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From science to business: translating live biotherapeutic products to the clinic

Maria Lluch-Senar

At Pulmobiotics, we engineer bacteria for the treatment of respiratory diseases. Here, we outline how we designed MycoChassis — an attenuated bacterium strain obtained by genome engineering of *Mycoplasma pneumoniae* (a human lung pathogen) — and discuss the challenges on the road to its clinical translation.

Respiratory disease

Complex respiratory diseases, such as antibiotic-resistant infections, lung cancer and asthma, require site-specific treatments that combine different mechanisms-of-action to avoid adverse effects following systemic administration. Lung-resident bacteria can be genetically engineered for the design of recombinant live biotherapeutic products (LBPs). Such engineered bacteria contain all the biological machinery necessary to simultaneously synthesize different proteins for combinatorial and site-specific therapies. Designing bacteria as therapeutics has low biosecurity concerns because the risk of DNA integration into the host genome is minimal compared with viruses, and their growth can be controlled using antibiotics and/or auxotrophic dependency modules or destruction circuits. Thus far, most LBPs are administered topically or orally (to colonize the digestive tract). Pulmobiotics is the first to design a nebulized LBP to treat respiratory diseases based on *Mycoplasma pneumoniae* (a human lung bacterium).

Biofilms in respiratory infections

Biofilms formed by *Pseudomonas aeruginosa* and *Staphylococcus aureus* are an important factor in several pulmonary chronic diseases (for example, cystic fibrosis) as well as in acute airways infection, such as ventilator-associated pneumonia (VAP). Biofilms are complex and dynamic structures formed by aggregates of microorganisms embedded in a polymeric matrix, which confer tolerance to antimicrobials and allow bacteria to evade host defense mechanisms. As a result, the effective minimum bactericidal concentrations of antibiotics required for biofilm eradication in vivo are very high and can cause adverse effects, such as renal and/or hepatic injury. Moreover, many lung pathogenic bacterial strains are resistant to antibiotics.

Biofilm formation is especially problematic if endotracheal tubes are used in patients who require mechanical ventilation (MV) in intensive care units (ICU). VAP is estimated to occur in 27% of all patients receiving MV[1]. For patients with SARS-CoV-2, the incidence rates of VAP exceed 50% overall[2]. Until now, most interventions aimed at reducing lung biofilms, including aerosolized antibiotics, have failed.

MycoChassis

Selecting a chassis that is present in the lung is key to ensuring a low inflammatory response, the survival of the administered bacteria and therefore, the efficacy of treatment. We selected *M. pneumoniae*, a mild human lung pathogen, which has several features that make it an ideal chassis for the development of treatments for respiratory diseases. *M. pneumoniae* has a small genome and is thus considered a model for systems biology. We characterized its virulence factors, deleting some of them using proprietary genetic tools ('SURE'[3]) to obtain a safe and attenuated strain (MycoChassis), and showed that MycoChassis does not cause lesions in the lungs of murine[4,5] models. MycoChassis cannot efficiently recombine or conjugate, thereby preventing horizontal transfer of DNA to or from other bacteria. Furthermore, it can be eliminated with common antibiotics, which provides a 'natural' safety kill switch. In addition, its UGA codon encodes for tryptophan instead of a stop codon, providing an intrinsic biocontainment mechanism. Importantly, MycoChassis lacks a cell wall, opening the opportunity to use it in combination with antibiotics that target the cell wall of pathogenic bacteria and facilitating the secretion of therapeutic proteins, such as interleukins.

MycoChassis targets biofilm-associated infections

Applying a proprietary strategy to deliver heterologous proteins, we designed two different products able to treat lung infectious diseases associated to biofilms. Our first product PB_VAP1 (Pulmobiotics VAP product 1) was engineered to express dispersin B and lysostaphin to target *S. aureus* biofilms. PB_VAP1 efficiently dissolves *S. aureus* biofilms in vitro, ex vivo and in vivo[4]. Our second product (PB_VAP2) expresses four different enzymes against *P. aeruginosa* infection[5]. PB_VAP2 showed efficacy in mouse and pig models. In addition, in collaboration with the Hospital Clinic of Barcelona, we showed that PB_VAP2 destroys biofilms formed in ETTs of patients with VAP, improving the activity of antibiotics commonly used in this setting[5].

From science to business

After more than a decade of effort, and with investment from public sources, we obtained proof-of-concept data supporting that MycoChassis could be used to treat respiratory diseases. Curiosity and responsibility motivated us to translate our technology to improve human health, which usually can be achieved either by licensing the technology to a pharmaceutical company that further develops it, or by starting a company and raise funding to carry out the development.

At that time, I was in a stable position, as an Associate researcher in the group of Luis Serrano, co-coordinating the part of the team focusing on synthetic biology, as well as several European projects. Despite being on track for a successful academic career, I seized the opportunity to become an entrepreneur and co-founded Pulmobiotics with Luis Serrano in March 2020 (yes, during the first COVID-19 lockdown) with a €2 million seed investment from Invivo Capital. Doing market analysis, establishing a business plan and building a pitch were challenging as a scientist and I was grateful for the support provided by the CRG Technology Transfer Office.

Although I attended several courses on different aspects of the business of science, you can only learn certain aspects of managing a

company when faced with them. For example, obtaining all the permits to work with genetically modified organisms (GMOs) as a company, establishing standardized protocols and creating a proper organization structure are important initial steps. Time management becomes crucial, and focus is key; often, you must sacrifice scientific curiosity to reach development milestones within the established deadlines. However, it is also quite motivating as an entrepreneur to build the mission and culture of a company. We created the company to cure people and to provide quality jobs for scientists, and it has become clear to me that a motivated team of excellent scientists with a shared vision is key for the success of the company.

Manufacturing, formulation and regulatory aspects

When we started Pulmobiotics, our main focus was to develop PB_VAP3, a treatment for VAP that combined the functional payloads of PB_VAP1 and PB_VAP2 to target complex biofilms formed by *P. aeruginosa* and/or *S. aureus*. The efficacy and safety profile of PB_VAP3 supported the decision to continue development towards clinical trials.

Manufacturing, formulation and regulatory compliance must be established before clinically testing a new pharmaceutical product. We showed that our engineered strains can be produced in small fermenters. However, scaling up is expensive and challenging, not only because of technical issues, but also because biosafety level 2 facilities are needed for the large-scale fermentation of MycoChassis strains. We are currently collaborating with AcuraBio on establishing conditions for the large-scale fermentation of our product under good manufacturing practices (GMP) conditions. We also had to prove that bacteria can be formulated for inhalation delivery to reach the lung. In collaboration with Quaypharma, we identified a formulation that allows storage of lyophilized MycoChassis strains for at least three months and a protocol to deliver the product with a nebulizer.

Although the US Food and Drug Administration (FDA) has approved several LBPs for clinical assays, the number of LBPs approved by the European Medicine Agency (EMA) remains low. In part, this difference is due to the lower number of LBPs being developed by European companies, and thus, a clear regulatory path has not yet been established. However, this may change with the increasing number of LBPs currently in development. We have recently received positive comments from the Innovation Office of the Spanish Agency of Medicines on the use of MycoChassis as a therapeutic delivery platform and useful feedback on our preclinical and clinical development plan for our VAP product. We are currently working on the pre-investigational new drug application (pre-IND) data package, also searching for a pharmaceutical company collaborator or venture capital funding to advance towards clinical development.

Expanding our technology platform

In the past three years, we showed that engineered MycoChassis can modulate the immune system by expressing cytokines and nanobodies[6], opening the possibility to treat additional diseases. Based on our market analysis, discussions with key opinion leaders in respiratory diseases, and with potential investors, Pulmobiotics has expanded its portfolio to include products for the treatment of lung cancer and asthma. We raised a total of €5.8 million (€2 million in seed funding and €3.8 million in grant funding); for example, we were awarded an European Innovation Council (EIC) Transition grant to develop a product based on MycoChassis for treating patients with lung cancer who do not respond to immune checkpoint inhibitors. Moreover, we were awarded a Private-Public Collaboration Project from the Spanish government to engineer a probiotic bacterium for the treatment of asthma in collaboration with the Center for Genomic Regulation (CRG) and the Institute for Research in Biomedicine (IRB).

We have also improved and expanded our genetic toolkit to edit the genome of other bacteria. Genetic payloads can be inserted in the genome to design stable LBPs (without resistance markers), instead of using replicative vectors that are lost in the absence of selection pressure. Currently, we are working on expanding our collaborator network to other companies interested in improving manufacturing or including new mechanisms of action in the probiotics they commercialize.

The human microbiome project revealed that probiotics can be used to restore equilibrium to treat diseases. Pulmobiotics goes a step further, rationally engineering probiotics to treat complex diseases.

Maria Lluch-Senar [1,2]
[1]Centre for Genomic Regulation (CRG), The Barcelona Institute of Science and Technology, Barcelona, Spain.
[2]Pulmobiotics S.L., Barcelona, Spain.
e-mail: maria.lluch@pulmobio.com

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Competing interests

M.L-S. is currently working in Pulmobiotics as Chief Scientific Officer (CSO) where she is co-author of patents and co-founder of the company.