Internship proposition 2025-2026

(One-page max)

Master 2 GP Medicine 4R (Repair, Replace, Regenerate, Reprogram)

Lab: **TaRGeT – Translational Research in Gene Medicine, UMR 1089**

Team: **Immunology for gene transfer**

Name and position of the supervisor: **Mickaël Guibaud, ingénieur hospitalier principal, CHU de Nantes**

Email of the supervisor: **mickael.guilbaud@univ-nantes.fr**

Candidate (if internship filled): **Clara AUGOT**

Title of the internship: **Immunomodulatory strategy to improve Adeno-associated serotype 8 vector efficacy and minimize toxicity**

Summary of the internship proposal:

Recombinant adeno-associated virus (rAAV) are very promising gene therapy vectors. In recent years, several treatments and numerous clinical trials of gene therapies using rAAVs to treat monogenic diseases have emerged. Nevertheless, the clinical use of rAAVs has raised unexpected toxicity issues due to the activation of the innate immune system in some patients. This immunity to AAVr capsids and transgene products not only compromises the efficacy of the therapy, but can also lead to major safety problems, in some cases even resulting in the death of the patient.

The activation of the innate immune system is generally due to recognition of viral PAMPs (Pathogen Associated Molecular Patterns) by the patient's PRRs (pattern recognition receptors). In the case of rAAVs, one of the most reported PRRs is Toll-Like Receptor 9 (TLR9), which recognizes the non-methylated CpG motifs of viral DNA. This may lead to AAV toxicity and possibly initiate cellular and humoral adaptive responses against the viral capsid and/or the transgene product. One strategy for limiting AAV immunotoxicity is to block the TLR9 pathway. We previously tested the impact of integrating a TLR9-inhibiting oligonucleotide sequence called DIMS (DNA Immunomodulating Sequence) in a rAAV9 viral vector on gene transfer efficacy and immunogenicity. The incorporation of the DIMS sequence enhanced gene transfer efficiency while mitigating hepatotoxicity.

In this Master 2 project, we aim to investigate the effect of the DIMS sequence included in an AAV8, another clinically relevant serotype, on transgene expression and hepatotoxicity in an immunogenic hepatic context. Two groups of Sprague Dawley rats will be injected with an AAV8 CAG GFP vector, either containing or lacking the DIMS sequence. The effects of the DIMS sequence will be evaluated based on transgene expression levels and the magnitude of both anti-AAV and anti-transgene immune responses.

From a technical standpoint, in addition to the ELISA and ELISpot assays for immune response monitoring, the project will include digital PCR, tissue immunolabelling, and confocal microscopy to assess transgene expression.

<u>Profile(s) linked to the project:</u>

☑ Experimental Biology *(Recherche expérimentale)*

☐ Clinical Research *(Recherche clinique)*

☐ Research in data analysis (Recherche en analyse de données)