PNEUMONP

FINAL REPORT

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Publishable Summary

1. Executive Summary

The main objective of **PneumoNP** (*Nanotherapeutics to Treat Antibiotic Resistant Gram-Negative Pneumonia Infections*) was the development of **a "theragnostic" (therapeutic + diagnostic) system** for the treatment of lung Gram-negative bacterial infections, i.e. a system capable to help both in **treating** and in **diagnosing bacterial infections**. The number of antibiotic resistant bacteria strains is rapidly increasing, thus new types of therapy are urgently required to avoid the use of standard antibiotics. As a **proof of concept**, PneumoNP focussed on infections caused by the bacterium *Klebsiella pneumoniae*. A **diagnostic kit** was developed to enable a rapid and precise identification of the bacteria strain causing the infection and avoid the use of wide spectrum antibiotics. In addition, the diagnostic kit allows monitoring the efficacy and efficiency of the therapy. For the treatment, two main **nanotherapeutic-based inhalable antibiotics** were developed. The therapeutic nanosystems consist of a nanocarrier (polymer nanoparticles or micelles) combined with an antimicrobial peptide (AMP). An **aerosol system prototype** was also developed to improve the antibiotic delivery to the lungs.

In an effort to promote collaborative research for bringing new antimicrobials to patients and to disseminate the results to the general audience and the industry, PneumoNP has been part of a **cluster with 2 other consortiums** working on antimicrobial resistance: FORMAMP (Innovative Nanoformulation of Antimicrobial Peptides to Treat Bacterial Infectious Diseases) and NAREB (Nanotherapeutics for antibiotic resistant emerging bacterial pathogens).

2. Summary description of project context and objectives

Antimicrobial resistance (AMR) is a global public health concern and the EU Commission considers the development of new effective antimicrobials or alternatives for treatment a current priority. The number of **antibiotic resistant bacteria** strains is increasing and **new types of antibiotic-based therapy** are urgently required. Gram-negative bacteria that cause pneumonia are one of the main sources of nosocomial infections, mainly in people with a weakened immune system. Apart from pneumonia, they can cause bacteraemia and other infections. **Early detection** of the infection source combined with the development of **effective therapies** to treat **multi-drug resistant (MDR) bacteria** caused infections will definitely radically improve the healing process of patients and avoid complications in the hospital.

In this context, the use of **nanomaterials**, i.e. chemical systems that are 10,000 times smaller than the diameter of a human hair and that show special properties due to their size, can assist to find innovative solutions in the development of new diagnostic systems and novel efficient therapies against diseases. The medical application of nanomaterials is part of the so-called "nanomedicine" and it is expected to gain importance to address biomedical problems. Currently, several nanopharmaceuticals are available on the market, especially for cancer treatment. However the full range of possibilities opened up by the use of nanoparticles in healthcare systems has not yet been fully realized. The nanoparticles can be manipulated to generate enormously versatile drug-carrying systems with the following characteristics:

i) Targeted delivery of drugs to their specific site of action in the human body, decreasing the risk of side-effects and increasing the therapeutic effect;

ii) Improved solubility and bioavailability of the drug;

iii) Facilitation of drug internalization in infected cells;

iv) Simultaneous loading of different drugs and imaging agents to combine synergetic therapy with diagnostic accuracy.

PneumoNP aimed at the **development of a theragnostic system for the treatment of resistant Gram-negative bacteria infections of the lung**, with a focus on *Klebsiella pneumonia*-caused infections.

The **main objectives** of PneumoNP were:

1) Development of a new **nanotherapy *via* inhalation**:

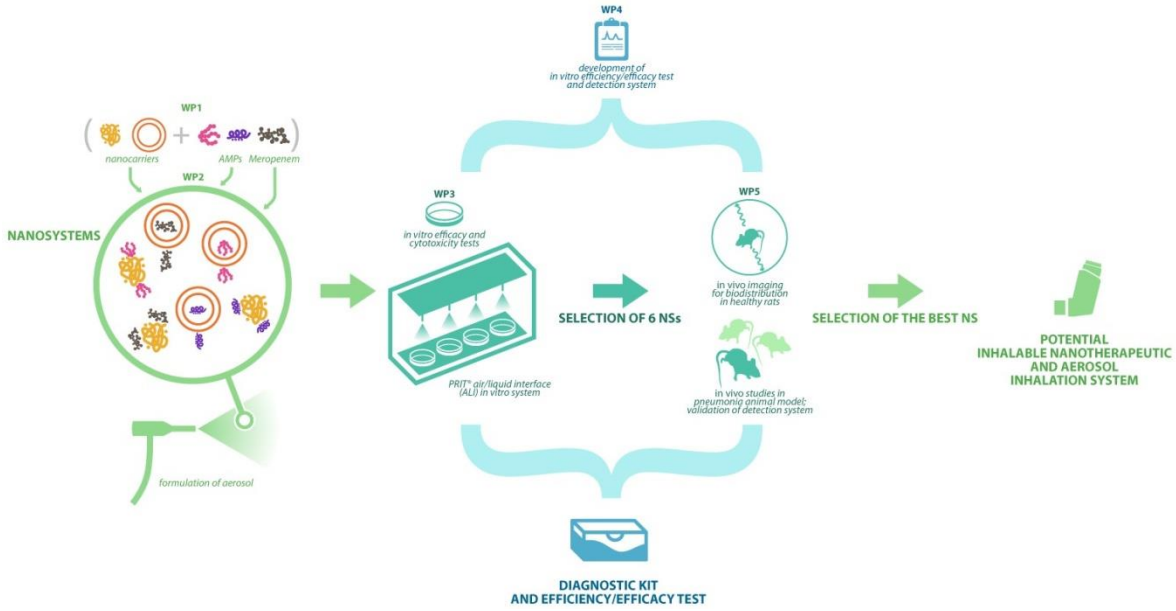
For the treatment a nanotherapeutic-based inhalable antibiotic has been developed. The therapeutic nanosystem is based on a **nanocarrier (NC)** combined with an **antimicrobial peptide (AMP)**. Three different types of NCs were tested with two AMPs to obtain a novel effective inhalable antimicrobial **nanosystem (NS)**. Nanotherapeutics offers many advantages in pulmonary drug-delivery, due to the huge surface area available in the lungs and their potential to achieve uniform distribution of drug dose among the alveoli. To improve this delivery to the lungs, an **aerosol system** was also developed.

2) Development of a **diagnostic kit** and an efficiency-efficacy test:

A diagnostic kit was developed (a) to enable a rapid and precise identification of the bacteria strain causing the infection and to avoid the use of wide spectrum antibiotics and (b) to monitor the efficacy and efficiency of the therapy.

In principle, this treatment could then be applied to any Gram-negative lung bacterial infection.

PneumoNP consisted of five technical Work Packages (WPs) and three cross-functional WPs.



WP1 focused on the preparation and characterization of **antimicrobial peptides (AMPs)** as therapeutic agents and of novel **nanocarriers (NCs)** based on polymeric nanoparticles (PNPs) or liposomes/micelles, for the delivery of AMPs. The main objectives of this work package were:

- Development of NCs based on 2 different families (polymeric NPs and liposomes/micelles) for the delivery of AMPs, choosing the most optimal ones in terms of stability, biocompatibility, cytotoxicity, delivery and targeting properties (see WP2).
- Labelling of the nanovehicles for *in vitro* and *in vivo* studies.
- Optimization of the synthetic process for scale-up progression to good manufacturing practice (GMP) production (1-5 g).
- Generation of the 2 types of AMPs at a lab scale and optimization for scaling-up around 100 mg for future production under GMP.

WP2 focused on the generation of **nanosystems (NSs)** combining a nanocarrier (NC) with a therapeutic agent such as an AMP, as well as formulation of the NSs for aerosol inhalation through an **aerosol prototype**. The main objectives of this work package were:

- Design and development of synthetic strategies for the coupling of NCs with AMPs.
- Evaluation of stability, availability and loading rations of the NSs.

- Optimization of generation of NSs in a 0.5 g scale towards good manufacturing practice (GMP) production.
- Formulation of NSs for aerosol applications and production of an aerosol prototype.

WP3 concentrated on *in vitro* studies to evaluate the antibacterial efficiency of NSs in different *Klebsiella pneumoniae* strains up to a pulmonary model. The main objectives of this work package were:

- Collection of diverse *K. pneumoniae* isolates in different European regions for identification of resistance markers and evaluation of activity of AMPs and clinical control API Meropenem towards these isolates.
- Selection of 5/6 combined NSs for *in vivo* studies based on *in vitro* efficacy and cytotoxicity studies.
- Evaluation of the combined NSs in the P.R.I.T® Air/Liquid Interface (ALI) culturing and exposure system under good laboratory practice (GLP) guidance.
- Genotoxicity testing for the final selected NS as an approval requirement under GLP compliance.

WP4 was dedicated to the development of a diagnostic system and an efficiency/efficacy test. The main objectives of this work package were:

- Development of a fast and quantitative real-time polymerase chain reaction (RT-PCR) test using Viable PCR to discriminate between live and dead bacteria to find out the efficacy/efficiency of the NSs.
- Detection of approximately 25 relevant resistance markers present in *K. pneumoniae* strains using the SMARTFinder-based technology to develop a new diagnostic kit.

WP5 consisted in evaluating the selected NSs *in vivo* (studies in healthy rats and proof of concept studies in infected rats). The main objectives of this work package were:

- Biodistribution/pharmacokinetic studies with optimized radiolabelled NCs, AMPs and combined NSs after inhalation in healthy rats using nuclear imaging.
- Initial screening for toxicity and bactericidal activity of selected NSs in *K. pneumoniae*-ESBL rat Pneumonia and further studies in the same model for the 2 most promising NSs.
- Final *in vivo* studies with the best NSs administered by inhalation in *K. pneumoniae*-ESBL rat Pneumonia.
- Establishment of a new model of rat Pneumonia with *K. pneumoniae*-KPC strain and intratracheal instillation for therapeutic and toxicity studies with the best antimicrobial NS

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3. Description of main S&T results/foregrounds

PneumoNP has 8 work packages. The technical work mainly focussed on the generation of AMPs, NCs and NSs (WP1 and WP2), *in vitro* toxicity and efficacy studies (WP3) and *in vivo* experiments (WP5), as well as preclinical validation of the multiplex PCR kit (WP4). The scientific and technological results of PneumoNP were generated in these technical WPs. The entire technical and scientific work in the project was supported by horizontal activities covered in WPs 6-8 (project management, dissemination/exploitation, scientific coordination).

The most important scientific and technological results can be summarized as follows:

a. A new diagnostic kit will be available in the market in the next months for rapid identification of bacterial strains that will help medical doctors within 3.5 hours selecting the best antibiotic for an effective treatment of bacteria.
b. Two promising AMPs were brought to preclinical trials and emergence of resistance to the new antibiotics was not detected.
c. The efficacy of nanotechnology at improving the stability and performance of active ingredients *in vivo* has been demonstrated. Importantly, the nanosystems used in the project are able to reduce toxicity to the active ingredients.
d. The project has also been pushing the development of manufacturing processes that will be translatable and scalable for future production. This will be crucial to include these new systems in the pharmaceutical sector, which is highly regulated and with very rigid quality controls. This is true for the nanosystems but also for the antimicrobial peptides that have been scaled-up.
e. At the end of the project two nanosystems that combine micelles and polymer nanoparticles with antimicrobial peptides have demonstrated to be the most promising systems for further development.

Main scientific and technological results related to PneumoNP WP by WP

WP1 – Preparation and characterization of NCs and synthesis of AMPs

Two **AMPs** were successfully developed by partners SetLance (SET) and Adenium (ADE). These AMPs have standard synthetic approaches in **automated synthesizer** using **solid phase synthesis** and both synthetic processes have been established, together with the methodology for characterization. Peptides already came from a selection of **stability and workability**. The solid-phase synthesis of these AMPs was set up at a lab scale and then optimized at large scale for future production under **Good Manufacturing Practice (GMP)**.

Regarding NCs, two types of **polymeric-based NCs** were generated by partner CIDETEC (CID). In addition, **liposomes and micelles** of different sizes and lipid compositions were prepared by Utrecht University (UU). All the NCs were characterized by different techniques to fulfil the requirements of **quality control** established at the beginning of the project. The NCs were prepared fulfilling the specifications of selected parameters such as morphology, physicochemical properties like size and charge, and stealth components to avoid immune response and non-specific adsorption of proteins. Optimization of the synthetic processes for scale-up progression to GMP production (gram scale) was also achieved.

The optimization of the synthetic process of NCs and AMPs taking into account a potential good manufacturing practice (GMP) scale-up process was a key aspect of the project. In fact, one of the great limitations for the **translation of new pharmaceutical products** from

research laboratories to the clinic is that these products are not designed in such a way that, since the beginning, specific quality guarantees are ensured. PneumoNP project covers the nanomedicine value chain from basic research (non-regulated environment) to preclinical (good laboratory practice), but a big effort has been dedicated to think in perspective to translation, trying to have in mind the requirements of clinical studies (good clinical practice) and of the market. Thus, a key result of PneumoNP is that the nanocarriers and the AMPs developed during the project, but also the nanotherapeutics (see WP2), have been generated in gram scale in a **controlled and reproducible way** for future production under GMP compliance.

Selected NCs were also functionalized with **fluorescent dyes** for *in vitro* studies and with **chelating agents** for further radio-labelling and *in vivo* studies (see WP5) at CIC biomaGUNE (CIC) where the procedures for the incorporation of radionuclides have been successfully implemented. Biocompatibility, cytotoxicity and genotoxicity studies of NCs were performed at the installations of partner Fraunhofer ITEM (see WP3). Furthermore, the NCs were designed to fulfil aerosol quality requirements and specifications for a proper use in inhalation and these experiments were performed by partner Ingeniatrics (INGEN).

WP2 – Production of antimicrobial NSs (NCs + APIs) and their formulation for inhalation

One of the main objectives of WP2 was the **development of synthetic strategies for the coupling of NCs with AMPs** in order to obtain stable and efficient nanosystems (NS). The polymeric nanoparticles were successfully loaded with AMPs using three different chemical strategies. Regarding liposomes, passive and remote loading strategies were used. Studies of stability, characteristics and loading ratios of the polymeric and liposomal NSs were successfully performed in **aqueous media**. Overall, a key result of WP2 was the demonstration that the AMPs could be loaded in **high amount and in a controlled way** into the NCs.

The **selection** of the final NSs to be tested *in vivo* was carried out on the basis of the evaluation of stability, availability and loading ratios of the NSs (WP2) together with *in vitro* toxicity and efficiency tests (WP3) and *in vivo* experiments (WP5).

Formulations of NSs were generated in such a way that they are **suitable for aerosol applications**. In addition, optimization of generation of NSs in > 0.5 g scale towards **GMP production** was achieved, in a similar way as discussed for NCs (see WP1). High loading capacity was achieved in selected systems. This is crucial in the case of antibiotics requiring high concentrations *in vivo*. This is not the case for oncologic therapies, for example.

Another key result was the development of an **aerosol prototype system** by INGEN. This system was fully engineered during the PneumoNP project and tested *in vivo*. The system

includes a **nebulisation system** and a **mist chamber**, for the delivery of NSs to lungs by inhalation both in biodistribution assays with healthy animals and in therapeutic/toxic tests with infected rats. The **construction and validation** of the different components were carried out by INGEN also with the help of CIC who optimized the parameters by using radiolabeled material. Nebulisation in the presence and absence of NCs was successfully performed: the mean drop sizes during nebulisation did not change significantly at the concentration tested. The final prototype was also accomplished with **safety cabinet requirements** for the *in vivo* tests with infected animals.

In spite of the fact that it was accomplished with the safety cabinet requirements and generates an aerosol mist with the proper characteristics, the administration efficiency of the drugs was not enough to reach the required antibiotic and NS doses *in vivo* (rats). The main reasons were not only imputable to the aerosol system (for example the big chamber), but also to other factors such as the fact that the rats were anesthetized, which resulted in low rat breath and limited time to drug exposure. These latter issues can be solved in the future by using animals trained to squeeze into special rooms (such as the ones at ITEM) or, in case of use in human patients, reducing the mist chamber size and removing the animal holders which obviously are not needed in humans.

WP3 – In vitro studies up to a pulmonary model

Fifty geographically diverse *K. pneumoniae* isolates from individual patients recovered from a variety of clinical samples have been completed. Antibiotics from various classes including Meropenem (MEM) were tested with six *K. pneumoniae* strains to check the antimicrobial drug susceptibility by minimum inhibitory concentration (MIC) and time-kill kinetics (TKK) assays. The AMPs developed in the project showed promising activity towards *Klebsiella pneumoniae* strains.

Model cells were used at ITEM to check whether the NCs and NSs were toxic. None of the tested compounds showed a cytotoxic effect under the applied test conditions. Importantly, *in vitro* efficiency tests were performed determining the MIC of 16 NSs, which allowed a first selection of 9 potential candidates (3 NSs for each antibacterial molecule). The candidates were further reduced to 5 NSs on the basis of TKK assays (WP3) and chemical stability/reproducibility studies (WP2). Screening of the 5 NSs with *in vitro* genotoxicity studies was also carried out through a mutagenicity testing, mouse lymphoma TK mutation assay, OECD 490. Genotoxicity testing for the final selected NS under GLP compliance was also performed.

A cell-based *in vitro* model including Air/Liquid Interface (ALI) exposure of human lung cells was also set up by ITEM, and the selected NSs were evaluated under GLP guidance in the so-called “P.R.I.T.® Air/Liquid Interface (ALI) culturing and exposure system”.

As a main conclusion of this WP, in the case of selected NSs, the nanocore did not reduce *in vitro* efficacy of the antibiotics, but reduced *in vitro* toxicity of the antibiotics. None of the selected systems showed mutagenicity.

WP4 – Development of a diagnostic system and efficiency/efficacy test

A prototype multiplex assay for *K. pneumoniae* resistance markers was developed. It is able to currently detect more than 30 proposed resistance markers present in *K. pneumoniae* strains using the 2SMARTFinder-based technology developed by partner Pathofinder (PAT). The ResistanceFinder 2SMART can detect 32 targets and an Internal Control within three hours, whereas conventional methods take up to 48 hours. The final composition of the marketable diagnostic assay has been established and the release onto market of the diagnostic assay is expected in Q3 2018.

WP5 – In vivo biodistribution/pharmacokinetic studies in healthy rates and proof of concept studies in infected rats

Result 1:

Nanocarriers (including liposomes, micelle-type NPs and PNPs), as well as antimicrobial peptides have been successfully **(radio)labeled** with positron or gamma emitters (radionuclids). In this way, all the chemical systems could be followed *in vivo* by advanced imaging techniques based on **nuclear imaging**, such as for example SPECT-CT. *In vivo* **biodistribution/pharmacokinetic studies** of radiolabeled AMPs, NCs and NSs were performed in healthy rats by SPECT after **intratracheal instillation and aerosol inhalation**. The biodistribution studies after intratracheal nebulisation performed with different imaging modalities in combination with complementary techniques have demonstrated that the use of the carrier results in a prolonged residence time of AMPs in the lungs.

The **nebulisation chamber** developed by partner INGEN was used in the biodistribution studies with liposomes in order to determine the percentage of nebulised dose that reaches the lungs. In parallel, a comparison with the commercially available **Penn-Century MicroSprayer** aerosolizer was carried out. As commented in the results section of WP2, administration through the aerosol device developed in the frame of the project did not allow reaching high concentration of the drug in the lungs. However, this will not impede the technology developed by INGEN to be applied to humans in the future.

The labeled particles were also used to assess the **regional lung distribution** of aqueous aerosol generated with the Penn-Century MicroSprayer. The SPECT-CT images indicated that the distribution in the lungs was not completely homogeneous and further showed that certain regions of the lungs are underexposed. Altogether, these results suggest that the Penn-Century MicroSprayer administration protocol is appropriate for nebulization of

aerosols in rat lungs, although sub-optimal results might be obtained when a uniform distribution of the administered aerosol is paramount.
Additionally, a method for the **determination of aerosol deposition in the lung**, based on the administration of Na[18F]F, has been developed and validated.

Result2:
Two *K. pneumoniae* infected rat models were generated (with two different resistant strains). A new model of rat pneumonia with the *K. pneumoniae*-KPC strain was established. This KPC pneumonia model as well as the ESBL pneumonia model were clinically validated by investigating the therapeutic response to parenteral treatment with Meropenem and Tigecycline (therapy schedules as used in patients with ESBL pneumonia or KPC pneumonia).

Result 3:
The maximum molerated dose (MTD) was determined *in vivo* in healthy rats for 5 selected NSs, including unloaded NCs and free AMPs at Erasmus Medical Centre (EMC). These experiments were necessary to identify the concentration ranges in which the treatments could be given to infected rats with *K. pneumoniae*-ESBL pneumonia.
Various treatment schedules were used differing in

- number of gifts (1 gift, 3 gifts, 10 gifts, or 20 gifts),
- frequency of gifts (12-hourly or 24-hourly)
- duration of treatment (12 h or 36 h, 5 days or 10 days).

Result 4:
Regarding therapeutic efficacy (TE), the data derived from the treatment schedule 10 gifts 24-hourly show that NSs were effective at MTD doses. Outcome parameter for therapeutic efficacy was a significantly longer rat survival time. 100 % rat survival could not be achieved due to 1) limitations caused by insufficient aerosol inhalation of the drug by rats under light anaesthesia 2) limitations in intratracheal instillation of the drug using the Microsprayer allowing a maximum volume of 100 µL only (exceeding this volume resulted in reflux). In future patient studies there will be no such restrictions.

Last but not least, a very positive aspect of the project is the **complementarity** and **multidisciplinarity** of the partners involved in the project. Each partner had a well-defined role and excellent expertise in their own area. This helped to face the challenges from different points of view, and we demonstrated to have sufficient flexibility to adapt the work to new requirements ensuing during the project.

In addition to these more technical conclusions, we learned about the importance of having a well-defined **risk analysis-based contingency plan**, which helped us to address problems and optimize the experiments when the results obtained were not as expected. In the course of the PneumoNP project, the animal testing had to be re-defined up to four times. This would not have been possible without the deep analysis of all technical parameters/results by each partner and the proactive, solution-oriented attitude of all partners involved.

PneumoNP was a challenging project as it tried to include three innovative aspects in the design of the new nanopharmaceuticals. New active ingredients, a new administration route (inhalation) and nanotechnology in the formulation of the drug were included.
Now that the positive effect of including nanotechnology for the formulation of active ingredients has been demonstrated, the use of those nanoparticles and micelles and the clinical development of the antimicrobial peptides should be studied in parallel. If the pharmaceutical companies are to include this product into their portfolio, the risk will have to be reduced and conventional administration routes will have to be used.
Klebsiella pneumoniae was chosen as an example of gram negative bacteria. Since a positive effect of the nanosystems developed could be observed, the procedure could also be widened to other bacteria and active ingredients.
With regard to the new diagnostic kit, there is still much work to be done for commercialisation and positioning the product in the market.

4. The potential impact (including the socio-economic impact and the wider societal implications of the project so far) and the main dissemination activities and exploitation of results

As described in the previous section, PneumoNP generated the following four results:
1) **a new inhalable drug system** composed of a **nanotherapeutic system (NS)** combining an antimicrobial peptide and a nanocarrier (NC);
2) **a new aerosol technology** specifically developed for the nanotherapeutic system;
3) **an innovative efficiency-efficacy test** to follow-up the treatment;
4) **a new diagnostic kit** for the rapid and multiplex identification of bacteria causing respiratory infections.

The main impacts of the project can be summarized as follows:
1. New therapy with novel administration route

PneumoNP aims at the **utilization of NCs to deliver antimicrobial peptides (AMPs) to the lungs**, clearly **impelling the nanotechnology use in medicine**. The use of nanovehicles for pulmonary delivery is an essential part of the project because the AMPs would not be able to reach the infected region *via* inhalation on their own. For this reason, in this project nanomedicine is expected to **assist in topic administration to the lower respiratory tract**. Regarding the **inhalation route** proposed in this project and compared to actual nebulizers that can only be used as co-adjuvants due to their low efficacy, the **nanotechnology-based inhalation system** includes NSs capable of better diffusing the drug into the lungs. The main goal is to obtain a **direct action on the affected area**, **enhancing the targeting** and allowing **accumulation of the drug where the bacteria are located**. As a consequence the antibiotic dose and the frequency of its administration can be reduced, **decreasing side effects** and accelerating the recovery of the patients. This inhalation route has not been much used so far and seems convenient for nanosystems.

2. Combining therapy and diagnostics

A radical improvement is expected in the **field of diagnosis**, since patients will be **rapidly diagnosed with the kit developed in PneumoNP**, enabling the identification of specific bacterial strains and **allowing immediate therapeutic intervention**. This will reduce mortality and the risk of generating antibiotic resistant strains, avoiding the utilization of wide spectrum activity antibiotics.

3. Research close to market

The pharmaceutical sector has experienced major changes due to patents expiring. In order to maintain their competitiveness pharmaceutical companies **need innovation**. In this sense, **nanomedicine** is a key component which may offer novel therapeutic approaches and profitable drugs against generic competition.

Although in the early technology readiness level (TRL), the PneumoNP project has been structured since the beginning to accomplish good laboratory practice (GLP) and take into account that the compounds should eventually be produced in **good manufacturing practice (GMP)** conditions. This is of utmost importance in order to fulfil the requirements of pharmaceutical companies which could be interested in the results and products of PneumoNP.

4. Socio-economic impact

Nanomedicine accounts for around 80 marketed products worldwide and the **nanomedicine market** is **expected to grow dramatically** in the next ten years.

The **urgent need of new antibacterials to treat multidrug resistant (MDR) bacterial infections** and the **lack of new agents reaching the market** indicates that not only human, but also economic consequences related to antimicrobial resistance are very serious. For this reason the **development of novel NSs** to treat infections caused by resistant bacteria could avoid enormous costs and obtain great benefits for the European pharmaceutical companies.

Use and exploitation of the results

At the end of the PneumoNP project, **three different products** were expected to be able to be commercialized by the corresponding SME. As mentioned before, the main objective of the project was the development of a theragnostic system for the treatment of resistant Gram-negative bacterial infections in lungs.

a. A new inhalable drug system composed of a nanotherapeutic system (NS) combining an antimicrobial peptide (AMP) or active pharmaceutical ingredient (API) and a nanocarrier (NC);
b. A new aerosol technology specifically developed for the nanotherapeutic system NS;
c. A new diagnostic kit for the rapid and multiplex identification of bacteria causing respiratory infections and an innovative efficiency-efficacy test to follow-up the treatment
d. A new bilateral *klebsiella pneumoniae* infection rat model with ESBL and KPC resistance strains

At the end of the project, a thorough analysis led to the following conclusions:

a. PneumoNP demonstrated the efficacy of nanotechnology improving the stability and performance of active ingredients *in vivo*. Two nanosystems that combine micelles and polymer nanoparticles with antimicrobial peptides demonstrated to be the most promising systems for further development. Importantly, the nanosystems used in the project are able to reduce toxicity to the active ingredients.

   The project was also pushing the development of manufacturing processes that will be translatable and scalable for future production. This will be crucial to include these new systems in the pharmaceutical sector, which is highly regulated and with very rigid quality controls. This is true for the nanosystems, but also for the antimicrobial peptides that have been scaled-up.

With the aim to reach faster clinical trials and reduce time to market, a new product has been selected and is under development that will consist of a repositioning of existing API.

b. A new aerosol technology for inhalation was developed in the frame of PneumoNP. The preliminary prototype was accomplished with safety requirements, however the administration efficiency of the drugs was not enough to reach the required antibiotic and NS dose *in vivo.* This was mainly due to the fact that the rats were anesthetized during *in vivo* experiments which resulted in low rat breath. This latter issue could be solved in the future by using animals trained to squeeze into special rooms (such as the ones at ITEM) or, in case of use in human patients, reducing the mist chamber size and removing the holders which are obviously not needed in humans.

c. PneumoNP will release a new diagnostic kit that will be available in the market in 2018 for rapid identification of bacterial strains that will help the medical doctors within hours to select the best antibiotic for an effective treatment of bacteria.

Communication and dissemination

The communication in PneumoNP had two main missions: (i) to raise awareness about the risks of antibiotic resistance, and (ii) to prepare the path for the exploitable results to enter clinical development.

For the later objective, efforts were done to speak to industrial partners and the pharma industry. It was important for the consortium to explain solutions developed in the project with regard to the challenges it addresses: the raise of antibiotic resistance, the need for quick and precise diagnostics, and the urge for new antibiotic drugs. The European Technology Platform (ETP) Nanomedicine provided support to the consortium in this regard.

PneumoNP aimed to build a proof of concept based on academic research and to translate this knowledge on projects that could enter clinical development. Even if this kind of project may be secretive due to intellectual property protection, PneumoNP will be able to release in 2018 most of its results as patents, scientific papers, or as products.

The Twitter account of the project, @PneumoNP, was quite active. In addition, two videos were prepared and released to explain the PneumoNP results.

PneumoNP (co-)organised two major workshops in the course of the project's duration.

The first one took place in July 2015 "Novel approaches to fight bacteria" co-organised by PneumoNP and its sister projects NAREB and FORMAMP as well as the IMI project TRANSLOCATION who hosted the event in Bremen, Germany.

The second workshop was connected to the Molecular Imaging Workshop (MIW2017) organised by PneumoNP partner CIC in San Sebastian (Spain) in November 2017 concentrating on the subject of “infection” and “lung imaging”. Both events attracted between 150 and 200 participants and PneumoNP partners contributed with several presentations and posters.

In addition, partners were very active in participating to numerous national and international events, such as ECCMID, FIGON Dutch Medicine Days or CLINAM.

For further information, please visit: www.pneumonp.eu.