Oligonucleotide therapy for treatment of erythropoeitic protoporphyria

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Erythropoeitic protoporphyria (EPP) is a rare genetic disease where patients suffer from extreme skin irritation under natural and artificial blue light [1]. EPP is caused by genetic variations on both alleles of the ferrochelatase (*FECH*) gene [2]: in one, a non-sense or missense mutation prevents synthesis of the FECH enzyme; in the other, an intronic single nucleotide polymorphism (SNP) causes mis-splicing of the pre-mRNA. Low levels of FECH leads to accumulation of its photoreactive substrate protoporphyrin IX (PPIX) in erythroid cells in the blood, in bone marrow and in the liver.

One strategy to treat EPP is to use splice-switching oligonucleotides (SSOs) - oligonucleotides complementary to the FECH pre-mRNA which bind close to the cryptic splice site and “switch” its splicing back to the functional FECH mRNA. The SSOs are composed of 2’-*O*-methoxyethyl (MOE) ribose units [3] linked by a phosphorothioate backbone, the same chemistry employed in recently approved drugs mipomirsen and nusinersen. We have further developed this chemistry with the recent introduction of stereochemically-pure phosphorothioate linkages [4].

A lead SSO sequence was identified by screening a stretch of *FECH* intron 3 in a “minigene” reporter assay. It is presently being investigated in mouse models for its distribution to various tissues, including bone marrow [5]. As delivery to organs other than liver and kidney is a fundamental limitation for oligonucleotide therapeutics, we are also investigating the conjugation of various short peptides to the SSO for enhanced uptake into hematopoietic compartments. The peptide library comprises sequences that are known bind to receptors on erythroid cells, or to locate to the cell nucleus. Conjugates are being tested in erythroid cells and we are quantifying delivery using a technique developed for chemically modified oligonucleotides - chemical-ligation qPCR (CL-qPCR) [6]. Progress on these various aspects of the project will be described.

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