1. EXCELLENCE

1.1. RADICAL VISION OF A SCIENCE-ENABLED TECHNOLOGY

Antimicrobial resistance (AMR) is an increasingly serious threat to the gains made in health and development and can compromise the achievement of the Sustainable Development Goals (SDGs), affecting health and food security, poverty and economic growth[1]. It has been estimated that more than 670,000 cases of infections with multidrug-resistant bacteria occurred in the EU in 2015, which caused about 33,000 deaths and 870,000 disability-adjusted life-years (DALYs).[2] 68% of the total DALYs were caused by infections with four pathogens: cephalosporin-resistant *Escherichia coli*, methicillin-resistant *Staphylococcus aureus* (MRSA), carbapenem-resistant *Pseudomonas aeruginosa* and cephalosporin-resistant *Klebsiella pneumoniae.* There is a gap between the burden of infections due to multidrug-resistant bacteria and the development of new antibiotics and other therapies to tackle this problem. **This project is concerned with chronic bacterial infections in the lungs**. For instance, progressive respiratory failure due to *P. aeruginosa* is the leading cause of morbidity and mortality in patients with cystic fibrosis. Other pathogens also play a key role in cystic fibrosis and hospital-acquired chronic and acute lung infections, with MRSA being among the most clinically relevant. The only available treatment for these infections is the use antibiotics, inhalable antibiotics being favoured over systemic ones as they can be deposited at a higher dose at the site of the infection reducing the risk of systemic side effects.[3] However, current antibiotics are often insufficiently effective because of AMR and contribute to its spread. The expectations for new antibiotics are not very good, either due to the likelihood that they will induce resistance as well. Moreover, a large number of chronic bacterial infections involve bacterial biofilms, which are inherently recalcitrant to antibiotics.[4] Thus, **alternative anti-infective approaches are urgently needed**, that are effective against bacterial biofilms and do not cause resistance themselves.

The Existing Paradigm: One very promising such approach is **photodynamic therapy (PDT),** which is based on the generation of cytotoxic reactive oxygen species (ROS) by the combined action of visible light, oxygen and a photosensitising drug (hereafter "the photosensitiser" - PS).[5] PDT is effective against multidrug resistant bacteria, is safe and is very unlikely to induce resistance itself owing to its multi-target mode of action. However, **application of PDT to the treatment of lung infections is considered an impossible task** because of the **need to deliver light to the whole of the bronchi surface** (~2m^2) **and possibly the 700 million pulmonary alveoli**. Irradiation of the lungs from the outside of the body cannot be considered due to the poor penetration of light in human tissues. The use of fibre optics or modified bronchoscopes would be highly invasive and costly and would not guarantee the whole illumination of the infected lung regions. In addition, delivery of the PS poses a number of challenges itself.

Our Vision: Light4Lungs will develop a **novel therapeutic scheme for the treatment of multidrug-resistant lung bacterial infections**, based on **inhalable light-emitting aerosol particles** containing long-decay phosphorescent compounds. The treatment will not require the delivery of PSs because the light-emitting aerosol will activate **endogenous** bacterial **photosensitising molecules** (particularly iron-free porphyrins) in the infected region of the lungs, producing **reactive oxygen species** that will selectively kill the lung-infecting bacteria, resulting in an effective management of the infection. Unlike antibiotics, light-emitting particles will not need to be internalized by the target bacteria for light emitted in their close vicinity will still reach their photosensitisers. Also, damage to the surrounding host tissue will be avoided because of its lack of self-photosensitising ability. The project encompasses the **design, synthesis, characterization, and testing** of the light-emitting material, its aerosol form, and the definition of the treatment parameters. The results of the project will be useful for patients with chronic multidrug resistant pulmonary bacterial infections, such as cystic fibrosis, and have a strong potential for extension to other pulmonary diseases, such as fungal infections and lung cancer, and eventually to other internal organs, having a profound impact on the fields of materials, photonics and healthcare. Hence Light4Lungs addresses a **clear and radical vision** in the treatment of recalcitrant respiratory tract infections enabled by a **new non-intrusive light-emitting technology** that challenges current paradigms in anti-infective treatments, photodynamic therapy, materials science and lighting technology.

[1] O'Neill. London: Wellcome Trust; 2015 (https://amr-review.org/).
[2] Cassini et al. Lancet Infect. Dis., 2019, 19, 56
[3] Smith et al., Cochrane Database Syst. Rev. 2018, 3. CD001021
[4] Costerton et al., Science 1999, 284, 1318
[5] Wainwright et al., Lancet Infect. Dis., 2017, 17, e49

The objectives, specific goals and expected results of the projects are given in the Table below:

LIGHT4LUNGS OBJECTIVES		
OBJECTIVES	**SPECIFIC GOALS**	**EXPECTED RESULTS**
1.- The **design** of the key parameters of the Light4Lungs therapeutic scheme	• To define the best biological models to test the aerosols • To define the properties of the aerosol light-emitting particles • To predict the *in vivo* action spectrum of *P. aeruginosa* and *S. aureus* photo elimination	• Definition of biological models, aerosol properties and methods • Photo-killing efficacy against *P. aeruginosa* and *S. aureus* in planktonic cultures and in biofilms • Calculated action spectrum *in vivo*
2.- The **synthesis, formulation and characterization** of the treatment components	• The **synthesis and characterization** of light-emitting particles • The **formulation** of aerosol forms of the light-emitting particles • The **method** for the activation of the light-emitting aerosol particles prior to inhalation	• Availability of particles with the required light-emitting properties • Availability of an aerosol form of the particles with the required light-emitting properties • Definition of the aerosol activation parameters
3.- The **assessment of the safety and efficacy** of the therapeutic scheme in *in vitro* and *in vivo* models	• Assessment of aerosol **biocompatibility** in ***in vitro* multi-cell 3D models** of lungs and ***in vivo* mouse models** • Assessment of **efficacy** of the aerosol treatment **against bacterial pathogens *in vitro*** in both planktonic phase and biofilms and **in clinically relevant lung tissue culture models** and ***in vivo* mouse models**	• Particles are expected to be non-toxic to the host tissue • Particles are expected to kill 99-99.9% bacteria both in planktonic phase and in biofilms • Particles are expected to kill 99-99.9% bacteria in mouse models of the infections

1.2. SCIENCE-TO-TECHNOLOGY BREAKTHROUGH THAT ADDRESSES THIS VISION

Light4lungs addresses the problem of AMR in the treatment of chronic lung infections.[1] Antibiotics will be replaced by breathable light sources that will excite bacterial endogenous PSs,[6] triggering a photodynamic effect that will kill the pathogenic bacteria.[5] Adoption of the Ligh4Lungs treatment scheme will therefore contribute to stop the spread of antibiotic resistance. The concept behind Light4Lungs is depicted in Figure 1. In a first step, novel light-emitting particles will be developed. Then an aerosol form will be developed, whereby the particles' emission will be activated prior to irradiation. Next, the active light-emitting particles will be delivered to the lungs where they will trigger the photodynamic effect on the pathogenic bacteria, killing them. **The key novelty of the Light4Lungs concept is the use of breathable therapeutic light sources, which do not exist either at a prototype level or in research to the best of our knowledge**. Thus, realization of a breathable source that can deliver a sufficiently high light dose to provide effective phototherapy in the lungs is the **first high-risk challenge** of the project. Herein, the consortium aims at making a new breakthrough and engineer nano- to micro-sized materials with outstanding long-lasting emission into biocompatible particles, capable of acting as local light sources to trigger photo-reactions that will kill the bacterial pathogens responsible for difficult-to-treat human infections. While light-emitting materials with persistent luminescence have been reported, their biocompatibility and light emitting performance are not fit for therapeutic purposes. [7] The **second high-risk challenge** is the antimicrobial efficacy of the new aerosol light-source. The state-of-the-art in aerosol drug delivery science,[8,9] will be superseded by developing new aerosol formulations and inhalers capable of delivering a sufficient number of light-emitting particles to the infected regions of the lungs. This will include the excitation source required to activate the light-emitting particles prior to inhalation. **New liquid light sources** will be a by-product of this project, to be used e.g. for other antimicrobial applications and, in a more distant perspective, lung cancer and infections and tumours of other internal organs. The list of breakthroughs of this project, in relation to the state of the art, is provided in the Table below:

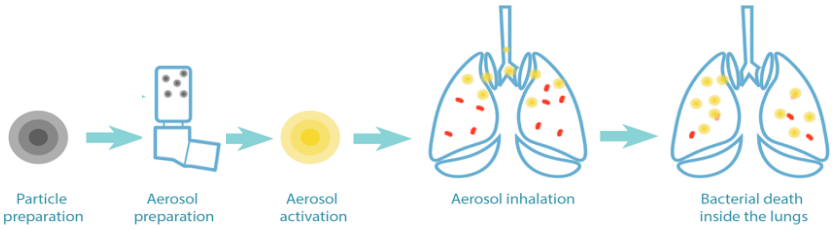


Figure 1. Light4Lungs 5-step concept

[6] Dai et al., Drug Resist. Updat. 2012, 15, 223
[7] Li et al. Chem. Soc. Rev. 2016, 45, 2090
[8] Borghardt et al., Can. Respir. J. 2018, 2018, 2732017
[9] Pleasants and Hess, Respir. Care 2018, 63(6), 708

LIGHT4LUNGS NOVELTIES		
CURRENT STATE OF THE ART	CHALLENGES FACED	LIGHT4LUNGS BREAKTHROUGHS
• Antibiotic therapy efficacy is limited by AMR[1] • Current management of bacterial lung infections combines intravenous and inhaled antibiotics[3]	• Progressive loss of therapeutic efficacy due to antibiotic resistance and resistance induction • Very limited availability of novel antibiotics	• Antibiotics will be replaced by breathable light sources that will trigger the photodynamic effect within pathogenic bacteria. • This technology will contribute to stop the spread of resistance
• Current PDT protocols require the delivery of exogenous PS to the pathogenic bacteria[4]	• PS selectivity for bacteria cannot be guaranteed and photo-damage to the surrounding host tissue cannot be totally avoided	• Exogenous PS will not be needed because *P. aeruginosa* and *S. aureus* contain endogenous PS. • Lack of self-photosensitising ability in healthy tissue ensures that only the pathogenic bacteria will be killed
• PDT principles and efficacy are well established but limited by light penetration in tissue. Therefore, light can be delivered non-invasively only to the skin[10] • Treatment of internal organs requires invasive light delivery devices associated to high costs due to hospitalization[10]	• It is not possible to deliver a sufficient and targeted light dose inside the infected zones of the lungs irradiating them from the outside • Lung irradiation by fibre optics or bronchoscopes is invasive and costly and does not assure the illumination of all infected areas	• Inhalation of the light source avoids the use of any "physical tether", such as optical fibres, which is patient compliant • Irradiation by the luminous aerosol can reach any lung district and more districts at a time, if required • A cost-saving and patient-compliant at-home therapy with the luminous aerosol will be developed
• Light-emitting materials with long lasting emission are used for signaling and bioimaging.[7]	• No materials fit for aerosol phototherapy yet • Light emitting performance insufficient for therapeutic purposes	• A biocompatible and therapeutically-apt highly-emitting aerosol will be developed

1.3. INTERDISCIPLINARITY AND NON-INCREMENTALITY OF THE RESEARCH PROPOSED

Light4Lungs is highly **interdisciplinary**, as it combines several different scientific areas from photonics to medicine, including materials chemistry, physical chemistry, photophysics, pharmaceutics, photobiology and microbiology. On one hand, a new nano- to micron-sized light source based on phosphorescent materials will be developed. This will require to put together expertise in optics, photonics and materials science. On the other hand, these new light sources must be made biocompatible, inhalable and active against bacteria associated with severe and recalcitrant lung infections. Cell biology, biophysics, pharmacology, clinical microbiology, photobiology and medicine are therefore also highly involved, too. Thus, only a strong synergetic consortium with experience across all disciplines will be able to reach the final goal of realizing a novel optical source which, at the same time, acts as a non-toxic vehicle of light and performs antimicrobial therapy in the lungs. The Light4Lungs consortium will overcome the challenge of bringing this scientific project to a real medical application by assembling multidisciplinary teams that will ensure the cross talk between disciplines and specialists throughout the project.

The proposal departs strongly from well-established paradigms by (1) **treating bacterial infections without drugs**, which will be replaced by our breathable light sources; (2) **using a photodynamic effect to kill bacteria without any externally-added PS**, since we will take advantage of the presence of endogenous PSs in the pathogenic bacteria; and (3) **using a breathable light source** to elicit the therapeutic action, avoiding the use of invasive physical tether to introduce light inside the lungs. **Due to its novelty and ambition, the project will be revolutionary in the use of light sources and biocompatible nanomaterials for medical applications, especially for non-invasive treatments**. On the one hand, it will be a breakthrough in medicine and microbiology, because it addresses the increasing phenomenon of AMR (identified by the World Health Organization as one of the major global threats to public health)[11], proposing a way to overcome it through light-triggered therapy. On the other hand, it will have an impact on medical physics and material chemistry, because it proposes a minimally invasive way to overcome the well-known difficulties of poor penetration of light in the human body. The possibility of an "untethered" light delivery to internal organs, such as the lungs, without the further need for online external excitation, has a ground-breaking nature, as it unleashes the possibility of defining effective, low-cost and minimally invasive light-driven treatments ("at home aerosol PDT") against infective diseases and possibly, in a future perspective, lung cancer.

1.4. HIGH-RISK, PLAUSIBILITY AND FLEXIBILITY OF THE RESEARCH APPROACH

1) High-risk vs plausibility: The project is based on the hypothesis that it will be possible to kill bacteria in the

[10] Agostinis et al., CA Cancer J. Clin. 2011, 61, 250
[11] World Health Org., Global Action Plan on Antimicrobial Resistance, 2015

lungs by the photodynamic effect using a revolutionary breathable light source and taking advantage of endogenous bacterial photosensitisers. Thus, a positive answer to the following questions is the key to its success:

- Will it be possible to produce light-emitting particles with the required luminescence properties?

Light-emitting materials with persistent luminescence is an ongoing vigorous research field. Based on recently-published data,[7] it is feasible to produce persistent-luminescent materials with ample freedom to modify their size, physico-chemical and light-emitting properties, the remaining questions being the amount of light that they can delivered per unit mass and their biocompatibility.

- Will it be possible to deliver them effectively and in sufficient number to the lungs?

Aerosol technology is a mature field and, in principle, we do not anticipate major problems for the aerosolization of our light emitting materials. Activation of the particles prior to inhalation will be achieved by exposing them to light of appropriate wavelength and intensity. Optimising these parameters seems feasible. Another question is how many particles will reach the infection sites. Literature reports indicate that the fraction of particles reaching the bronchi and alveolar region depends on a number of parameters, such as particle size and flow rate, which can be controlled.[8]

- Will they be able to deliver a sufficient light dose to the bacteria?

Photodynamic killing of bacteria (>99.9%) requires typically light doses of 1-10 J/cm^2, depending on the particular PS and bacterial strain.[6] Light4Lungs should be able to reach theses doses using the light-delivery strategy depicted in Fig. 2, where the number of the photons (n) emitted by a single particle will undergo a magnifying process by packing them into nanospheres, and these in turn in larger aerosol particles, being ultimately proportional to the number of aerosol particles inhaled. This leads to a final estimated number of photons "breathed" in a single aerosol session in the range of ~$10^{19\text{-}20}$, starting from a conservative estimate of n ~$10^{3\text{-}4}$.[12] .This corresponds to ~5-50 J at a wavelength of 405 nm. Aerosol delivery to the whole bronchi surface (about 2m^2 in adults)[13,14] 2-4 times/day for a period of 4-24 weeks (as in, e.g. clinical trials),[13,15] would correspond to a dose of 2-5 J/cm^2, close to the anticipated needs. As science evolves, it can be expected that particle-emission and aerosol-delivery efficiencies will increase, thereby shortening the duration of the treatments.

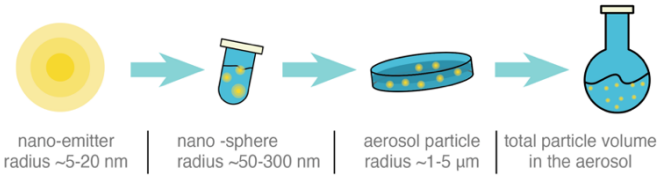


Figure 2. Scheme of the bottom-up scaling structure of the light-emitting aerosol (e.g. case of nebulized liquid)

- Will the concentration of endogenous photosensitisers be enough to kill the bacteria at the available light dose?

Regarding the use of endogenous PSs to kill the bacteria at the available light dose, the scarce available data on bacterial photo-killing without the use of external PSs are encouraging.[6] For example, 10 J/cm^2 upon excitation at 405 nm were associated with up to 95% killing rate of high-cell density *S. aureus* and *P. aeruginosa* cultures,[16] and 20 J/cm^2 at 415 nm induced 90% colony forming units (CFU) inactivation for *P. aeruginosa* in mouse models.[17,18] These light doses are comparable to those we expect to deliver with the aerosol emitting particles.

- Will the technology be safe for the patients?

Current data indicate that particles at the nanoscale (≤200 nm) can cross the cellular barrier and are eliminated by the usual systems, while micron scale particles between 1 µm and 5 µm are efficiently taken up by macrophages, and particles larger than 6 µm are rather exhaled.[19] To a large extent, the toxicity of particles depends on their surface properties and these can be widely modified by attaching organic ligands and/or biomolecules to them.[20] On the other hand. many studies have shown that antimicrobial blue light does not cause significant damages to host cells.[21]

Thus, none of the above questions can be answered with certainty with the current state of the art, therefore the project entices several high-risk steps. Nevertheless, data provided in the text above strongly indicates the project **plausibility.**

2) Flexibility: The project has been designed methodologically in a step-wise fashion bearing in mind the need to adapt to potential bottlenecks and progress with alternative routes, thereby minimising the risks and maximising the potential to reach the expected goals. The main potential roadblock concerns the **ability to prepare light emitting nanoparticles capable of producing light of sufficient quality** in terms of spectrum, duration and intensity. Fortunately, on one hand, the field of persistent luminescence materials is progressing rapidly, especially regarding maximization of the number of emitted photons per unit particle by tuning on the nanoparticle semiconductor properties like composition, bandgap levels, electronic properties, passivation of surface traps or addition of electro-

[12] Basu, Theory of Optical Processes in Semiconductors, Oxford Science Pub., 2003
[13] Wenzler, Clin. Microbiol. Rev. 2016, 29(3), 581
[14] Folkesson et al., Nat. Rev. Microbiol. 2012, 10(12), 841
[15] European Public Assessment Report EMA/CHMP/676680/2014
[16] Guffey et al., Photomed. Laser Surg. 2006, 24(6), 684
[17] Dai et al., Antimicrob. Chemother. 2013, 57, 1238
[18] Amin et al., Lasers Surg. Med. 2016, 48(5), 562
[19] Carvalho et al. Int J Pharm 2011, 406, 1
[20] Bartczak et al. Toxicol. Res., 2015, 4, 169
[21] Dai, Virulence. 2017, 8, 649

active surface ligands. On the other hand, improved aerosol preparation technology will help modify the number and density of nanoparticles per unit inhaled volume.

The second potential roadblock is that **the concentration of endogenous photosensitisers in the pathogenic bacteria could be too low to elicit a sufficient antimicrobial photodynamic effect at the highest light dose** that we may be able to deliver. In this case, the light-emitting particles will be slightly modified with photosensitisers matching the endogenous ones to supply the additional photodynamic power needed to kill the bacteria, at the risk of losing some selectivity. Nevertheless, this risk can be minimised by also attaching ligands to the surface of the nanoparticles to favour their rapid and preferential binding to the bacteria, e.g., adding positively-charged moieties that favour rapid electrostatic binding. In addition, it must be borne in mind that the duration of the light glow is limited and can be tailored with great flexibility to minimise damage to the host tissue, taking advantage of the fact that particle internalisation by mammalian cells is much slower that binding to the bacterial cell wall. Nanoparticle size and surface modification can also be widely modulated to modify the efficiency, selectivity and kinetics of elimination from the lung surface, thereby reducing further the risk of side effects.[22]

2. IMPACT

2.1. EXPECTED IMPACTS

SCIENTIFIC AND TECHNOLOGICAL CONTRIBUTIONS TO THE FOUNDATION OF A NEW FUTURE TECHNOLOGY	
FIELD	**IMPACT**
Pharmacotherapy and the pharma industry	New opportunities will arise on the synergistic and/or exclusive use of antibiotics and light-driven therapy. This scenario may, in the future, be applied to the case of lung tumours, and other internal organs, where a mixed treatment aerosol plus systemic or local photosensitizer plus anti-tumour agents can be envisaged, raising the potential for important new synergies
Therapeutic schemes development	The development of new minimally invasive therapeutic schemes, based on self-administration of the luminous aerosol for pathologies that now require hospitalization, with a significant decrease in costs for patient management and a rise in patient quality of life
Therapeutic use of light	A new public consciousness about the therapeutic use of non-ionizing radiation will also arise ("light can heal")
Material science and nanotechnology	To develop a breathable light source, the partners will have to design new biocompatible nanomaterials, capable of both intense and long-lasting photon emission, whose optical characteristics can be extended to infrared or UV emission with appropriate modifications
Nanomedicine	The inherently versatile nature of these materials will find new applications in nanomedicine (theranostic vectors and probes, optical sensors). Consequently, industries in the fields of biomedicine, photonics, pharma, aerosol formulation, etc will find interest in the project.
Healthcare	Considering that many iatrogenic pathologies are originated by antibiotic-resistant bacteria, this project will also contribute to a **decreased iatrogenic pathology incidence** and a new social perception of a "better healthcare system".

POTENTIAL FOR FUTURE SOCIAL OR ECONOMIC IMPACT AND/OR MARKET CREATION
The target of this project are lung bacterial infections, accounting for a significant portion of healthcare-associated infections in Europe and worldwide.[13] As the therapeutic principle is based on the use of visible light instead of antibiotics, a key impact will be in the fight against antibiotic resistance whose importance is both social and economic,[1,2] as it is associated with ineffective and repeated treatments, with a corresponding increase in healing time and costs. The absence of external photosensitisers/drugs paves the way to a wide scope of potential applications for the light treatment of internal organs. The European scientific activity will also benefit from the developments of this new instrument as well as of the novel biocompatible nanomaterials. We envision the creation of an SME based on the technology developed at the end of the process that pushes the technology further and eventually reaches the market.
LEADING RESEARCH AND INNOVATION CAPACITY ACROSS EUROPE
Light4Lungs aim at excellence and impact will permeate all of the actions from the outset of the project. Thus, we will recruit young excellent researchers initiating a highly inter-disciplinary work, supported by specific exchange initiatives between partners. Many of the consortium members collaborate for the first-time in a FET proposal. High-tech SMEs will be invited to participate in an open workshop during the project, whereby we expect to be able to engage them to participate in future developments of this technology. Light4Lungs will also raise public awareness toward the therapeutic use of non-ionizing electromagnetic radiation, endorsing new, more effective and sustainable therapeutic schemes which will contribute in improving life quality and increase EU leadership in health services. Light4Lungs will highlight to the European scientific community the importance of light-driven therapies, besides the well-established use of ionizing radiations. This will contribute to an increased leadership in the use of light in medicine by creating a scientifically-proven common ground where research institutions and main local stakeholders, (i.e. biophotonic/biomedical enterprises, The European Pharmaceutical Aerosol Group - EPAG, no-profit organizations -

[22] Rijt et al., Eur Respir. J. 2014, 44, 765

SCIENTIFIC AND TECHNOLOGICAL CONTRIBUTIONS TO THE FOUNDATION OF A NEW FUTURE TECHNOLOGY
e.g. the Cystic Fibrosis Foundation, EU-level Health Associations, and health authorities) can meet and establish new synergies for EU leadership in non-invasive therapies and quality of healthcare services (see also WP8 description). The new know-how will contribute to raise European nanotechnology competitiveness and to develop a knowledge-intensive industry, capable of revolutionizing the practice of medicine: spin-outs and in general high-tech SMEs in the fields of biomedical photonics, nanotechnology, aerosol formulation and delivery will greatly benefit from the new material defined in this project, whose biocompatibility, light-excitation / emission properties and delivery into the human body may be adapted to other specific regions and diseases (e.g. intestine instead of lungs, tumour or fungal infections instead of bacterial pathogens).
OTHER INNOVATIONS
Liquid light sources are a likely extension of our project, which opens the way to a new delivery technology for the diagnostic and treatment of internal organ diseases by direct ingestion or injection (e.g. stomach, intestine). In the vacuum or gas industries, light-emitting aerosols can be used to detect gas leakages.

2.2. MEASURES TO MAXIMISE IMPACT

2.2.1. DISSEMINATION AND EXPLOITATION OF RESULTS

Light4Lungs envisions a coordinated strategy for the communication, dissemination and exploitation of project outcomes for which a **Plan for the Use and Dissemination of the Project Results** (PUDPR) will be designed and agreed upon at the project onset: **(1) <u>Dissemination</u>**: The Plan will assess, define and prioritise the needs and expectations of the different target audiences; include the key messages; and determine the most appropriate tools and actions to be used and developed during the project implementation for the appropriate dissemination of the results. A specific WP will tackle this task as well as the actual implementation of the plan, the correct monitoring o f activities with appropriate KPIs, and the correct inclusion of the acknowledgement to the EC funding. The plan will serve the Consortium to maximize project results communication and dissemination amongst all concerned audiences during project implementation and beyond, making available all non-confidential outcomes, generated data, and protocols developed. This is considered of special importance for the scientific community, as the most interested audience in the use of the results, but also for both the Health Authorities, the HTA regulatory agencies, and the companies or industrial partners who can be interested in the further development of the therapies to reach the market. For this the Consortium will emphasize the importance of producing **high-quality peer-reviewed publications** in high-impact journals, of contributing to **books and reviews** in the fields of interest, of presenting the project and its outcomes in **international conferences, events, webinars and meetings**, and of undertaking individualized or **small groups presentations** to target audiences. **<u>Data Management</u>**: A policy of "**gold" open access publications** will be pursued, using the "green" open access only whenever this is not possible, in order to maximize the dissemination of non-confidential information. Additionally, all data arising from the project will be inserted in a **database** accessible via the project website (see below) with three access levels, with data available for: (i) the Consortium; (ii) the scientific community; and (iii) public data. Data will be set searchable by queries and readable with the major data analysis programs.

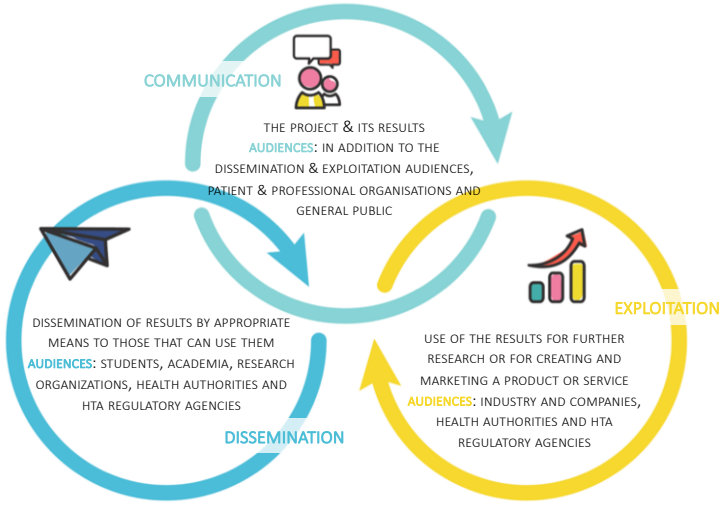


Figure 3. Light4Lungs strategy for communication, dissemination and exploitation

(2) <u>Exploitation</u>: The Plan will encompass the dissemination strategy whilst ensuring the preservation of all partners exploitation interests and securing the potential patentability of the therapies and innovations under development. It will include, in alignment with all considerations included in the CA, the procedures to establish the partners' contributions in all patentable project outcomes, define ownerships in each case, elucidate exploitation interests, and define the project after-life plan for the project results survival and progress towards market. The plan will also establish regular market and technology watches, which outcomes will be used for the strategic revision and update of the plan. Half-way to the project life, once project outcomes are more clearly defined and characterised, a patentability analysis will be undertaken in order to pave the way towards the future protection of the project key assets, followed by the corresponding patent applications. CSL/i2c will contribute to the task and assist in the generation of a "bespoke licensing package" attractive to pharma and device companies who might be interested in the further development of the project innovations and to whom it will be presented during the second half of the project.

2.2.2. COMMUNICATION ACTIVITIES

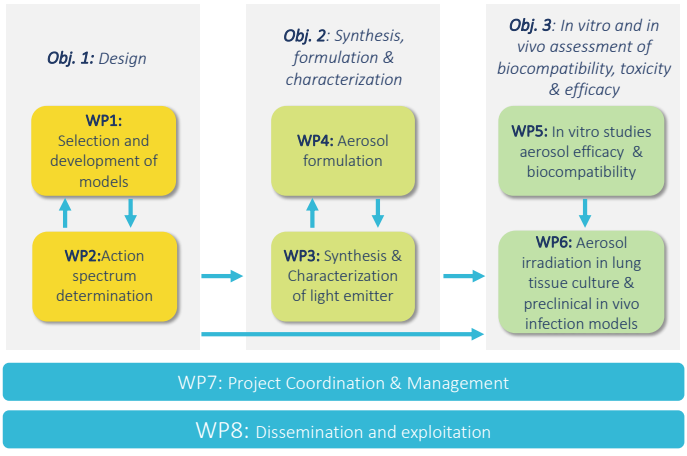
Communication activities will be implemented in accordance with the PUDPR**, including** the early development of a **project website** in which all project publishable information, related news, and educational and dissemination documents will be posted. Also, as mentioned above, it will have access to a **database** where all publishable results will be shared addressing the different profiles defined. The current **logo** will be revisited for improvement and a **brand manual** and the corresponding **templates** will be developed in order to produce all communication and dissemination materials in a consistent format. A project **leaflet** will be produced to present the project with updates whenever required. Additionally, **press releases** will be published to communicate the attainment of specific milestones and to inform about the project, with room for adapting to each partner local needs. Each partner will be encouraged to present the project in local events targeting audiences of interest, to participate in the European Researchers' Nights, and to contribute to **educational activities** like seminars, short courses and trainings addressed to students and researchers. The young researchers of the consortium will participate in the 2020 summer school of the European Society for Photobiology. The consortium will also be present at the FET annual conference exhibiting the results of the project in a stand. Special care will be taken in the communication to patients, health organisations, SMEs, and NGOs such as the European Patients' Forum (www.eu-patient.eu), the European Public Health Association (https://eupha.org) and EU-level Health Professional Organizations, with one specific **workshop** in the project second year, addressing them to present the project, its objectives, and to gather feedback on their expectations, needs and visions on the therapies. The workshop will also be open to the general public. Regular updates will be provided to participants in order to maintain engagement (as part of the engagement strategy detailed in the PUDPR). Finally, the general public will be regularly addressed via the website, the press releases and the project leaflet, but also by using **social networks**, such as Twitter, LinkedIn, YouTube or ResearchGate. The relevance of participating in other networks such as the European Enterprise Network or technology networks will also be assessed. Specific **indicators** will be used to monitor the actions including but not only: number of website visits, registered users of the database, number of publications in high-impact peer-reviewed journals, number of participants in educational activities, number of participants in the workshop, and number of followers in the social networks and other related statistics.

MEANS/TARGET GROUPS	HEALTH AUTHORITIES & HTA REGULATORS	ACADEMIA & RTD ORGS.	INDUSTRY & COMPANIES	STUDENTS	PATIENT ORGS.	GENERAL PUBLIC
PRESS RELEASE	X	X	X	X	X	X
LEAFLET	X	X	X		X	X
WEBSITE & DATABASE	X	X	X	X	X	X
SOCIAL MEDIA	X	X	X	X	X	X
EVENT	X				X	
GENERATED DATA & PROTOCOLS		X	X	X		
SCIENTIFIC PUBLICATIONS	X	X	X	X		
EDUCATIONAL ACTIVITIES		X		X		
CONFERENCES & PRESENTATIONS		X	X	X		

Figure 4. Communication matrix

3. IMPLEMENTATION

3.1. RESEARCH METHODOLOGY AND WORK PLAN



This project wants to establish a proof-of-principle for the antimicrobial efficacy of the luminous aerosol in **three** successive **steps**. **(1) definition of the treatment component properties**; **(2) development of the light-emitting particles and their aerosol formulation** and **(3) assessment of antimicrobial efficiency**. Light delivery to the target bacteria will be maximized by designing an aerosol of a specific particle size to achieve high deposition on the relevant airway walls.[23] The Light4Lungs work plan has been designed accordingly, following the development sequence of the therapies envisioned, as summarized in the PERT graph. Specific methods follow:

Action spectrum determination: The best estimate for the action spectrum of photokilling *in vivo* will be obtained by **(1) measuring** the relevant **optical properties** of endogenous bacterial PS, bacterial planktonic cultures, biofilms, and bronchial tissue; **(2)** then, the obtained data will be inserted into a specifically developed **theoretical model** of light – biofilm and light-tissue interaction to provide a first estimate of the action spectrum. **(3)** Light of the relevant wavelengths will then be used in **experimental systems** of increasing complexity (*in vitro* planktonic and sessile bacterial cultures) to measure the relative spectral efficacy for bacterial killing. This will generate the best estimate for the action spectrum, which will be crucial to the accurate design of the aerosol light-emitting properties.

Synthesis of the light-emitting particles: Light-emitting will be developed using highly luminescent doped colloids

[23] Inhalation Drug Delivery: Techniques and products, Ed. P. Colombo, D. Traini and F. Buttini, Wiley-Blackwell, 2013

i.e. $SrAl_2O_4$ or rare earth metals (europium, yttrium, erbium).[24] [25] Their light-emission capacity will be maximized optimizing their phosphorescence spectrum, quantum yield, decay lifetime and particle size, as well as minimizing self-absorption.[26] If necessary, a metallic core will be introduced to enhance the emission through plasmonic resonance. The single particles will then be embedded into **silica spheres** by the reverse microemulsion method (Figure 2) and the surface will be coated with suitable ligands to enhance biocompatibility and minimize toxicity.

Characterization of the light-emitting particles: The **emission spectra and lifetime** of the light-emitting particles and nanospheres will be characterised by optical emission spectroscopies[27] in aqueous suspensions, **bacteria** grown in planktonic phase **and biofilms.** Their ability to **photo-excite bacterial PSs** and elicit the **production of singlet oxygen** will be assessed by optical emission spectroscopies as well.

Aerosol formulation and characterization: Aerosol generation methods will be investigated in order to obtain optimum particle agglomeration, delivered light dose, aerodynamic particle size and formulation stability. These properties will then be improved by choice of pharmaceutical excipients. **Activation of the light emitting particles** will be achieved using appropriate optical sources to excite their phosphorescence either prior to being used in a dry powder inhaler or during passage through a mouthpiece or a holding chamber of the type used as "spacers" for certain pharmaceutical aerosols.

***In vitro* measurements of aerosol efficacy:** Bacterial cultures (planktonic phase and biofilms) will be **irradiated** by the luminous aerosol itself. The efficacy of light irradiation on bacterial biofilm will be measured by optical microscopy techniques. Biofilm growth will be determined over time following aerosol treatment. The real-time amount of bacterial biomass will be determined based on optical transmission measurements. Time-lapse videos will be collected followed by an image analysis procedure to quantify instantaneous biomass.

Biocompatibility / toxicity studies: The ***in vitro* toxicity/biocompatibility** of the aerosol particles will be tested in suitable **airway models** of the host respiratory tract. Monolayers of cells from different airway regions (i.e. trachea, bronchi, alveoli, etc.) will be treated in submerged conditions for quick-screening purposes. In a second step**, air-liquid interface (ALI) cultures** including various cell types of the lung will be established on microporous membranes and feeding the basal side with medium while the apical side will be in contact with the aerosol formulation to be tested. In addition, cell systems cultured at the ALI will be covered with mucus/surfactant fluids to gain more insight into their role in modulating nanoparticle cytotoxicity.[28] **Aerosol toxicity/biocompatibility** will be assessed starting with **classic cytotoxicity approaches** (MTS and LDH assays, cell cycle alterations, ROS production, interleukin and inflammation mediator release, study of cell death mechanism) and moving on to **more sophisticated evaluations**. Gene expression profile analysis will be performed by using genome-wide human oligonucleotide microarray platforms and will be validated by quantitative real-time PCR (qRT-PCR).[29] In addition, morphological and ultra-structural alterations will be evaluated using confocal as well as transmission electron microscopy. In co-culture models, particular effort will be addressed to the study of particle clearance by immune cells and of nanoparticle penetration in lung epithelial cells.

Lung models (UNILIV) - Three **clinically relevant models of bacterial lung infection** will be prepared and irradiated by the aerosol: **(i) cell model:** a novel Triple Cell Co-Culture (TCC) model that closely replicates the human epithelial airway barrier that both *P. aeruginosa* and *S. aureus* first encounter during colonisation of the respiratory tract.[30] The model consists of a layer of human-derived lung epithelial cells (A549), co-cultured with human monocyte-derived macrophages and dendritic cells.[31] **(ii) mouse model:** *S. aureus* invasive lung infection model and *P. aeruginosa* chronic lung infection model.[32] **(iii)** The **Artificial Sputum Media** model, which closely reflects the lung respiratory epithelial barrier structure and the formation of biofilm respectively. Specific measurements for *in vivo* biocompatibility of the aerosol treatment will be performed by e.g. weight and temperature measurement and full repertoire of lung immune response during treatment, including: leukocyte cellular infiltration patterns, inflammatory markers, histo-pathology, apoptosis and necrosis, tissue barrier disruption.

Sex analysis will be taken into proper consideration when performing **both *in vitro and in vivo*** experiments. Whenever possible, equal proportions of XY and XX karyotypes will be chosen in both cases.

TABLE 3.1A: LIST OF WORK PACKAGES

WP	WORK PACKAGE TITLE	LEAD PART.	LEAD PART. ACRONYM	P/M	START MONTH	END MONTH
WP1	Selection and development of models	1	IQS-URL	23	1	24
WP2	Action spectrum determination	2	UNIFI	35	3	26
WP3	Synthesis & Characterization of the light emitter	1	IQS-URL	84	5	42

[24] Castelló Serrano et al., RSC Adv., 2014, 4, 15040
[25] Ma et al., Chem. Comm., 2011, 47, 7071
[26] Castelló Serrano et al., J. Mater. Chem., 21, 2011, 17673
[27] Nonell and Flors, Singlet Oxygen: Applications in Biosciences and Nanosciences Vol. 2, The Royal Society of Chemistry, London, 2016, pp 7-26.
[28] Paur et al., J. Aerosol Sci. 2011, 42, 668
[29] Moret et al., Arch. Toxicol. 2015, 89, 607
[30] Rothen-Rutishauser et al., Am. J. Respir. Cell Mol, Biol, 2005, 32(4), 281
[31] Lehmann et al., Eur J. Pharm. Biopharm. 2011. 77(3), 398
[32] Fothergill et al., Nat. Comm. 2014, 5, 4780

WP	Work Package Title	Lead Part.	Lead Part. Acronym	P/M	Start Month	End month
WP4	Aerosol formulation	6	CSL/i2c	50	10	44
WP5	*In vitro* studies of aerosol efficacy & biocompatibility	5	UNIPD	51	10	36
WP6	Aerosol irradiation in lung tissue culture & preclinical *in vivo* infection models	7	UNILIV	62	13	48
WP7	Project Coordination & Management	1	IQS-URL	46	1	48
WP8	Dissemination & Exploitation	8	WEDO	40	1	48
	Total person- months			**391**		

TABLE 3.1B: WORK PACKAGE TABLES

WP #	1			Lead Part. Short Name		IQS-URL	
Work Package Title	Selection and development of models						
Part. No	**1**	2	3	4	5	6	7	8
Part. Short Name	**IQS-URL**	UNIFI	SU	ICIQ	UNIPD	CSL/i2c	UNILIV	WEDO
Part Person-Months	**6**	3	2	3	3	3	3	
Start Month	1			End month			24	

OBJECTIVES: WP1 aims at defining:

- The *in vitro* models (bacterial strains, lung tissue culture, mammalian cells) and relative methods for irradiation and biocompatibility/toxicity methods
- The *in vivo* preclinical models (mouse) and relative irradiation and biocompatibility/toxicity methods
- The aerosol model and the methods to obtain its required light-emission parameters (action spectrum studies) and to characterize its emission and chemo-physical properties

DESCRIPTION OF WORK: **T1.1** (**UNIFI**, SU, **UNIPD**, CSL/i2c, UNILIV): (i) to identify the *in vitro* models: a panel of reference and clinical isolates of *P. aeruginosa* and *S. aureus*; mammalian cell types for *in vitro* biocompatibility/toxicity studies; TCC and ASM models; (ii) to define the methods for *in vitro* investigation of aerosol antimicrobial activity and for *in vitro* biocompatibility/toxicity studies. **T1.2 (UNILIV**, UNIFI, UNIPD, CSL/i2c**)**: to identify the most suitable *in vivo* preclinical infection models and relative protocols for irradiation and *in vivo* biocompatibility/toxicity studies. **T1.3** (**IQS-URL**, **UNIFI**, SU, **ICIQ**, **CSL/i2c**, UNILIV): to identify (i) the required chemo-physical / optical properties of the light-emitting particles (including methods for action spectrum determination); (ii) the most suitable aerosol model, including potential vehicles and carrier systems; (iii) the methods to measure aerosol quality and chemo-physical / optical properties

DELIVERABLES: **D1.1** Report on the chosen *in vitro* models (bacteria strains, mammalian cells, TCC, ASM) and relative analysis methodologies and *in vivo* models (mouse) and relative analysis methodologies (M13); **D1.2** Report on aerosol required chemo- and photo-physical properties (including method to obtain action spectrum) (M13); **D1.3** Report on (i) chemo-/photo-physical and optical characterization methods and (ii) irradiation and biocompatibility/toxicity study protocols, *in vitro* and *in vivo* (M24)

WP #	2			Lead Part. Short Name		UNIFI	
Work Package Title	Action spectrum determination						
Part. No	1	**2**	3	4	5	6	7	8
Part. Short Name	IQS-URL	**UNIFI**	SU	ICIQ	UNIPD	CSL/i2c	UNILIV	WEDO
Part Person-Months		**20**	10				5	
Start Month	3			End month			26	

OBJECTIVES: WP2 will identify the best light emission spectrum for the aerosol particles, for optimizing its antibacterial efficacy, including:

- Determination of *in vitro* bacterial photokilling efficiency, following photo-physical characterization of the endogenous photosensitizer(s)
- The study of the optical properties of the biofilm for the chosen strains and of *ex-vivo* lung tissue samples;
- The final determination of the action spectrum for *in vivo* light photodynamic action.

DESCRIPTION OF WORK: **T2.1 (UNIFI)**: Irradiation of *in vitro* bacterial cultures, following photo-physical analysis of endogenous photosensitizers. **T2.2 (SU)**: Analysis of biofilm chemo-physical and optical properties. **T2.3 (UNIFI**, UNILIV**)**: semi-theoretical modelling of infected lung tissue optical properties, to be merged with data from T2.1 and T2.2 to give the final *in vivo* action spectrum for bacterial photokilling.

DELIVERABLES: **D2.1** Report on *in vitro* bacterial killing efficacy (M18); **D2.2** Report on optical properties of the bacterial biofilm and of *ex-vivo* lung tissue (M24); **D2.3** Report on action spectrum for *in vivo* phototherapy (M26)

WP #	3			Lead Part. Short Name			IQS-URL	
Work Package Title	Synthesis & characterization of the light emitter							
Part. No	**1**	2	3	4	5	6	7	8
Part. Short Name	**IQS-URL**	UNIFI	SU	ICIQ	UNIPD	CSL/I2C	UNILIV	WEDO
Part Person-Months	**42**			42				
Start Month	5			End month			42	

OBJECTIVES: WP3 will work for:

- The synthesis of phosphorescent particles according to requirements defined in WP1;
- The characterization of the physicochemical and photophysical properties of the particles, alone and while interacting with bacteria in planktonic phase and biofilm.

DESCRIPTION OF WORK: **T3.1 (ICIQ)**: to design, synthesize and characterize the nano-*emitter* and *sphere* composition and dimensions; **T3.2 (IQS- URL)**: to characterize the physico-chemical and light-emitting properties of the nano-*emitter/sphere*, alone and while interacting with bacteria in planktonic phase and biofilm

DELIVERABLES: **D3.1** Protocol for the synthesis and excitation of phosphorescent particles (M24); **D3.2** Report on the physico-chemical and photophysical characteristics of the particles (M36)

WP #	4			Lead Part. Short Name			CSL/I2C	
Work Package Title	Aerosol formulation							
Part. No	1	2	3	4	5	**6**	7	8
Part. Short Name	IQS-URL	UNIFI	SU	ICIQ	UNIPD	**CSL/I2C**	UNILIV	WEDO
Part Person-Months		17				**33**		
Start Month	10			End month			44	

OBJECTIVES: WP4 will:

- Define a protocol for a pharmaceutically acceptable aerosol formulation and phosphorescence excitation that preserves the aerosol stability and the particles' light-emitting properties;
- Produce the aerosol and characterise its light-emission and physico-chemical properties, also assessing their preservation upon bacteria cell and biofilm binding to the aerosol particles.

DESCRIPTION OF WORK: **T4.1 (CSL/i2c**, UNIFI): investigation of the most suitable aerosol excipients; development and adaption of pharmaceutical methods for the characterisation of the aerosol chemo-physical and light-emission properties; **T4.2 (CSL/i2c,** UNIFI): formulation of the light-emitting aerosol, following the definition of a protocol and experimental setup for aerosol phosphorescence excitation; characterization of the aerosol chemo-physical and light-emitting properties, both with the aerosol *per se* and in interaction with bacteria cells and biofilms.

DELIVERABLES: **D4.1** Report on the stability and compatibility of light-emitting particles with pharmaceutical excipients and on aerosol excitation methods (M30); **D4.2** Report about: (i) the operating procedure for aerosol formulation, generation and utilisation in *in vitro* and *in vivo* experiments; (ii) aerosol light-emission and physico-chemical properties, per se and in interaction with bacterial cells and biofilm (M44)

WP #	5			Lead Part. Short Name			UNIPD	
Work Package Title	*In vitro* studies of aerosol efficacy & biocompatibility							
Part. No	1	2	3	4	**5**	6	7	8
Part. Short Name	IQS-URL	UNIFI	SU	ICIQ	**UNIPD**	CSL/I2C	UNILIV	WEDO
Part Person-Months		13	13		**25**			
Start Month	10			End month			36	

OBJECTIVES: WP5 will

- Obtain the dose-effect curve for *in vitro* irradiation of the selected bacteria strains with the light-emitting aerosol.
- In vitro study of aerosol biocompatibility in human cells of the respiratory tract.

DESCRIPTION OF WORK: **T5.1 (UNIPD, SU,** UNIFI): measurement of the light dose- dependent antimicrobial efficacy of the aerosol *in vitro* (both planktonic phase and biofilm), following development of specific setup for irradiation with the aerosol; **T5.2 (UNIPD):** (i) investigation of the cellular uptake of the aerosol particles in co-cultures of epithelial cells, macrophages and dendritic cells, following exposure to light-activated and non-activated aerosol particles of submerged and air-liquid interface cultures (mimicking the different district of the airway apparatus); (ii) assessment of the aerosol biocompatibility/toxicity evaluated for different exposure times and doses by multiple tests (see: Methodology section).

DELIVERABLES: **D5.1** Report on the dose-effect curves for *in vitro* irradiation with the light-emitting aerosol (both for bacterial pathogens in planktonic form and biofilm) (M36); **D5.2** Report on the aerosol biocompatibility in the selected mammalian cell models (M36)

WP #	6			Lead Part. Short Name		UNILIV	
Work Package Title	Aerosol irradiation in lung tissue culture & preclinical *in vivo* infection models						
Part. No	1	2	3	4	5	6	**7**	8
Part. Short Name	IQS-URL	UNIFI	SU	ICIQ	UNIPD	CSL/I2C	**UNILIV**	WEDO
Part Person-Months	9	9			9		**35**	
Start Month	13			End month			48	

OBJECTIVES: WP6 will work to:

- Obtain the dose-effect curve for bacterial irradiation and killing with the light-emitting aerosol in the selected in vitro and *in vivo* lung infection models
- Study the in vivo (mouse) biocompatibility of the light-emitting aerosol.

DESCRIPTION OF WORK: Aerosol treatment efficacy and biocompatibility will be tested, following the definition of proper experimental setups. **T6.1 (UNILIV,** UNIPD, IQS-URL, UNIFI**)**: irradiation of the *in vitro* TCC model and ASM lung infection model and controls and measurement of the dose-effect response. **T6.2 (UNILIV,** UNIPD, IQS-URL, UNIFI**)**: irradiation of the *in vivo* acute and chronic lung infection models (mouse) + controls; measurement of the dose-effect response. **T6.3 (UNILIV):** *in vivo* (mouse) biocompatibility study of the aerosol treatment, including analysis of the possible build-up effects due to repeated treatments.

DELIVERABLES: D6.1 Report on **(i)** the *in vitro* photokilling dose-effect curve (TCC and ASM models) and bacterial CFUs at various time points post aerosol exposure; (ii) *in vivo* photokilling dose-effect curve (acute and chronic lung infection models-mouse), bacterial CFUs in time post aerosol exposure, host survival and immune responses (M24); **D6.2** Report on *in vivo* biocompatibility of the aerosol treatment (M48)

WP #	7			Lead Part. Short Name		IQS-URL	
Work Package Title	Project coordination and management						
Part. No	**1**	2	3	4	5	6	7	8
Part. Short Name	**IQS-URL**	UNIFI	SU	ICIQ	UNIPD	CSL/I2C	UNILIV	WEDO
Part Person-Months	**23**							23
Start Month	1			End month			48	

OBJECTIVES:

- To provide the overall scientific direction and drive the progress of the project, steering efforts towars the project's objectives and milestones completion.
- To set-up a project management structure that ensures an efficient operational management including administrative, financial and legal issues, and appropriate liaison with the European Commission.
- To provide procedures and tools for ensuring that all results are delivered on time, with an adequate quality level and within cost, including quality control procedures on deliverables.
- To enable the appropriate communication and team work dynamics for a successful project completion.

DESCRIPTION OF WORK: T7.1 Day to day coordination and management (**IQS,** WeDo) including: liaison with the EC, work plan control and update, schedule control and implementation of corrective actions, timely delivery of project outcomes, decision making, conflict resolution and consensus building, meeting organization and minutes production and distribution, and development of communication tools (mailing lists, knowledge management systems); **T7.2** Reporting, financial and legal management (including partnership management, Grant Agreement and Consortium Agreement implementation and amendment); **T7.3** Quality assurance of all project outcomes and deliverables, and risk management (identification, assessment, registry and follow-up, contingency and mitigation plans); **T7.4** Ethical surveillance.

DELIVERABLES: D7.1 Project handbook – reference manual summarizing key provisions of the Grant Agreement and the Consortium Agreement in lay terms (M2) **D7.2** Data Management Plan (M6)

WP #	8			Lead Part. Short Name		WEDO	
Work Package Title	Dissemination & exploitation						
Part. No	1	2	3	4	5	6	7	**8**
Part. Short Name	IQS-URL	UNIFI	SU	ICIQ	UNIPD	CSL/I2C	UNILIV	**WEDO**
Part Person-Months	5	3	3	3	3	5	3	**15**
Start Month	1			End month			48	

OBJECTIVES: WP8 aims to:

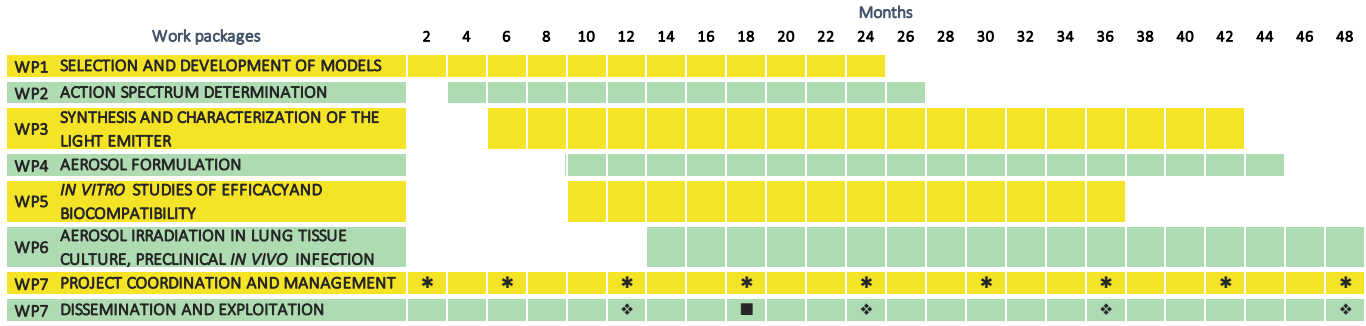
- Design a plan for the optimal communication within and outside the project, and the dissemination of project results and knowledge generated to all relevant stakeholders and the general public
- Design and produce all communication tools required to implement the plan
- Undertake extensive dissemination activities according to plan, both at the European and the local levels
- Devise sustainable models to ensure the continuation of the therapies' development towards the market

DESCRIPTION OF WORK: T8.1 (WeDo, all partners) Development of the PUDPR, which will outline and describe the project strategy for the communication and dissemination of the project and its results, identifying target

audiences, defining suitable means and tools in each case, establishing appropriate actions (including trainings and educational activities), and describing the open access policy. **T8.2 (WeDo)** Development of the dissemination and communication tools, focused on the refinement of the logo, the development of the brand manual and the templates derived from it (for presentations, deliverables, posters, etc), of a project website, of the project leaflet and press releases, as well as any other required tool, and the set up and maintenance of the project profiles in the social networks of interest. **T8.3 (WeDo**, all partners) Implementation of the dissemination and outreach actions with a starting point on the PUDPR, defined to target and engage identified audiences; including but not only the publication in high-impact peer-reviewed journals; the presentation at conferences, events, and meetings; the participation in and/or the organization of educational and training activities (lectures, summer schools); and the organization of a workshop addressed to patient and professional organizations, SMEs and the general public in the second year. **T8.4 (WeDo**, IQS-URL, UNIFI, CSL/i2c, and other partners) Exploitation and long-term sustainability activities, including the strategic planning, ownership and exploitation interests, the patentability assessment, regular technology and market watches, the development of a licencing package, and the engagement with interested pharma and device companies.

DELIVERABLES: D8.1 Website Launch (M3); **D8.2** Plan for the Use and Dissemination of the Project Results v1 (M5); **D8.3** Plan for the Use and Dissemination of the Project Results v2 (M48)

LIGHT4LUNGS GANTT



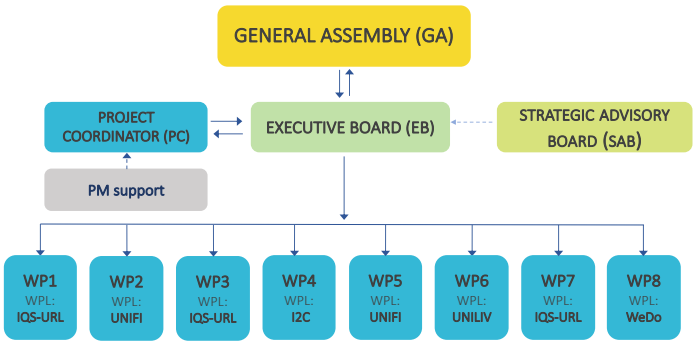
✱ General Assembly Meetings (GAM) | ❖ Outreach event | ■ Workshops

TABLE 3.1C: LIST OF DELIVERABLES (BY DUE DATE)

DEL NO	DELIVERABLE NAME	WP NO	LEAD PART. SHORT NAME	TYPE*	DISS LEVEL#	DUE
D7.1	Project handbook	7	WEDO	R	CO	M2
D8.1	Website Launch	8	WEDO	DEC	PU	M3
D8.2	Plan for the Use and Dissemination of the Project Results v1	8	WEDO	R	CO	M5
D7.2	Data Management Plan	7	WEDO	R	CO	M6
D1.1	Report on the chosen *in vitro* models (bacteria strains, mammalian cells, TCC, ASM) and *in vivo* models (mouse) and relative analysis methodologies	1	IQS-URL	R	CO	M13
D1.2	Report on aerosol required chemo- and photo-physical properties (+ method to obtain action spectrum)	1	IQS-URL	R	CO	M13
D2.1	Report on *in vitro* bacterial photokilling efficiency	2	UNIFI	R	CO	M18
D1.3	Report on **(i)** chemo-/photo-physical and optical characterization methods; **(ii)** irradiation and toxicity/ biocompatibility study protocols, *in vitro* and *in vivo*	1	IQS-URL	R	CO	M24
D2.2	Report on optical properties of the bacterial biofilm and of *ex-vivo* lung tissue	2	UNIFI	R	CO	M24
D3.1	Protocol for synthesis and excitation of phosphorescent particles	3	IQS-URL	R	CO	M24
D2.3	Report on action spectrum for *in vivo* phototherapy	2	UNIFI	R	CO	M26
D4.1	Report on the stability and compatibility of light-emitting particles with pharmaceutical excipients and on aerosol excitation methods	4	CSL/I2C	R	CO	M30
D6.1	Report on **(i)** *in vitro* photokilling dose-effect curve (TCC and ASM models) and bacterial CFUs at various time points post aerosol exposure; **(ii)** *in vivo* photokilling dose-effect curve (acute and chronic lung infection models-mouse), post aerosol exposure bacterial CFUs, host survival and immune responses	6	UNILIV	R	CO	M32
D3.2	Report on the physico-chemical and photophysical characteristics of the particles	3	IQS-URL	R	CO	M36

DEL NO	DELIVERABLE NAME	WP NO	LEAD PART. SHORT NAME	TYPE*	DISS LEVEL#	DUE
D5.1	Report on the dose-effect curves for *in vitro* irradiation with the light-emitting aerosol (both for bacterial pathogens in planktonic form and biofilm)	5	UNIPD	R	CO	M36
D5.2	Report on the aerosol biocompatibility in the selected mammalian cell models	5	UNIPD	R	CO	M36
D4.2	Report about: **(i)** operating procedure for aerosol formulation, generation and utilisation in *in vitro* and *in vivo* experiments; **(ii)** aerosol light-emission and chemo-physical properties, per se and in interaction with bacterial cells and biofilm	4	CSL/I2C	R	CO	M44
D6.2	Report on *in vivo* biocompatibility of the aerosol treatment	6	UNILIV	R	CO	M48
D8.3	Plan for the Use and Dissemination of the Project Results v2	8	WEDO	R	CO	M48

3.2. MANAGEMENT STRUCTURE, MILESTONES AND PROCEDURES



Light4Lungs has an adapted simplified management structure to warrantee the strategic direction and coordination required to attain its ambitious goal within time and resources, whilst avoiding unnecessary burdens on the administrative and financial fronts. Includes: **(1) General Assembly (GA)** (with one senior representative per partner with capacity to represent their organizations and authority to make decisions, chaired by the Project Coordinator): is the ultimate decision-making body of the project, monitoring progress, ensuring attainment of milestones and approving deliverables, and dealing with critical issues affecting the project such as amendments of the work plan or the associated effort/budget allocation, or changes in partnership. The GA will have a face-to-face meeting every 6 months, requiring 2/3 of attendees for quorum. Each partner will have one vote and simple majority will suffice for decision adoption. In case of a tied vote the Chair will have an additional vote. **(2)** The **Executive Board (EB)** (formed by the 5 WP Leaders and chaired by the Project Coordinator) is the operative body in charge of the day-to-day progress monitoring as reported by the WP Leaders, dealing with minor work amendments and changes at the WP level, with capacity to require any WP Leader to undertake actions to resolve arising matters at the WP level, and undertaking regular risk assessments and strategic analysis. Monthly teleconferences will be organized, with 2/3 of attendees required for quorum. Each partner will have one vote and simple majority will suffice for decision adoption. In case of a tied vote the Chair will have an additional vote. **(3) IQS-URL (Prof Santi Nonell)** will be the **Project Coordinator (PC)**, responsible for the project interface with the Commission as well as with other projects and initiatives, and the overall scientific leadership of Light4Lungs. He will be providing guidance, coordinating efforts, contributing to an efficient conflict resolution, facilitating communication, enabling decision-making processes and consensus building. The PC will drive risk management procedures, and -with the support of the **Project Manager** (PM)- manage the quality control procedures for all project deliverables, ensuring its timely submission. Also, with the support of the PM, will be responsible of the financial management (budget management, payments control, and justifications) and assist the GA in any budget rearrangements. Finally, with the assistance of the PM, the PC will be leading the meetings organizations and the minutes generation and distribution. **(4)** Each WP will have a **WP Leader (WPL)**, responsible for the day-to-day management and coordination of the activities included; working towards the satisfactory resolution of conflicts and arising problems, the timely submission of deliverables, the early identification and management of arising risks, the progress reporting and the proposal of roles, efforts and/or budget redistributions within their WPs to better respond to upcoming needs. Regular WP teleconferences will be encouraged in order to maintain coordination, effectively steer efforts and ensure communication. **(5)** Finally, Light4Lungs will have a **Scientific Advisory Board (SAB)**, a consultative body formed by a pool of independent experts who will provide additional expertise and know-how to assist the Consortium in its strategic decisions. The SAB will attend one of the GA annual meetings for this purpose and all members will sign a non-disclosure agreement to ensure confidentiality. Invited SAB members are: **Prof Stuart Elborn** and **Prof Michael Tunney** (Queen's University of Belfast, UK) ***clinical experts in lung infections and the use of inhaled antibiotic treatments;*** and **Prof Michael R. Hamblin** (Wellman Center of Photomedicine, Massachusetts General Hospital, Harvard Medical School) and **Dr Kristjan Plaetzer** (Universität Salzburg) ***experts in antimicrobial photodynamic therapy.***

TABLE 3.2A: LIST OF MILESTONES

MIL NO	MILESTONE NAME	RELATED WPS	DUE MONTH	MEANS OF VERIFICATION
Mi1	Definition of models	WP1	M13	D1.1

MIL NO	MILESTONE NAME	RELATED WPS	DUE MONTH	MEANS OF VERIFICATION
Mi2	Definition of action spectrum	WP2	M26	D2.3
Mi3	Luminous aerosol stability assessed	WP4	M30	D4.1
Mi4	Aerosol efficacy evaluated in the *in vitro* lung infection models	WP6	M32	D6.1
Mi5	Phosphorescent particles synthesised and physico-chemical and photophysical characteristics assessed	WP3	M36	D3.1, D3.2
Mi6	Completion of the *in vitro* biocompatibility assessment and efficacy evaluation	WP5	M36	D5.1, D5.2
Mi7	Completion of the *in vivo* biocompatibility assessment and efficacy assessment	WP6	M48	D6.2
Mi8	Successful completion of the project	All	M48	All Dels

TABLE 3.2B: CRITICAL RISKS FOR IMPLEMENTATION

RISK PROB	RISK DESCRIPTION	WPS	RISK MITIGATION MEASURES
HIGH	**R1:** Insufficient quality of the light emitting particles in terms of spectrum, duration and intensity	WP1 WP2 WP3	**(1)** Modify the particle composition and dimensions; **(2)** Increase the number of particles in the nanosphere; **(3)** include a metal core to enhance the emission by plasmonic effects; (**4**) modify the particle surface with electroactive ligands
HIGH	**R2:** too low photon emission per inhaled volume of aerosol	WP4	**(1)** increase the photon emission per nano-*emitter* and/or nano-*sphere* (see previous points for WP3). Protocol modifications for: **(2)** aerosol synthesis, to increase the number of aerosol particles / dm^3; **(3)** external aerosol excitation timing and dosage
MEDIUM	**R3:** non-satisfactory *in vitro* bacterial photo-inactivation (e.g. high strain-dependent effect, low antibiofilm activity)	WP2 WP5	**(1)** see measures for R2; **(2)** modification of irradiation protocol; **(3)** addition of exogenous PS to the particle surface (see 1.4 point 2)
LOW	**R4:** non-negligible aerosol toxicity (*in vitro*)	WP5	**(1)** re-formulation of particle shielding; **(2)** modification of aerosol formulation (e.g. excipients); **(3)** modification of irradiation protocol timing and dosage
MEDIUM	**R5:** low aerosol efficacy (lung tissue culture models)	WP6	See mitigation measures for R3
LOW	**R6:** high *in vivo* aerosol toxicity (mouse model)	WP6	Modification of aerosol: **(1)** particle shielding; **(2)** formulation; **(3)** irradiation timing / fractionation. **(4)** reduction of particle dimensions to favour exhalation
MEDIUM	**R7:** low *in vivo* aerosol efficacy (mouse model)	WP6	**(1)** addition of exogenous PS to the particle surface (see 1.4 point 2); **(2)** modification of irradiation protocol timing and dosage

3.3. CONSORTIUM AS A WHOLE

The Consortium responds perfectly to the requirements of this project, being composed of complementary partners, with pre-existing collaborations ranging from 2 to 10 years, that fit all the necessary steps to achieve the final proof of concept for the proposed photo-therapy of lung infections with a luminous aerosol in preclinical models. (1) The study of the theoretical and experimental action spectrum for light therapy and the best light emission characteristics needed will be performed by **UNIFI**. (2) The modelling, synthesizing and study of light-emitter "particle" will be performed by the **ICIQ**, expert in material science, and **IQS-URL**, expert in photochemical/photophysical processes and singlet oxygen. (3) The formulation of suitable aerosols for *in vitro* and preclinical studies out of those particles will be performed by **CSL/i2c,** acting in the field of innovative aerosol-driven drug-delivery solutions. (4) Testing of the aerosol biocompatibility will be performed by **UNIPD**, expert in toxicology and drug delivery, and *in vitro* aerosol efficacy will be performed by **UNIFI**, expert in recalcitrant and antibiotic resistant infections. (5) Testing of the aerosol in biofilms will be performed by **SU**, expert in biofilm response studied upon external chemo-physical stimuli. (6) Testing of the aerosol efficacy in infected preclinical models will be done by **UNILIV**, expert in aerosol-driven drug-delivery and drug efficacy studies in different lung infected models including preclinical studies. (7) **WeDo** will bring in the expertise required to assist the Project Coordinator in its managerial duties in the project, but mainly to develop and deploy the PUDPR, for an effective dissemination but most importantly for identifying protection and future strategies to get the therapies to the market. The presence of the 2 SME (CSL/i2c, WeDo) gives added value in terms of both the technological development of the new aerosolised light source and therapeutic schemes and of a broad vision towards their future exploitation.

3.4. RESOURCES TO BE COMMITTED

TABLE 3.4A: SUMMARY OF STAFF EFFORT

	WP1	WP2	WP3	WP4	WP5	WP6	WP7	WP8	TOTAL
IQS-URL	**6**		**42**			9	**23**	5	**85**
UNIFI	3	**20**		17	13	9		3	**65**

	WP1	WP2	WP3	WP4	WP5	WP6	WP7	WP8	TOTAL
SU	2	10			13			3	**28**
ICIQ	3		42					3	**48**
UNIPD	3				**25**	9		3	**40**
CSL/I2C	3			**33**				5	**41**
UNILIV	3	5				**35**		3	**46**
WEDO							23	**15**	**38**
TOTAL	**23**	**35**	**84**	**50**	**51**	**62**	**46**	**40**	**391**

TABLE 3.4B: OTHER DIRECT COST TABLES

IQS-URL	COST (€)	JUSTIFICATION
TRAVEL	12.000,00	Annual 3.000 euro to attend to project related meetings (GAM, workshop, conferences, events, etc)
OTHER GOODS AND SERVICES	60.000,00 10.000,00 10.000,00 16.000,00 3.000,00	Reagents IP protection Open Access Publications Estimated to cover SAB members contributions/travels Audit certificate
TOTAL	**111.000,00**	

UNIFI	COST (€)	JUSTIFICATION
TRAVEL	12.000,00	Annual 3.000 euro to attend to project related meetings
OTHER GOODS AND SERVICES	95.000,00 10.000,00 10.000,00 3.000,00	Reagents IP protection Open Access Publications Audit certificate
TOTAL	**130.000,00**	

SU	COST (€)	JUSTIFICATION
TRAVEL	12.000,00	Annual 3.000 euro to attend to project related meetings
OTHER GOODS AND SERVICES	65.000,00 10.000,00	Reagents Open Access Publications
TOTAL	**87.000,00**	

ICIQ	COST (€)	JUSTIFICATION
TRAVEL	12.000,00	Annual 3.000 euro to attend to project related meetings
OTHER GOODS AND SERVICES	60.000,00 10.000,00	Reagents Open Access Publications
TOTAL	**82.000,00**	

UNIPD	COST (€)	JUSTIFICATION
TRAVEL	12.000,00	Annual 3.000 euro to attend to project related meetings
OTHER GOODS AND SERVICES	126.000,00 10.000,00	Reagents Open Access Publications
TOTAL	**148.000,00**	

CSL/I2C	COST (€)	JUSTIFICATION
TRAVEL	12.000,00	Annual 3.000 euro to attend to project related meetings
OTHER GOODS AND SERVICES	22.000,00 10.000,00	Reagents Open Access Publications
TOTAL	**44.000,00**	

UNILIV	COST (€)	JUSTIFICATION
TRAVEL	12.000,00	Annual 3.000 euro to attend to project related meetings
OTHER GOODS AND SERVICES	194.000,00 10.000,00 3.000,00	Reagents Open Access Publications Audit certificate
TOTAL	**219.000,00**	

WEDO	COST (€)	JUSTIFICATION
TRAVEL	12.000,00	Annual 3.000 euro to attend to project related meetings
OTHER GOODS AND SERVICES	10.000,00 10.000,00 10.000,00	Patentability analysis Workshop organization Dissemination tools (website, brochures, newsletters,)
TOTAL	**42.000,00**	