Splice-switching oligonucleotides for erythropoeitic protoporphyria

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Erythropoeitic protoporphyria (EPP) is a genetic disease affecting in average 1/100.000 individuals and causing acute light photosensitivity in patients, who quickly develop skin irritation upon exposure to blue light coming from natural or artificial sources [1]. EPP onset requires two independent genetic events on both alleles of the ferrochelatase (*FECH*) gene affecting the production of FECH protein [2]: in one allele, a non-sense or missense mutation prevents the synthesis of the functional enzyme; in the other, an intronic single nucleotide polymorphism (SNP) causes aberrant splicing of the pre-mRNA. Low levels of FECH lead to accumulation of its photoreactive substrate protoporphyrin IX (PPIX) in erythroid cells in the bone marrow and in the blood, as well as in the spleen and in the liver, leading in worst cases to liver failure.

We are investigating several approaches to restore FECH production. One strategy to treat EPP is to use splice-switching oligonucleotides (SSOs) - oligonucleotides specially designed to bind the pre-mRNA and sterically force the production of a functional *FECH* pre-mRNA. An active sequence was identified *in vitro* after screening and tested *in vivo* in an in-house disease model, where it was able to partially restore correct splicing in the liver and the spleen but failed to have an effect in the last disease-relevant tissue, bone marrow. Several peptide- and lipid- conjugates were therefore designed for a better bone marrow vectorization and are being tested *in vivo* in the same model. In an independent manner, we are screening several approved small molecule drugs known to have an effect on the heme biosynthesis pathway to identify candidates able to increase FECH protein production through a splicing-independent mechanism that could be repurposed in an EPP context.

Finally, we are working within this project with a technique developed for chemically modified oligonucleotides - chemical-ligation qPCR (CL-qPCR) [3] – to quantify the amount of SSOs delivered within the cells *in vitro* or in tissues *in vivo*. The technique is currently being exploited as a platform to investigate the effect of oligonucleotide chemistry, length or conjugation on uptake, activity and subcellular localization.

[1] Lecha M. et al., *Orphanet J of Rare Diseases*, **2009**, *4:19*
[2] Gouya L. et al., *Nat Gen*, **2002**, *30*
[3] Boos J. et al., *Nucl Acid Res*, **2013**, *41(15)*