Résumé du projet de thèse *(1 page maximum, en anglais)*
Indiquer la participation de chaque co-directeur et structure dans la gestion du projet. Please indicate explicitly the specific contribution of each supervisor to the PhD project.

Trinucleotide repeat expansions are the cause of two dozen neurodegenerative, neurological and developmental disorders. One of these, myotonic dystrophy type 1 (Steinert disease, or DM1) is due to the expansion of a CTG triplet in the 3' UTR of the *DMPK* gene. We previously showed that the expression of a recoded TALE Nuclease (TALEN) in human DM1 cells induced a moderate to large contraction of the CTG repeat tract in 68% of the clones analyzed [1]. The present proposal is articulated around three axes of research: i) increase TALEN efficacy; ii) investigate new avenues of vectorization; iii) transduce the TALEN in transgenic mice model for DM1.

1) Heterodimerization. Presently, each TALEN arm encodes 15 DNA-binding motifs and the wild-type FokI nuclease domain. This domain is known for its ability to form homodimers, which can potentially induce a DSB through self-dimerization of one single arm. The off-targets identified during the expression of TALEN in human cells may indeed arise from this mechanism. Therefore, our approach entails replacing the wild-type FokI domain with different FokI domains that lacks homodimerization capacity, aiming to enhance specificity [2]. This novel TALENs will undergo testing in DM1 cells to evaluate its efficacy in contracting CTG repeats. To comprehensively evaluate the specificity of the re-engineered TALEN, we will employ whole-genome sequencing using PacBio and compare CTG repeat lengths to the genome of the cell line before TALEN induction, as previously described.

2) Optimizing non-viral delivery methods. We want to develop a viable alternative to recombinant AAV (rAAV) transduction, in case a future patient would develop an immune response against the virus, or to avoid problems linked to the potential toxicity of the virus and/or unwanted integration in the host genome. Lipid-mediated delivery of mRNA could be an alternative to viral transduction [3]. Recent advances in modified mRNAs as well as in delivery methods have greatly improved the efficacy of such an approach. As a result, only a few months after the start of the Covid-19 epidemic, two vaccines based on modified mRNA were made available and showed improved efficacy as compared to more classical DNA-based or protein-based vaccines. Protocols to make such modified mRNA are readily available and published.

3) Transgenic mice. In the final phase of this project, the TALEN will be expressed in transgenic mice. Geneviève Gourdon and her colleagues have developed a mouse model that faithfully reproduces somatic and intergenerational CTG repeat instability, as well as phenotypic features resembling DM1 [4–6]. These mice carry 20, 55, 320 or 1000 CTG triplets. To express the TALEN in DM1 mice, we will utilize rAAV or lipidic particles, depending on the outcome of aim #2. TALEN efficacy will first be tested after intramuscular injection into the tibialis anterior (TA) muscle of hemizygous mice. The size of the CTG repeat will be analysed by PCR and small pool PCR one month after injection and the results will be compared to age-, CTG repeat- and sex-matched controls. By using different mice, we aim to assess the effectiveness of the nuclease for various CTG repeat lengths. Additionally, we will analyze the distribution and number of nuclear RNA foci formed by the large repeat expansions within mutant *DMPK* transcripts, a prominent molecular marker of DM1, that should be suppressed or alleviated with CTG repeat length reduction.

Our two laboratories have the required expertise needed for this project and may get help from multidisciplinary teams and platforms on the Pasteur campus, if required. The Richard lab will be in charge of designing the new TALENs and expressing them in human DM1 cells, as well as setting up non-viral delivery methods. The Gourdon lab will be in charge of all mice experiments.

1. Bétemps, L. *et al.* TALEN-induced contraction of CTG trinucleotide repeats in myotonic dystrophy type 1 cells. 2023.10.14.562330 Preprint at https://doi.org/10.1101/2023.10.14.562330 (2024).
2. Guilinger, J. P. *et al.* Broad specificity profiling of TALENs results in engineered nucleases with improved DNA-cleavage specificity. *Nat. Methods* **11**, 429–435 (2014).
3. Zuris, J. A. *et al.* Cationic lipid-mediated delivery of proteins enables efficient protein-based genome editing *in vitro* and *in vivo*. *Nat. Biotechnol.* **33**, 73–80 (2015).
4. Gourdon, G. *et al.* Moderate intergenerational and somatic instability of a 55-CTG repeat in transgenic mice. *Nat Genet* **15**, 190–192 (1997).
5. Gomes-Pereira, M. *et al.* CTG trinucleotide repeat 'big jumps': large expansions, small mice. *PLoS Genet* **3**, e52 (2007).
6. Huguet, A. *et al.* Molecular, physiological, and motor performance defects in DMSXL mice carrying >1,000 CTG repeats from the human DM1 locus. *PLoS Genet* **8**, e1003043 (2012).