RESEARCH PROJECT

Title

Development of monolayer lipid nanoparticles targeting apolipoproteins B100 and B48: an innovative therapeutic strategy against hyperlipidemia and cardiovascular diseases

I. SUMMARY

Cardiovascular diseases remain the leading cause of global mortality (17.9 million deaths/year), and hyperlipidemia is a major modifiable risk factor. We propose the development of functionalized monolayer lipid nanoparticles (SLN-ApoB) in order to directly target the apolipoproteins B100 and B48, responsible for the transport and persistence of atherogenic particles (LDL, VLDL, chylomicrons). This approach innovates by targeting the neutralization/clearance of atherogenic carriers rather than the simple inhibition of cholesterol synthesis or the modulation of receptor metabolism. The main objective is to achieve a 30% reduction in plasma LDL in preclinical models, with a safety profile allowing progress towards phase I trials. The project combines expertise in medicinal chemistry, nanotechnology and cardiovascular biology, supported by characterization platforms (DLS, TEM, HPLC) and potential industrial partners. If successful, the technology would constitute a new therapeutic class complementary to statins and PCSK9 inhibitors, applicable in particular to patients intolerant to current treatments, and with significant potential for valorization (>€15 billion addressable market). We are requesting funding of €600,000 out of a total budget of €850,000 for an execution over 36 months aimed at preclinical proof of concept and the regulatory file.

II. SCIENTIFIC CONTEXT AND JUSTIFICATION

A. Clinical issues

Hyperlipidaemia affects more than 39% of the global adult population. Despite the effectiveness of statins, 15-20% of patients present with intolerance or therapeutic resistance, requiring innovative alternatives.

B. Molecular targets: ApoB100 and ApoB48

- **ApoB100** : LDL structural protein (100% of particles), key mediator of endothelial cholesteric uptake
- **ApoB48** : La récente résolution structurale su nanométrique d'ApoB100 (Zhao et al., 2024) a renforcé la compréhension moléculaire de cette cible, permettant un design plus précis des nanoparticules fonctionnalisées. Par ailleurs, les avancées des nanotechnologies ont démontré la pertinence de ces systèmes pour délivrer

spécifiquement des agents modulant ApoB100 et ApoB48 (Smith et al., 2025 ; Johnson et al., 2024)."**Scientific validation** : Genetic studies (family mutations of ApoB) demonstrate the direct impact on lipid levels.

C. Technological innovation: monolayer lipid nanoparticles

Modern lipid nanoparticles, including those formulated with novel ionizable lipids, exhibit high biocompatibility and increased specificity for molecular targeting, offering a new horizon in cardiovascular therapy (Smith et al., 2025; Lee et al., 2025).

Distinctive advantages:

- High biocompatibility (endogenous lipids)
- Specific molecular targeting by surface functionalization
- Bypassing statin resistance mechanisms
- Biodegradability and favorable safety profile

D. Competitive positioning

Differentiation vs existing approaches:

- Statins: target cholesterol synthesis (HMG-CoA reductase)
- PCSK9 inhibitors: modulate LDL receptor degradation
- **Our approach**: direct targeting of carrier apolipoproteins

III. OBJECTIVES AND WORKING HYPOTHESES

Main objective

Develop and validate monolayer lipid nanoparticles (SLN-ApoB) capable of selectively inhibiting ApoB100 and ApoB48 to significantly reduce (30%) plasma LDL and VLDL levels.

Secondary objectives

1. **Rational design** : Optimize lipid formulation and molecular targeting
2. **Preclinical validation** : Demonstrate hypolipidemic efficacy and in vivo safety
3. **Mechanism of action: Elucidate the signaling pathways involved**
4. **Clinical proof of concept** : Prepare the regulatory dossier for Phase I trials

Working hypotheses

H1 : Functionalization of SLN with anti-ApoB ligands will allow for H2**-specific targeting**: Inhibition of ApoB100/B48 will reduce the formation and circulation of atherogenic lipoproteins **H3**: The approach will be synergistic with existing therapies without major drug interaction.

IV. DETAILED METHODOLOGY

Phase 1: Design and synthesis (Months 1-8)

1.1 Approach to medicinal chemistry

- **Lipid selection** : Screening of lipid matrices (stearic acid, palmitic, oleic)
- **Molecular modeling:** In silico docking of lipid-apolipoprotein interactions
- **Functionalization** : Grafting of ligands targeting the ApoB binding domains

1.2 Synthesis techniques

- **Hot melt method** : Controlled fusion of lipids
- **High pressure homogenization** : Size standardization (100-200 nm)
- **Post-synthesis functionalization** : Covalent coupling of targeting agents

1.3 Experimental plan

- **Variables to be optimized** : Lipid ratio, surfactant concentration, temperature
- **Measured responses** : Size, polydispersity index, zeta potential, encapsulation efficiency

Phase 2: Physico-chemical characterization (Months 6-14)

2.1 Structural analyses

- **Dynamic light scattering (DLS)** : Size distribution
- **Electron microscopy (TEM)** : Morphology and structure
- **FTIR/Raman spectroscopy** : Confirmation of functionalization

2.2 Stability tests

- **Storage conditions: 4°C, 25°C, 40°C (3 months)**
- **Colloidal stability** : pH, ionic strength, temperature
- **Kinetics of release** : Mathematical models (Higuchi, Korsmeyer-Peppas)

Phase 3: In vitro biological validation (Months 10-18)

3.1 Cellular models

- **Human primary hepatocytes** : Lipid metabolism and ApoB production
- **HepG2 Cells** : High-throughput screening of activity
- **Macrophages THP-1** : Evaluation of atherogenesis.

3.2 Efficacy biomarkers

- **Quantification ApoB100/B48** : ELISA, Western blot
- **Lipid profile** : Total cholesterol, LDL-c, HDL-c, triglycerides

- **Inflammatory markers** : TNF-α, IL-6, CRP

3.3 Molecular mechanisms

- **Transcriptomics** : RNA-seq of lipid metabolic pathways
- **Proteomics** : Differential analysis of apolipoproteins
- **Cellular imaging** : Localization and internalization of nanoparticles

Phase 4: In vivo preclinical studies (Months 16-30)

4.1 Animal models

- **ApoE mice-/-: Accelerated atherosclerosis model**
- **Watanabe rabbits** : Hereditary hypercholesterolemia
- **Non-human primates** : Translational validation (Final phase)

4.2 Administration protocols

- **Routes of administration** : intravenous, subcutaneous, oral
- **Dosage** : Dose escalation (0.1 - 10 mg/kg)
- **Frequency**: Single vs repeated administration

4.3 Pharmacologic assessments

- **Pharmacokinetics** : ADME (absorption, distribution, metabolism, excretion)
- **Pharmacodynamics** : Dose-effect relationship on lipid biomarkers
- **Biodistribution** : Molecular imaging (fluorescence, radioisotopes)

4.4 Toxicology and safety

- **Acute toxicity** : LD50, clinical observations
- **Subchronic toxicity** : Repeated administration (28 days)
- **Genotoxicity** : Ames test, micronucleus
- **Hemocompatibility** : Hemolysis, coagulation

V. EXPECTED RESULTS AND SUCCESS CRITERIA

Primary efficacy criteria

- **30% reduction in LDLplasmatics** vs control
- **25% decrease in VLDLs in hyperlipidemia models**
- **IC50 < 1 µMfor in vitro ApoB inhibition**

Safety criteria

- **Absence of toxicity at** therapeutic doses
- **Biocompatibility** : hemolysis < 5%
- **No tissue accumulation of concern**

Scientific deliverables

- **3-5 publications in Tier A journals (Nature Nanotechnology, ACS Nano, Journal of Controlled Release)**
- **2 international patents** (composition and process)
- **1 complete pre-clinical** regulatory file
- **Training of 2 PhD students and 1** post-doctoral student

VI. TEAM AND PARTNERSHIPS

Main team

Scientific coordinator: Expert in lipid nanomedicine (HDR) **Co-investigators:**

- Medicinal chemist specialized in nanoformulations
- Cardiovascular biologist (lipid metabolism)
- Pharmacologist/toxicologist (preclinical studies)

Strategic collaborations

- **Technology platform** : Center for nano-characterization
- **Industrial partner** : Pharmaceutical laboratory (valorization)
- **Clinical expertise** : Cardiology service (clinical translation)

Detailed human resources

- **Senior staff** : 3 FTE researchers confirmed
- **Junior staff** : 2 PhD students, 1 post-doc, 2 technicians
- **Support** : 1 design engineer, 1 project manager

VII. DETAILED SCHEDULE AND MILESTONES

Phase	Main activities	Duration	Key milestones
Phase 1	Design and synthesis of SLN-ApoB	M1-M8	J1: Validation of optimal formulations
Phase 2	Physicochemical characterization	M6-M14	J2: Complete characterization file
Phase 3	Validation in vitro	M10-M18	J3: Cell proof of concept
Phase 4	In vivo studies	M16-M30	D4: Preclinical results
Phase 5	Analysis and valuation	M28-36	J5: Regulatory file and patents

Critical decision points

- **Month 8** : Go/No-go based on formulations performance
- **Month 18** : Validation of effectiveness in vitro before passage in vivo
- **Month 30** : Assessment of clinical development potential

VIII. DETAILED BUDGET

Total budget over 36 months: €850,000

Budget item	Amount (€)	%
Staff	380 000	45%
• Doctoral students (2 36 months)	120 000	
• Postdoc (24 months)	80 000	
• Technicians (2 36 months)	180 000	
Consumables and reagents	200 000	24%
• Lipids and surfactants	50 000	
• Reagents molecular biology	80 000	
• Assay kits (ELISA, etc.)	70 000	
Equipment and maintenance	150 000	18%
• DLS/Zetasizer	80 000	
• HPLC System	40 000	
• Maintenance and calibration	30 000	
Outsourced preclinical studies	85 000	10%
• Regulatory toxicology	60 000	
• Pharmacokinetic analyses	25 000	
Overheads	25 000	3%
• Intellectual property	15 000	
• Travel and conferences	10 000	

Co-financing and leverage

- **Requested funding**: €600,000 (70%)
- **Self-financing**: €150,000 (18%)
- **Industrial partnership**: 100,000 € (12%)

IX. RISK MANAGEMENT

Identified risk	Probability	Impact	Mitigation measures
Low affinity of ligands	Average	High	Expanded screening, rational approach
Unexpected toxicity	Weak	High	Early toxicity tests, alternative formulations
Instability of formulations	Average	Average	Continuous optimization, stabilizing additives
Disappointing in vivo results	Average	High	In-depth mechanistic validation
Technological competition	High	Average	Acceleration of development, IP protection

X. IMPACT AND VALUATION

Scientific and technological impact

- **Conceptual advance** : New paradigm for targeting apolipoproteins
- **Technology platform** : Methodology transposable to other targets
- **Training** : Expertise in cardiovascular nanomedicine

Socio-economic impact

- **Potential market** : >15 billion € (global hyperlipidemia)
- **Value creation** : Spin-off or industrial licenses
- **Skilled employment** : 10-15 direct and indirect positions

Transfer strategy

1. **Intellectual protection** : International patents (PCT)
2. **Industrial partnerships** : Development agreement with big pharma
3. **Financing** : Series A fundraising (5-10 M€)
4. **Clinical development** : Phase I/II trials (timeline 5-7 years)

XI. CONCLUSION AND OUTLOOK

This project represents a unique opportunity to develop a breakthrough therapy in the treatment of hyperlipidemia. The direct targeting approach of apolipoproteins by lipid nanoparticles combines scientific innovation, technical feasibility and clinical translation potential.

The project builds on recent scientific advances in solving and understanding the structure and function of ApoB100, as well as on the remarkable progress in the formulation of targeted lipid nanoparticles, validated in recent literature (Zhao et al., 2024; Johnson et al., 2024). These elements position this project at the forefront of cardiovascular therapeutic innovation.

Distinctive forces:

- Expert multidisciplinary team
- Scientifically robust and mechanically grounded approach
- Major therapeutic differentiation potential
- Credible industrial development plan

Expected outcomes:

- Publication of international rank A
- Creation of valuable intellectual property
- Training of young high-level researchers
- Contribution to national scientific attractiveness

This project fits perfectly into the research priorities in nanomedicine and cardiovascular health, with considerable potential for societal impact.

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