COLLEGE ED BIOLOGIE DOCTORAL SANTE PAYS DE LA LOIRE

Year 2023 /2024

THESIS TOPIC

Subject N° (to be completed by the ED):	**FUNDING:** ☒ **Requested** ☐ **Acquired**	**Funding origin:**
Thesis title: **Self-assemblies from prodrugs composed of conventional Anticancer drugs and antimicrobial peptides: a revolution in the lung cancer TreAtment, with MICrobiota as a main actor**		3 keywords: Lung cancer Microbiota Nanomedicines
Unit / team: **MINT INSERM 1066 CNRS 6021**		
Supervisor's name: **Dr. Elise Lepeltier**		Phone number: +33244688535 Email address: elise.lepeltier@univ-angers.fr

Socio-economic and scientific context (approximately 10 lines):

Lung cancer is the third most common cancer in France and the deadliest (National Cancer Institute 2021). Around 85% of these cancers are the so-called "non-small cell lung cancer" (NSCLC) form, with smoking as the main risk factor. Current treatments are difficult, involving surgery, radiotherapy and intravenous chemotherapy, with significant side effects and a limited effectiveness. It is therefore necessary to find new therapeutic strategies. In this context, nebulization as an administration route would be a major progress: local administration of chemotherapy without the inconvenience of the repeated injections required for the intravenous route, with an ultimate ideal, an outpatient treatment.

Moreover, according to literature, it has become difficult to ignore the role of the microbiota in a number of physiological processes, including tumour development and biological response to cancer treatments. In 2017, a study demonstrated the inactivation of gemcitabine, a classical anticancer drug, by colon microbiota. The therapeutic efficacy of the anticancer drug was recovered after co-administration of an antibiotic. In this context, the local microbiota must be considered as a real biological barrier and must be studied to develop anticancer treatments of tomorrow, notably for lung cancers.

Working hypothesis and aims (approximately 8 lines):

The objectives of this project will be to create innovative, excipient-free nanomedicines combining anticancer properties and antimicrobial properties, via the self-organization of amphiphilic prodrugs and to provide preclinical evidence that this therapeutic strategy is effective on a murine orthotopic lung cancer model, after an administration by nebulization.
The chosen antimicrobial agent belongs to the class of antimicrobial peptides (AMP): essential components of the innate immune system of animal and plant species, they have many advantages such as being biodegradable, biocompatible, with a possible broad-spectrum activity and some mucolytic properties. An AMP will be thus covalently coupled to a classical hydrophilic anticancer agent, such as gemcitabine. The resulting conjugate will be an amphiphilic prodrug, potentially capable of spontaneous self-assembly in water. A second amphiphilic conjugate, composed of a hydrophilic polymer such as polyethylene glycol (PEG) covalently linked to a hydrophobic anticancer agent such as paclitaxel, could also be co-nanoprecipitated, to overcome the different biological barriers encountered during a pulmonary administration.

Grandes étapes de la thèse (env. 12 lines) :

This PhD thesis will be performed in MINT laboratory, supervised by Dr. Elise Lepeltier, in collaboration with the Hospital of Angers (pneumology department with Dr. Justeau and infectious disease department with Dr. Pailhories) and with the Institut Curie (LIP led by Dr. Didier Decaudin).

WP1. Microbiota and lung cancer (M1-M36)

Task 1.1 Characterization of the lung microbiota in lung cancer patients (M1-M36): Hospital of Angers

There is clearly a need for experimental data to establish a potential link between lung cancer and lung microbiota composition. Thus, throughout this PhD thesis, the microbiota of around 150 NSCLC patients will be characterized by 16S ribosomal RNA and shotgun sequencing from bronchial aspiration, to establish an extensive database: this study will be performed by the Hospital of Angers and closely followed by the PhD student.

Task 1.2 Evaluation of the anticancer drug degradation by the lung microbiota (M1-M6)

The anticancer drugs chosen should be used by oncologists, hydrophilic and have double bonds to promote self-association. One candidate already identified is the gemcitabine (Gem). The potential degradation of this molecule in contact with the lung microbiota will be characterized by LC-MS.

WP2. Synthesis, formulation and characterization of the prodrugs AMP-anticancer drug (M7-M16)

Task 2.1 Synthesis of amphiphilic prodrugs and purification (M7-M12)

The chosen AMP will be mainly hydrophobic, with a broad-spectrum activity: one candidate has been already identified, Macropin I. The synthesis of Macropin-Gem conjugate will be performed by Merrifield synthesis, via a Fmoc strategy, using a microwave-assisted peptide synthesizer. An acetyl-lysine will be added on the N-terminal side of the sequence, to obtain a cleavable bond in the tumour environment. Moreover, PEG-paclitaxel conjugate will be synthesized in solution, with an ester bond.

Task 2.2 Formulation (M13)

The suspensions will be formulated by nanoprecipitation. Macropin-Gem alone or co-nanoprecipitated with PEG-paclitaxel will be studied.

Task 2.3 Physico-chemical characterization (M14-M16)

The Critical aggregation concentration (CAC) will be determined by fluorescence spectroscopy. The self-assemblies will be characterized by ^{1}H NMR diffusometry (DOSY) to identify the hydrodynamic diameter. Stability after nebulization will be studied.

WP3. *In vitro* biological efficacy of the different suspensions (M17-M22)

Task 3.1 Antimicrobial activity of the conjugates (M17-M18)

The antimicrobial activity of the prodrugs will have to be validated on the lung microbiota: the minimum inhibitory concentration (MIC) will have to be evaluated on strains identified from Task 1.1.
Task 3.2 *In vitro* biological efficacy of the different suspensions (M18-M22)
The cell viability of the different suspensions will be determined by classical MTT and LDH assays on A549 and on NCI-H460 (NSCLC) cancer cells.

WP4. *In vivo* proof of concept (M23-M33)
Task 4.1 Efficacy on an orthotopic lung cancer model (M23-M33)
NCI-H460 (NSCLC) cancer cells, prepared in Matrigel®, will be injected into the left lung of a nude mouse. The intravenous and the pulmonary routes by nebulization will be compared. The tumour volume will be evaluated by MRI over minimum 27 days after suspension administration. The toxicity will also be checked by monitoring the mass of the animals.
Task 4.2 Efficacy on PDX lung cancer models (M23-M33) performed by Institut Curie
Additionally, the suspensions will be intravenously administered to different patient derived xenograft models (PDX) from NSCLC tumours (Institut Curie, Dr. Didier Decaudin). To mimic the local lung microbiota, an intravenous injection of a bacterial strain known to specifically colonize tumour tissues, such as E. coli Nissle 1917, could be performed in parallel.

Scientific and technical skills required by the candidate (2 lines):
The candidate should have a good background in organic chemistry, in particular in peptide synthesis and in nanomedicines, should have experience already in nanoparticle formulation and characterization. Some skills in cell and bacterial culture would be an advantage.

3 publications from the team related to the topic (last 5 years):
- Ladaycia A., Passirani C., **Lepeltier E.***, Microbiota and nanoparticles: description and interactions, *European Journal of Pharmaceutics and Biopharmaceutics,* **2021**, 169, 220-240, **IF 5.6**

- Ladaycia A., Loretzt B., Passirani C., Lehr CM, **Lepeltier E.***, Microbiota and cancer: *in vitro* and *in vivo* models to evaluate nanomedecines, *Advanced Drug Delivery Reviews,* **2021**, 170, 44-70 **IF 17.9**

- Guyon L., **Lepeltier E.***, Gimel J.-C., Calvignac B., Franconi F., Siegler B., Lautram N., Chrétien D., Bourgaux C., Pigeon P., Saulnier P., Jaouen G., Passirani C., Importance of Combining Advanced Particle Size Analysis Techniques To Characterize Cell-Penetrating Peptide–Ferrocifen Self-Assemblies, *Journal of Physical Chemistry Letters*, **2019**, 10(21), 6613-6620 **IF 6.9**

National and international collaborations:

- **Dr. Didier Decaudin, LIP Institut Curie, Paris: oncologist, specialist of PXE lung cancer models**
- **Prof. Thierry Urban, CHU Angers: pulmonologist, specialist of lung cancer**
- **Dr. Hélène Pailhories, CHU Angers: infectious disease department, specialist of lung microbiota**
- **Prof. Claus-Michael Lehr, Saarbrücken, Germany: specialist of coculture models, notably of the lung**