An RNA based approach based on ExSpeU1 for correction of CFTR splicing defects: analysis of efficacy in primary bronchial cells

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Background. Aberrant splicing represents a common disease-causing mechanism in CF and exon skipping is one of the most frequent defect. We recently reported that modified U1 core spliceosomal components (named ExSpe U1) , when specifically engineered to bind in the intron downstream the 5'ss rescues splicing of defective exons. This therapeutic activity can be observed in CFTR exon 13 and 5, where the modified U1s efficiently correct different splicing defects present either at the 5'ss or at the exonic splicing regulatory elements.

Hypothesis. The molecular mechanism of ExSpeu1 -mediated splicing enhancement and effect in vivo are largely unknown.

Essential Methods. In this project we have evaluated the efficacy and safety of the novel therapeutic approach based on ExSpeU1 in the correction of CFTR splicing defects in primary bronchial cells. The analysis was conducted on three splicing mutations 711+5G->A, 1898+3A->G and c.2657 +5G>A variants that affect respectively exons 5 , 13 and 16.

Results. In vitro analysis with minigene assays identified for each mutation a panel of active ExSpeU1s. However a low ExSpeU1 correction efficacy for exon 13 and no expected mutation in 711+5G->A primary cells led us to focus specifically on the c.2657 +5G>A mutation associated to exon 16 skipping. Lentiviral-mediated delivery of ExSpeU1s molecules in FLIP IN cells that constitutively express a splicing competent cDNA showed that exon 16 splicing correction result in rescue of normal of CFTR protein. This results highlight the potential therapeutic efficacy of ExSpeU1s delivered by lentiviral vectors to correct the c.2657 +5G>A splicing mutation.

Spin off for research. We have filed a patent for ExSpeU1 as therapeutic tools for splicing correction that apply to several rare diseases, including CFTR

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