical cohort with a minimal number of drop-outs, while symptom activity was monitored using high-end e-medicine technology. Questionnaires and physiological measurements including lung function, inflammation, responsiveness and immune status data were obtained as planned.

Since the monitoring period officially ended in March 2020 and databases with clinical data are finalized, we are diligently working on the analysis of cohort characteristics and disease activity. This data will be combined with the metagenomic and host immune data obtained by other partners while unsupervised analyses will be used to identify asthma clusters characterized by distinct patterns of microbiome/immunological configurations.

NKUA and MUL are in regular contact with asthma patients for sample collection. What are patients' expectations of the CURE Project? What are their feelings towards phage therapy as a potential solution for asthma treatment?

During the intense one-month and one-year follow-up, we established a very close relationship with the CURE participants. A key point of participants' recruitment and adherence was that they were aware from the beginning of the purpose and obligations of their participation during the study. Frequent communication with members of the study team provided asthmatic patients the safety of a close follow-up from a group of experts. Moreover, we continued providing telemedicine guidance and in person clinical evaluation, where needed, during the COVID-19 pandemic.

At the end of the study when we asked CURE participants to evaluate their participation in the project, the vast majority stated that they were extremely satisfied from the monitoring and from their potential contribution to a "novel" therapeutic approach. The vast majority were not aware that phage therapy has existed for more than a hundred years, for example to treat bacterial infections, and were excited about participating in a project that aimed at transforming the way we treat respiratory diseases.

From a clinical perspective, what are your thoughts and personal expectations of CURE?

I am confident that CURE will transform phage therapeutic technology in the Western world and will kick off new projects for implementing phage therapy for patients with asthma. Recognition and description of specific metagenomic profiles in conjunction with disease characteristics will identify "the" specific and suitable strains for each individual, contributing to "precision medicine" for patients with respiratory allergy – associated diseases.

In Vitro Microfluidics Airway Epithelial Cells

For CURE, the **Laboratory of Allergy and Clinical Immunology (NKUA)**, in collaboration with the **Laboratory of microSENSES (UWA)**, is developing a portable, custom-made Trans Epithelial Electrical Resistance (TEER) measurement system based on microfluidic technology. This system measures how strong the resistance of the epithelium is to external threats and of course, the effect of bacteriophages on this.

CURE will use the in vitro microfluidic platform to make a model of the airway mucosal epithelial surfaces, under different conditions.

Thus, we will analyse interactions between three different components: bacteria, phages and epithelial cells. The tripartite interaction model, along with the microfluidic device, can be used to check the effect of different types of phages in combination with bacteria and cells from the nose (upper airway), measuring the effect of phage treatment on epithelial resistance and the inflammatory profile of the cells. Differences between healthy and asthmatic cells could be identified using the aforementioned biological system of interaction, thus establishing new patterns of interconnectivity between bacteria, phages and epithelial cells characterising health and disease.

CURE Outcomes: Publications

Bacteriophage Deficiency Characterizes Respiratory Virome Dysbiosis in Childhood Asthma

On August 4th 2020, the first paper was released in pre-publication form, revealing an association between changes in respiratory microbiome and the disease state of asthma.

The results show that preschool children with asthma have reduced levels of bacteriophages in their airways compared to their non-asthmatic counterparts. Given that bacteriophages are important regulators of the microbiome, their underrepresentation may lead to an ecological imbalance of the respiratory tract, comprising the resilience of the respiratory system towards asthma exacerbations. At the same time, the level of certain viruses is higher in asthma patients, increasing the chances of a symptomatic infection or sustained inflammation.

The publication is available here.

Interactions of Bacteriophages and Bacteria at the Airway Mucosa: New Insights into the Pathophysiology of Asthma

On January 26th 2021, a focused review from CURE was published.

Recent studies indicate that the microbial composition of asthmatic airways, across the spectrum of disease severity, differ significantly compared to healthy individuals. The levels of bacteriophages differ too, since people with asthma have a lower level of bacteriophages compared with non-asthmatic individuals[8].

The CURE publication reviewed the importance of the interaction between phages, bacteria, and respiratory epithelium, in asthma pathogenicity and development. The relationship between airway epithelial cells, bacterial symbionts and resident bacteriophages should be considered as a functional and interdependent unit with direct implications for the respiratory system and overall homeostasis. While the role of epithelial cells in asthma pathophysiology is well-established, this tripartite interaction should be scrutinized, both to better understand asthma as a system disorder and to explore potential interventions.

The publication is available here.

[8] Megremis, S., Constantinides, B., Xepapadaki, P., Bachert, C., Neurath-Finotto, S., & Jartti, T. et al. (2020). Bacteriophage deficiency characterizes respiratory virome dysbiosis in childhood asthma. https://doi.org/10.1101/2020.08.04.236067

Partners' contribution to COVID-19 research

Despite the impact of the COVID-19 pandemic, CURE has managed to continue delivering on its planned projects and activities. Currently, CURE is at its peak.

In addition to documented CURE objectives, our partners also undertook efforts to understand the complexities of the SARS-CoV-2 infection in relation to respiratory diseases such as asthma and allergies.

The National and Kapodistrian University of Athens (N.K.U.A), clinical partner of CURE, participated in international efforts to manage allergy and asthma during the COVID-19 pandemic. By taking part in the ARIA and the European Academy of Allergology and Clinical Immunology (ARIA-EAACI) statements[9], a World Allergy Organization's initiative on acute asthma management during COVID-19[10] and other projects, it has helped provide a greater understanding on the wider impact of COVID-19 on people with pre-existing health conditions, particularly allergies and asthma.

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Furthermore, the Pediatric Asthma in Real Life (PeARL) think tank, led by Prof. Papadopoulos of N.K.U.A and coordinator of the CURE Project, has promoted practice adjustments and disease burden analysis for children with asthma[11] and conducted a study evaluating asthma management in children before and during the pandemic[12].

SIAF
Swiss Institute of Allergy & Asthma Research
University of Zurich

The Swiss Institute of Allergy and Asthma Research (SIAF) was involved in the publication of 30 articles related to COVID-19 infections, in collaboration with Zhongnan Hospital of Wuhan University and EAACI. The articles focused on immunopathological changes in COVID-19, including patients that already suffer from other pathologies and respiratory syndromes[13].

In one of the review articles, researchers explored the immune response and immunopathological changes in patients alongside deteriorating clinical conditions such as cytokine storm, acute respiratory distress syndrome, autopsy findings and changes in acute-phase reactants, and serum biochemistry in COVID-19.

[9] (1) Bousquet, J., Akdis, C., Jutel, M., Bachert, C., Klimek, L., & Agache, I. et al. (2020). Intranasal corticosteroids in allergic rhinitis in COVID-19 infected patients: An ARIA-EAACI statement. Allergy, 75(10), 2440-2444. https://doi.org/10.1111/all.14302
(2) Klimek, L., Jutel, M., Akdis, C., Bousquet, J., Akdis, M., & Bachert, C. et al. (2020). Handling of allergen immunotherapy in the COVID-19 pandemic: An ARIA-EAACI statement. Allergy, 75(7), 1546-1554. https://doi.org/10.1111/all.14336
(3) Bousquet, J., Jutel, M., Akdis, C., Klimek, L., Pfaar, O., & Nadeau, K. et al. (2020). ARIA-EAACI statement on asthma and COVID-19 (June 2, 2020). Allergy, 76(3), 689-697. https://doi.org/10.1111/all.14471
(4) Pfaar, O., Klimek, L., Jutel, M., Akdis, C., Bousquet, J., & Breiteneder, H. et al. (2021). COVID-19 pandemic: Practical considerations on the organization of an allergy clinic—An EAACI/ARIA Position Paper. Allergy, 76(3), 648-676. https://doi.org/10.1111/all.14453

[10] Levin, M., Ansotegui, I., Bernstein, J., Chang, Y., Chikhladze, M., & Ebisawa, M. et al. (2020). Acute asthma management during SARS-CoV2-pandemic 2020. World Allergy Organization Journal, 13(5), 100125. https://doi.org/10.1016/j.waojou.2020.100125

[11] Papadopoulos, N., Custovic, A., Deschildre, A., Mathioudakis, A., Phipatanakul, W., & Wong, G. et al. (2020). Impact of COVID-19 on Pediatric Asthma: Practice Adjustments and Disease Burden. The Journal Of Allergy And Clinical Immunology: In Practice, 8(8), 2592-2599.e3. https://doi.org/10.1016/j.jaip.2020.06.001

[12] Papadopoulos, N., Mathioudakis, A., Custovic, A., Deschildre, A., Phipatanakul, W., & Wong, G. et al. (2021). Childhood asthma outcomes during the COVID-19 pandemic: Findings from the PeARL multi-national cohort. Allergy, 76(6), 1765-1775. https://doi.org/10.1111/all.14787

[13] Azkur, A., Akdis, M., Azkur, D., Sokolowska, M., Veen, W., & Brüggen, M. et al. (2020). Immune response to SARS-CoV-2 and mechanisms of immunopathological changes in COVID-19. Allergy, 75(7), 1564-1581. https://doi.org/10.1111/all.14364

Another review article entitled "A compendium answering 150 questions on COVID-19 and SARS-CoV-2"[14], contains answers to several questions formulated by young clinicians and scientists on COVID-19 and allergy. It provides a comprehensive overview of COVID-19 and allergic diseases on topics like virology, immunology, diagnosis, management of patients with allergic disease and asthma, treatment, clinical trials, drug discovery, vaccine development, and epidemiology.

The Biomedical Research Foundation, Academy of Athens (BRFAA), and particularly the Andreakos lab, in collaboration with the General Chest Diseases Hospital of Athens, Sotiria, conducted a study on the mechanisms underlying the severity of respiratory illness observed in patients infected with SARS-CoV-2, compared to other viral infections[15]. They found that, in sharp contrast with how influenza works, SARS-CoV-2 infections do not induce timely and sufficient production of type I and III interferons (INF), the key mediators of antiviral immunity (Figure 1). This untuned response is often accompanied by the production of pro-inflammatory cytokines, linked to acute respiratory failures. The findings are very valuable, since type I and III IFN are currently in clinical protocols against COVID-19.

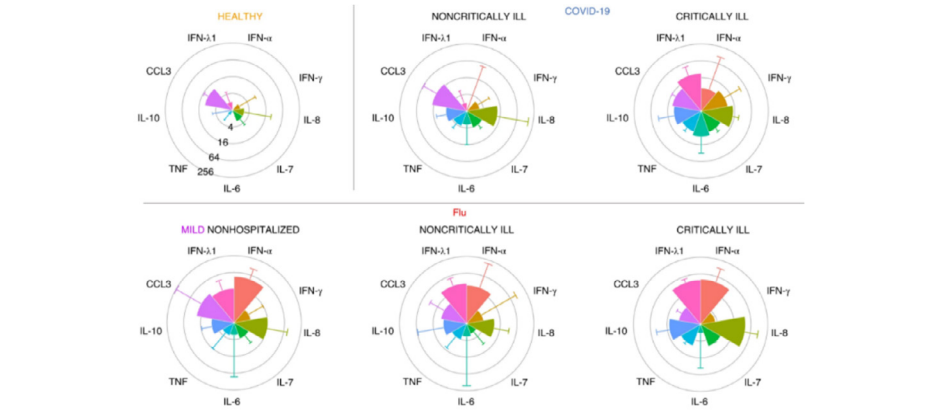

Figure 1: Analysis of blood serum levels of interferons and pro-inflammatory cytokines and chemokines revealed impaired production of both type I (IFN-α) and III (IFN-λ) interferons in COVID-19 patients when compared to influenza patients of various disease severity.

Currently, the Andreakos lab is conducting more research in the field, focusing on the importance of allergy and asthma in the susceptibility to SARS-CoV2 infections, the analysis of patients' response to different therapeutic protocols, the follow-up of patients in convalescence, as well as the immune response induced after COVID-19 vaccination.

[14] Riggioni, C., Comberiati, P., Giovannini, M., Agache, I., Akdis, M., & Alves-Correia, M. et al. (2020). A compendium answering 150 questions on COVID-19 and SARS-CoV-2. Allergy, 75(10), 2503-2541. https://doi.org/10.1111/all.14449

[15] Galani, I., Rovina, N., Lampropoulou, V., Triantafyllia, V., Manioudaki, M., & Pavlos, E. et al. (2020). Untuned antiviral immunity in COVID-19 revealed by temporal type I/III interferon patterns and flu comparison. Nature Immunology, 22(1), 32-40. https://doi.org/10.1038/s41590-020-00840-x

The University of Manchester, led by Prof. R. Tucker Gilman, conducted research on the impact of COVID-19 in refugee camps[16]. Using an agent-based model, they studied the effects of feasible interventions that could slow down SARS-CoV2 infections in the Moria refugee camp in Greece. First, they divided the camp into sectors which reduced peak infections by up to 70% and delayed the spread of the virus. Furthermore, the use of face masks, coupled with timely isolation of infected individuals, reduced the overall incidence of infections, even eradicating the epidemic at the beginning (Figure 2).

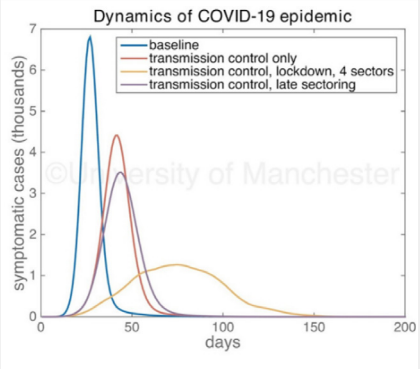

Figure 2: The projected total number of people infected over time without interventions (blue), with transmission control (e.g., face masks; red), and with transmission control and movement restrictions together (orange). The purple line shows the result if movement restrictions are not imposed until after 1% of the population shows symptoms.

The research and its findings provide an evidence base for successful intervention strategies in similar camps. This in turn could help other camp managers plan for and mitigate the impacts of COVID-19 and future pandemics.

[16] Gilman, R., Mahroof-Shaffi, S., Harkensee, C., & Chamberlain, A. (2020). Modelling interventions to control COVID-19 outbreaks in a refugee camp. BMJ Global Health, 5(12), e003727. https://doi.org/10.1136/bmjgh-2020-003727

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