

## Gene therapy of 5' splice site mutations: near future or still a long way to go?

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Pathogenic mutations localized within the canonical splice sites are increasingly recognized as a widespread mechanism through which sequence alterations cause disease. In the Human Gene Mutation Database ([www.hgmd.org](http://www.hgmd.org)), single base pair substitutions within exon/intron boundaries of a total of 2,768 human genes constitute ~10% of a total of 73,411 mutations causing human inherited diseases. In order to correct aberrant splicing patterns caused by 5' splice site mutations, we designed artificial U1 snRNAs that specifically fitted aberrant splice donor sites and confirmed in HeLa cells that only some of them were able to specifically correct the mutation. As integrating retroviral vectors are the only gene delivery system that is capable of stably introducing foreign DNA into the genome of hematopoietic stem cells, we transferred mutation-adapted U1 snRNA expression cassettes into lentiviral vectors. Transduction of primary fibroblasts from patients assigned to the Fanconi anemia (FA)-C group carrying a single point mutation within in the 5' splice site of *FANCC* exon 2 on both alleles with a lentiviral vector harboring a mutation-adapted U1 snRNA expression cassette was able to correct the characteristic MMC-induced G2/M arrest. These results demonstrate that the retroviral delivery of engineered U1 snRNAs is capable of restoring a functional FA pathway in primary cells of FA patients. This novel form of genetic therapy for stem cells ensures that the corrected protein is still subject of the physiological gene expression control mechanisms.