# KR-12 LOADED SMART POLYMERIC NANOPARTICLE AGAINST MULTI-DRUG-RESISTANT KLEBSIELLA PNEUMONIAE

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#### INTRODUCTION

Antimicrobial resistance (AMR) is a growing global health crisis projected to cause up to 10 million deaths annually by 2050. Klebsiella pneumoniae, a multidrug-resistant (MDR) pathogen, is a leading cause of nosocomial infections and demonstrates high resistance to antibiotics including third generation cephalosporins, carbapenems and colistin. Innovative therapeutic strategies are urgently needed to combat such pathogens. Among these, antimicrobial peptides (AMPs), such as LL-37 and its potent derivative KR-12, have emerged as promising alternatives due to their ability to selectively disrupt bacterial membranes. However, direct AMP delivery faces challenges including short-life, degradation, toxicity, and limited bioavailability.

To address delivery barriers, recent advances in nanobiotechnology, especially in the development of biocompatible polymeric nanoparticles, have shown potential for the treatment of bacterial pulmonary infections. Inhalable AMP formulations are of particular interest due to their ability to localize treatment directly to the site of infection, bypass systemic side effects, and enhance peptide stability in the hostile pulmonary environment.

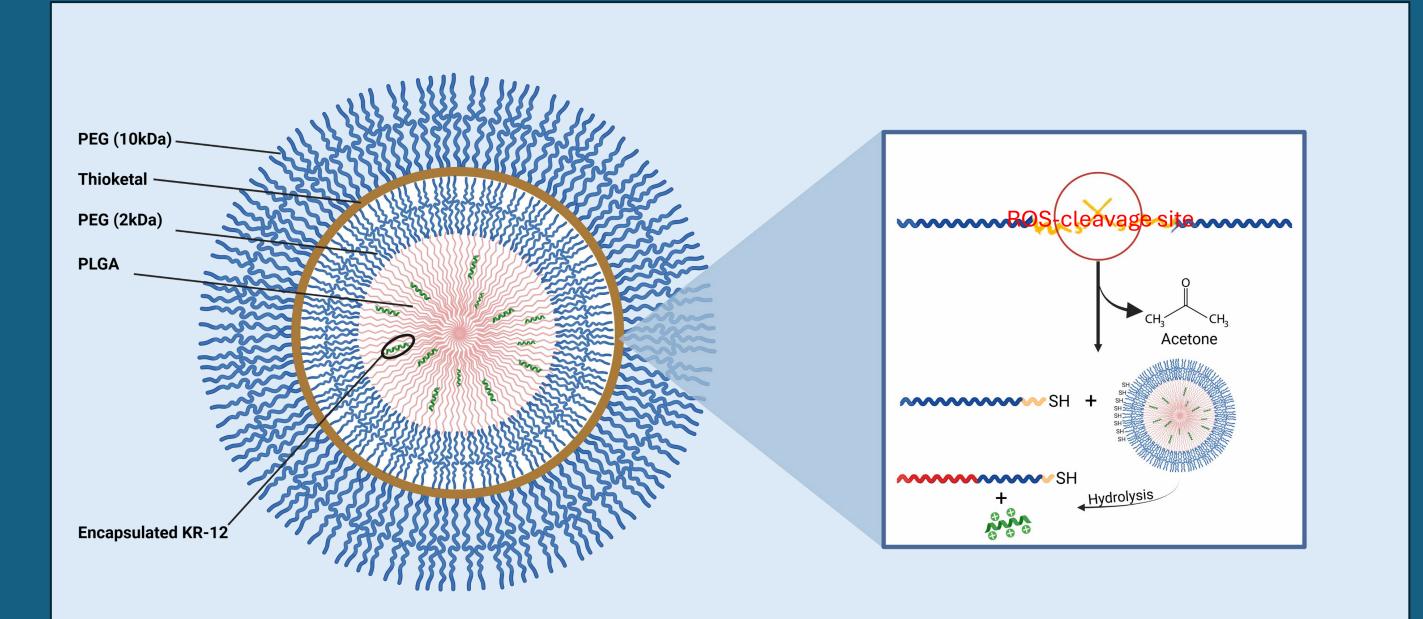
#### **HYPOTHESIS**

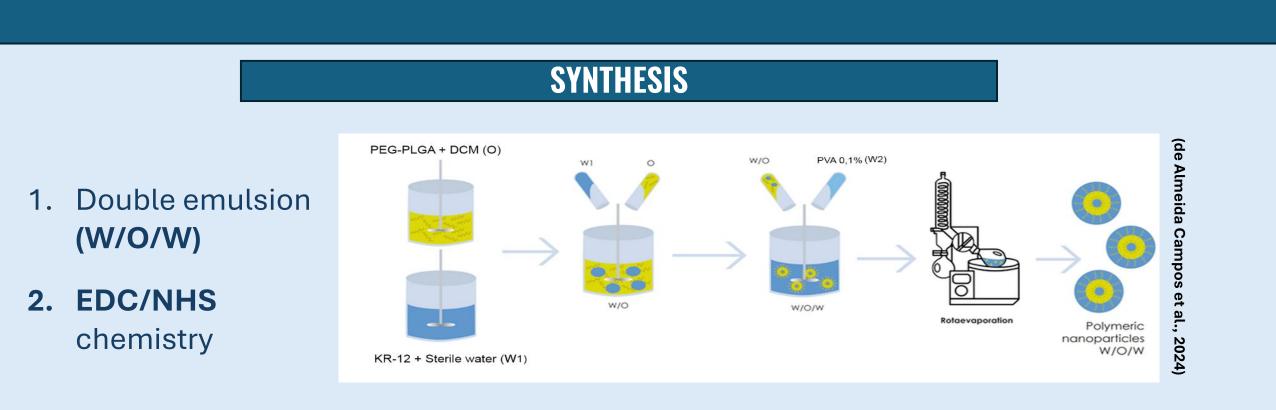
A ROS-responsive PEG-PLGA nanoparticle crosslinked with thioketal can efficiently encapsulate and protect the antimicrobial peptide KR-12, and selectively release it at infection sites in the lungs, enhancing antibacterial activity against Klebsiella pneumoniae, while minimizing toxicity and unspecific release

#### **OBJECTIVES**

- Design a nanoparticle-based delivery system for KR-12, targeting lung epithelial cells.
- Evaluate the cytotoxicity of the KR-12 loaded nanoparticles on human lung epithelial cells.
- Assess the antibacterial efficiency of nanoparticle-derived KR-12 against Klebsiella pneumoniae in vitro and in vivo, and compare it to the free peptide.

#### **OBJECTIVE 1**





#### **CHARACTERIZATION**

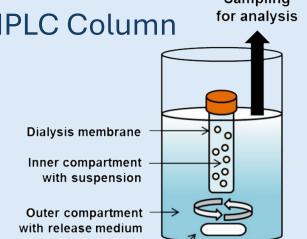
- **TEM**: Morphology, diameter **DLS**: Hydrodynamic diameter, polydispersity index, zeta potential
- **Encapsulation efficiency** & loading capacity

## **STABILITY TEST**

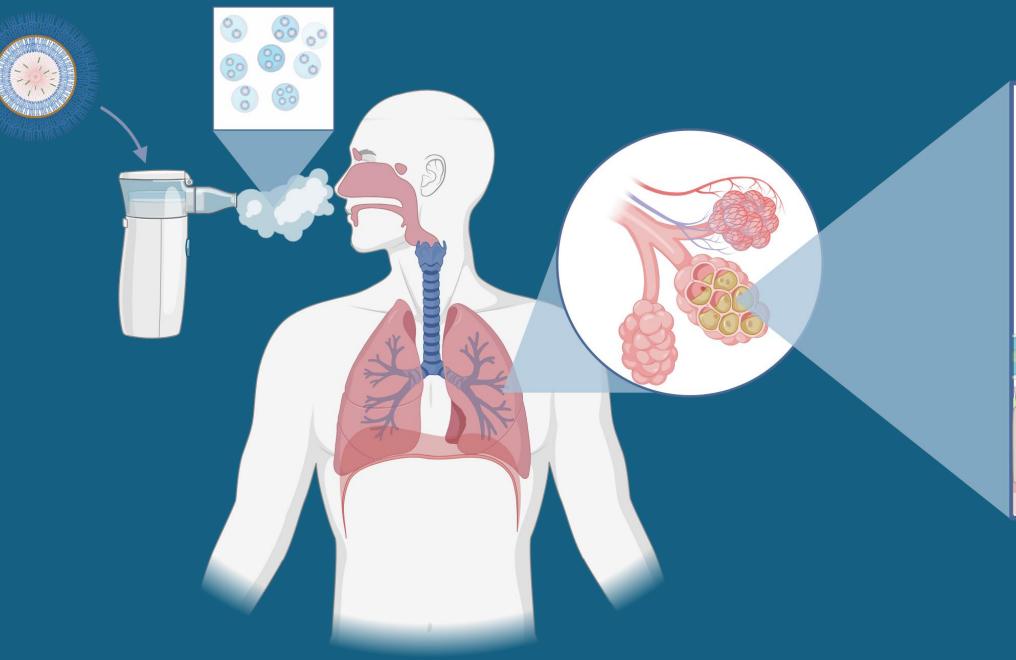
- NaCl dilutions 25-300mM **DMEM** with & without **10** % **FBS** Incubation 37°C
- **DLS** at 0 48 hours
- Hydrodynamic diameter
- Polydispersity index Aggregation State

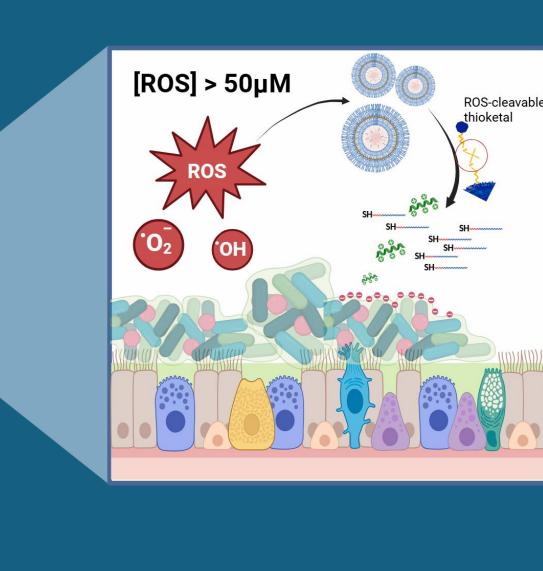
#### IN VITRO DRUG RELEASE

- H<sub>2</sub>O<sub>2</sub> dilutions
- 0/5/30/100/200/500 μM - MWCO3.5kDa
- C18 HPLC Column



(Kim et al., 2021)

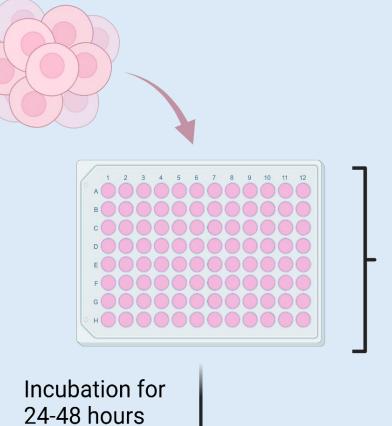




## **OBJECTIVE 2**

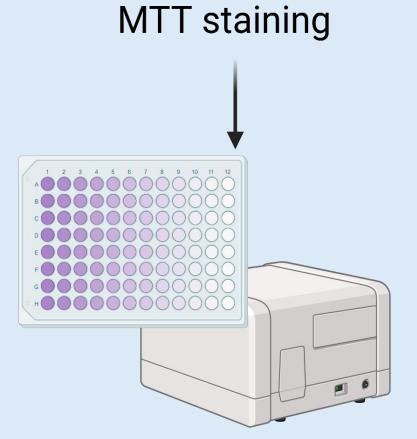
BEAS-2B cells

(1x104 cells/well)



Growing concentrations from [MIC] of formulations:

- KR-12
- unloaded PEG-PLGA-TK-
- mPEG PEG-PLGA-KR-12-TK-mPEG
- PEG-PLGA-KR-12-TK-mPEG
- (ROS presence) Control:
- Triton X-100 (100% death)
- Non-treated cells



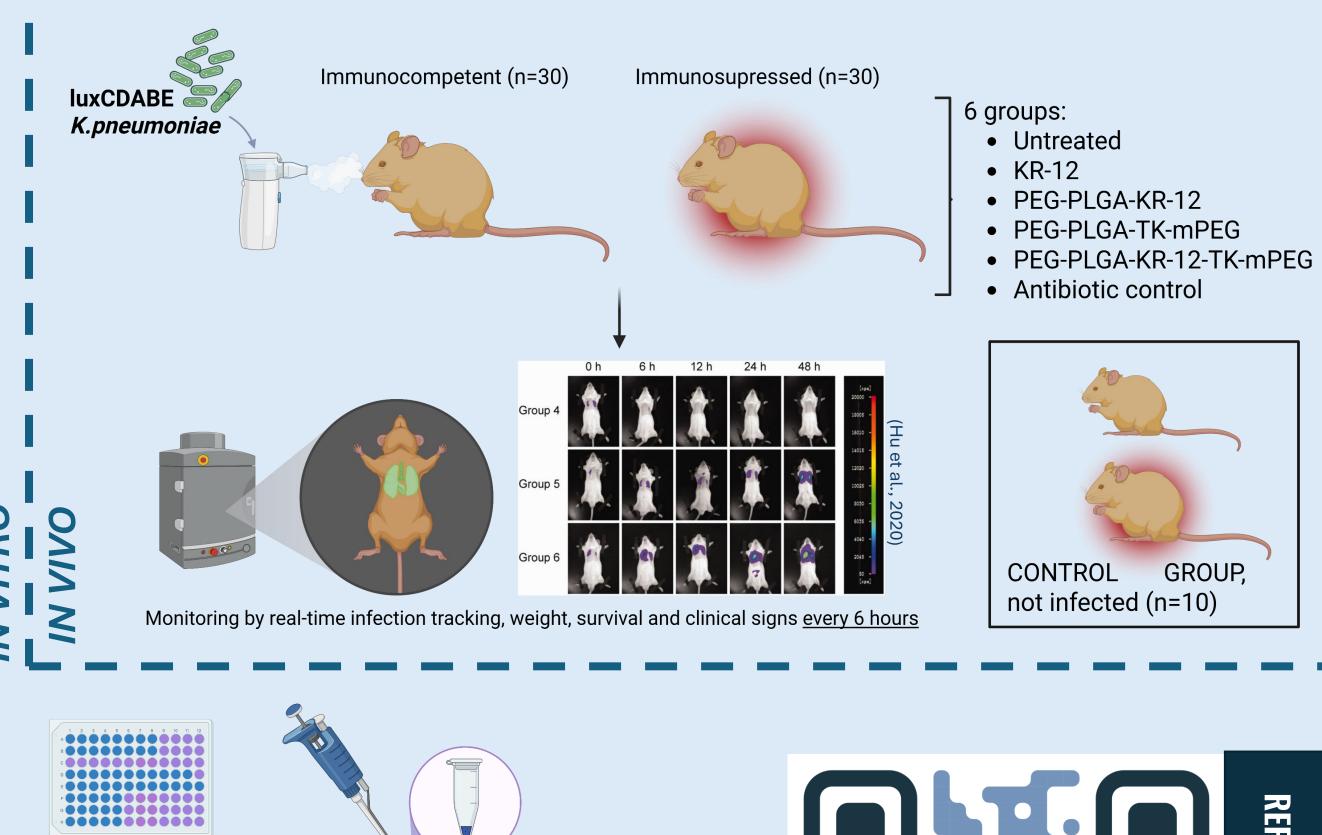
Microplate Lecture Spectrophotometer at 570 nm

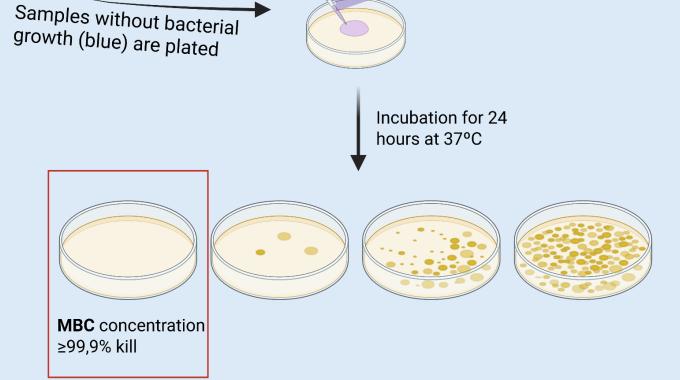
#### Serially diluted formulations • KR-12 • PEG-PLGA-KR-12 Carbapenem-Resistant Unloaded PEG-PLGA-TK-mPEG Carbapenem-Sensitive • PEG-PLGA-KR-12-TK-mPEG Klebsiella pneumoniae Aminoglycoside (positive control) (1×10<sup>6</sup> CFU/mL) Three different media (+ $H_2O_2$ ): • Standard: CAMHB • **Physiological:** CAMHB + 15% human serum + 150 mM NaCl + 1 mM Ca<sup>2+</sup>/Mg<sup>2+</sup> • Pulmonary: physiological medium + 0,5% porcine type-II mucin Incubation for 24 hours VITRO 30 µL of Resazurin/well Incubation for 4 hours at Lecture at 570 nm by Microplate Spectrophotometer or visual determination

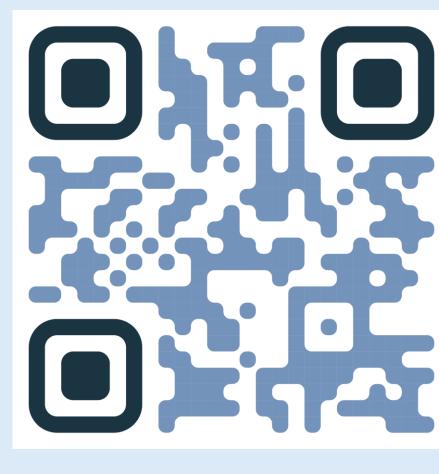
**OBJECTIVE 3** 

remains blue)

•••••• MIC is the lowest concentration of antimicrobials that inhibits microbial growth (bacteria not metabolically active --> well



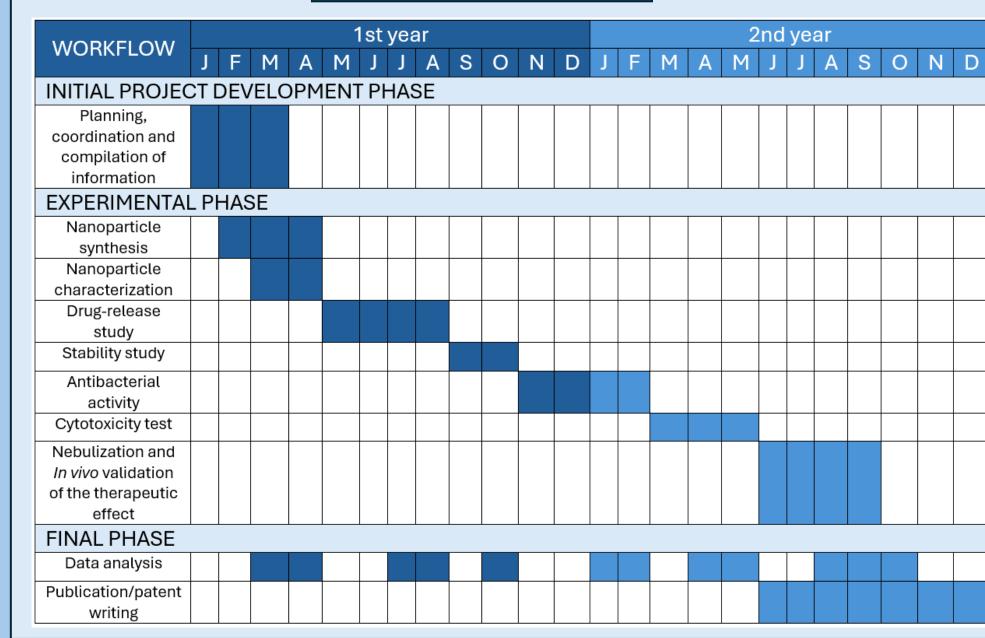




REFERENCES 20 **FULL** SCR

GROUP,

#### TIMELINE **EXPECTED RESULTS BUDGET** 4.000 INVENTORY



- 18.090 **PUBLICATION AND COMMUNICATION** 1263 **CELL CULTURES** 5.963,36 **EXTERNAL SERVICES** 21.740 **FUNGIBLE** 48.000 **HUMAN RESOURCES** 10.000 20.000 30.000 40.000 50.000 60.000 Euros (€)
- ✓ Nanoparticle with a diameter of 150-200 nm with thioketal **cleavage** around  $[H_2O_2] = 50-100 \mu M$
- ✓ MIC values ~ 8-16 µg/mL
- ✓ MBC values ~ 16-32 µg/mL
- ✓ Lack of toxicity in BEAS-2B cells
- ✓ In vivo infection clearance with low to null mortality rate. No nanoparticle accumulation.