Development of easy outcome measures for the *in vivo* screening of therapeutical agents targeting DUX4 in FSHD.

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FacioScapuloHumeral muscular Dystrophy (FSHD) is characterized by a muscle dysfunction progressing according to a rostro-caudal axis. Its molecular mechanism leads to the ectopic expression in skeletal muscle of *DUX4*, a gene encoding a transcription factor normally expressed in germline and early embryogenesis. Potential therapeutic agents targeting DUX4 are currently developed in the field, among those antisense oligonucleotides (*ASOs*) patented at UMONS. *In vivo* proof-of-concept studies are now unavoidable as a next step towards clinical trials. However, several hurdles such as DUX4 toxicity and its stochastic expression make the generation of an animal model recapitulating all the pathophysiological aspects of FSHD very challenging. Transgenic mouse models have been described, each of them possessing their own advantages and limits.
In this context, our laboratory has established two naked DNA injection methods allowing DUX4 expression in mouse hind limb muscles. The transgene is injected either hydrodynamically into a limb vein (IVHD) or intramuscularly followed by an electroporation (IMEP). Different transgene expression patterns were observed with a *LacZ* reporter (i) IMEP: strong and localized, allowing an easy readout (ii) IVHD: $LACZ^{+}$-fibres scattered throughout different muscles, a pattern closer to DUX4 expression in FSHD.
In the short-term goal to easily screen therapeutic molecules targeting DUX4 *in vivo*, we first focus our attention on the IMEP model. In the Tibialis Anterior muscles of mice injected with a DUX4 expression vector, muscle lesions are clearly observed after one week. The concomitant injection of the *pcDNA-his-LACZ* reporter vector facilitates the location of muscle areas that express the transgene. We also optimized a quantification method allowing to rapidly determine the damaged surface areas by color thresholding. Dose-response and time-course analyses were performed. We also confirmed *DUX4 mRNA* expression by 3' RACE and started to study induction of DUX4 target genes. The testing of *ASOs* targeting *DUX4 mRNA* is ongoing.

Mots clefs: FSHD, DUX4, models, therapeutic testing, antisense oligonucleotides