

# Alternative Splicing Modification as a Treatment For Genetic Disorders

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## Abstract

Alternative splicing is a co-transcriptional mechanism that regulates eukaryotic gene expression affecting the majority of human genes. In this mechanism, different sequences can be identified and removed from the pre-mRNA. Using alternatives splicing, multiple mRNA combinations of joined sequences can be produced from a single gene, increasing the coding potential of the genome.

Malfunctions of alternative splicing events can affect the natural expression of different transcripts. Several strategies have been developed to regulate alternative splicing and the mechanisms underlying the functional and physiological implications of these tools are diverse. Collectively, these strategies are intended to improve the treatment of human genetic diseases.

## Short Communication

Alternative splicing is a co-transcriptional mechanism that regulates eukaryotic gene expression. Even when at the beginning the extent of this process was underestimated, it is currently established that almost 90% of the human genes undergo some splicing event, increasing the coding potential of the genome [1]. Malfunctions of this mechanism can lead to different diseases, including neural disorders, rare conditions and cancer [2]. Unfortunately, due to the transitive nature of gene expression it has been difficult to establish a precise correlation between one mutation and the correspondent alternative splicing event. On top of this, each alternative splicing event can be regulated differently according to the cell context, developmental stage or specific requirements [3].

The general mechanism that regulates alternative splicing is currently established. In this mechanism, exonic or coding sequences are included while intronic or non-coding elements are excluded from the mature messenger RNA. This decision is determined by a large protein complex called spliceosome, conformed by more than 300 proteins and ribonucleoproteins [4]. The catalytic core of the spliceosome are the snRNPs (small nuclear ribonucleoproteins) U1, U2, U4, U5 and U6. The auxiliary factors, responsible for the fine regulation of this mechanism include two major groups: the SR proteins and the hnRNP family [5,6].

Over the last two decades, molecular tools have been developed in order to correct or redirect aberrant splicing events [7]. The mechanisms by which these tools operate are also diverse. The development of strategies to regulate alternative splicing has focused on modifying or modulating this process using nucleic acids or nucleic acids analogs such as short oligonucleotides, designed either to silence or to enhance gene expression (Figure 1). Nucleic acids analogs could be single stranded antisense oligonucleotides designed as a complementary molecule that targets a specific mRNA to regulate its expression both in vitro and in vivo. Frequently, these oligonucleotides include a sequence that is complementary for a regulatory element contained in the mRNA. These elements are usually targets for auxiliary proteins, like SR and hnRNPs. In this regard, an oligonucleotide directed to regions located at or close to a splice site can mask normal or aberrant splicing events leading either to exon exclusion or inclusion. Modulatory oligonucleotides have been modified mainly to increase their resistance to nucleases being more effective due to a longer life into the cell. The modified molecules just described are known as peptide-nucleic acids (PNAs), phosphorodiamidate morpholino oligos (PMOs), 2'-OMe (2'-ortho-methyl) or splice-switching oligonucleotides (SSOs). All these molecules are effective in vitro and some of them have reached

clinical stages for their trial in the treatment of diseases like different types of atrophies (Table 1). On top of this, it has also been necessary to develop some strategies to introduce the nucleic acid or analogous molecule into the cell, such as cell-penetrating peptides (CPPs), which are modified molecules based on antimicrobial peptides (Figure 1). CPPs are a group of efficient non-viral delivery vectors that mediate the entry of a variety of molecules used in gene modulation, both in vivo and in vitro and several splicing-regulatory oligonucleotides have been conjugated to CPPs like Penetratin, and Transportan with high efficiency [8].

More recently, a microbial derivative named Spliceostatin was depicted as an alternative splicing modulator and it has also demonstrated effective anti-proliferative and anti-cancer activities [9]. Spliceostatin was identified as a metabolite from *Pseudomonas* sp. No. 2663, but since this initial discovery, similar molecules have been isolated from different bacterial strains. The precise mechanism of action for this molecule is not completely known, but it has been demonstrated that the compound interacts with an essential component of the spliceosome, SF3b (Figure1).

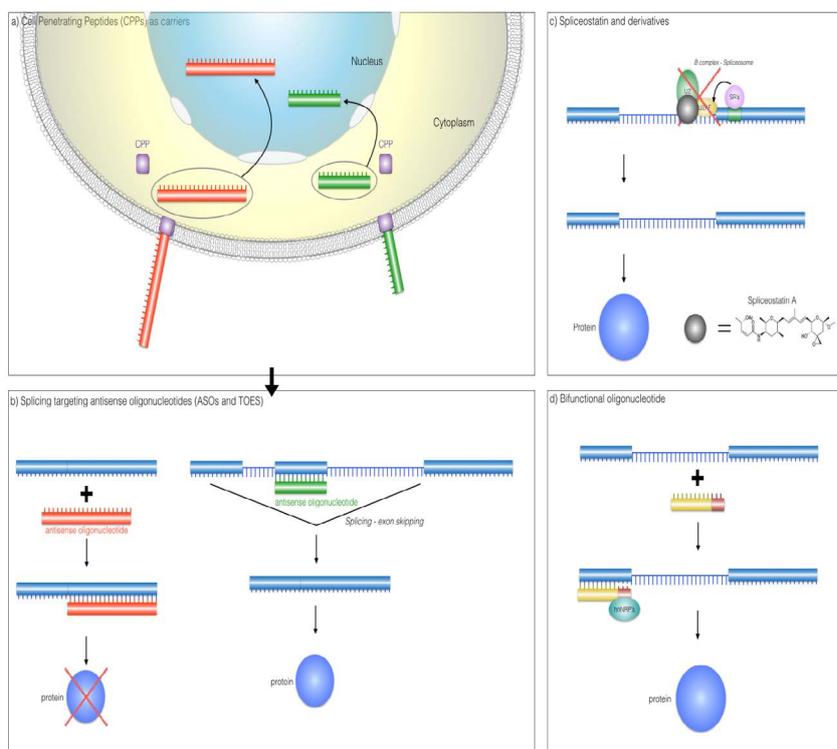
Alternative splicing has emerged as a new drug target. Given the importance of this mechanism, it becomes logical that different compounds possessing the ability to modulate certain splicing events or stages in the splicing reaction have been developed and modified [10]. However, the specificity of these compounds remains a problem. Microbial derivatives such as CPPs or spliceostatin have been shown to function as potent vehicles for splice correction and have been used to correct aberrant splicing events that occur in several genetic disorders and cancers. The discovery of these molecules promotes the possibility to find still other natural or synthetic microbial metabolites that could interact with the spliceosome and alter molecular pathways that regulate splice-site selection. The development of specific substances that possess not only the ability to regulate splicing, but that could also

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**Figure 1:** Different tools applied to redirect alternative splicing events. A) Cell penetrating peptides (CPPs) can be used as carriers for different oligonucleotides that affect splicing events. B) Antisense oligonucleotides are designed to block specific sequences in the pre-mRNA in order to correct aberrant events. C) Spliceostatin and similar molecules interact with SF3b, preventing splicing to occur. D) Bifunctional oligonucleotides possess one small sequence complementary to the pre-mRNA and another region that recruits splicing regulatory factors (like SR or hnRNP proteins) restoring appropriate protein production.

Study	Condition	Interventions	Status	Outcome
Efficacy and Safety Study of EGCG/Tocotrienol in 18 Patients With Splicing-mutation-mediated Cystic Fibrosis (CF)	Cystic Fibrosis	Dietary Supplement: EGCG, Tocotrienol or EGCG + Tocotrienol	Unknown	Changes in nasal chloride secretion as assessed by TEPD, with assessment of mean changes in TEPD by drug compared to baseline and the proportion of patients with a chloride secretion response by drug compared to baseline. Pulmonary function testing: forced expiratory volume in 1 sec [FEV1], forced vital capacity [FVC], and maximal expiratory flow25-75 [MEF25-75]
Effect of Treatment With Metformin in Type 2 Diabetes Patients on Alternative Genes Splicing	Type 2 Diabetes	Drug: Metformin	Completed	Comparison of expression of isoforms A and B of the insulin receptor using quantitative RT PCR
Sodium Channel Splicing in Heart Failure Trial	Atrial Fibrillation, Atrial Flutter, Heart Failure	-	Completed	Amount of sodium channel splice variants: ACE mRNA, Ang II mRNA, HIF-1α mRNA
DNA Changes That Affect Vitamin D Metabolism in Patients With Colorectal Cancer Receiving Vitamin D Supplements	Colorectal Cancer	Dietary Supplement: cholecalciferol. Genetic: PCR, polymorphism analysis, protein expression analysis, RT-PCR, western blotting. Other: HPLC, laboratory biomarker analysis, pharmacological study. Procedure: adjuvant therapy, immunoscintigraphy	Completed	Identification of CYP24 single nucleotide polymorphisms (SNPs). Effect of CYP24 SNPs on baseline serum vitamin D3 metabolites (25-D3, 24,25-D3, and 1,25-D3), and parathyroid hormone levels (PTH). Effect of CYP24 SNPs on serum vitamin D3 metabolites and PTH levels during cholecalciferol treatment. CYP24 splicing, protein expression, and enzyme activity at baseline and during cholecalciferol treatment. Relationship between serum cholecalciferol pharmacokinetic parameters and CYP24 SNPs, splicing variants, and enzyme activity
The Safety and Tolerability of Kinetin, in Patients With Familial Dysautonomia	Familial Dysautonomia	Dietary Supplement: Kinetin	Recruiting	Change in Safety blood labs, in vital signs and in ECG
CMV Disease and IRIS in HIV-1 Infected Persons	HIV Infections, Cytomegalovirus	Genetic: IE gene	Completed	-
Prevalence of a Non-Expressing 11B Mutation in Aka Peoples of the Central African Republic	Zinc Fingers Proteins	-	Completed	-

Aryl Hydrocarbon Receptor Interacting Protein (AIP) Gene Mutations in Acromegaly	Acromegaly	-	Unknown	Number of acromegalic patients with AIP mutation. Number of acromegalic patients with aggressive tumor with AIP mutation
Family Study of Melanoma in Italy	Melanoma, Dysplastic Nevi, Melanocytic Nevi	-	Recruiting	Defining the clinical spectrum and natural history of familial melanoma and susceptibility states over multiple generations
A Study of RO6885247 in Adult and Pediatric Patients With Spinal Muscular Atrophy (MOONFISH)	Muscular Atrophy, Spinal	Drug: RO6885247 or placebo	Suspended	Pharmacodynamics: SMN protein levels in blood. Effect of RO6885247 on muscle electrophysiology, as assessed by Compound Muscle Action Potential (CMAP). Effect of RO6885247 on Electrical Impedance Myography. Pharmacodynamics: <b>In vivo</b> splicing modification of SMN2 mRNA in blood
Pharmacogenetic Study in Castration-resistant Prostate Cancer Patients Treated With Abiraterone Acetate	Pharmacogenetic Study	Drug: Abiraterone Acetate	Recruiting	Relationships between candidate-gene polymorphisms specifically related to AA pharmacology: CYP17A1, SLCO2B1 and SLCO2B3 (13 single nucleotide polymorphisms) and the clinical efficacy of AA in terms of progression-free survival.
The Effects of Linezolid and Vancomycin on Inflammation and Cellular Signaling Vents	Sepsis	Drug: Linezolid or Vancomycin	Completed	IL-6 and MCP-1

**Table 1:** Clinical trials associated to splicing events.

show specificity for a certain step or event of splicing still represents a challenge. Finally, alternative splicing has been studied in only few eukaryotic models and it is becoming a necessity to analyze some simple eukarya in order to gain insights into the details of splicing that could be later implemented to regulate some human splicing events. Future efforts are still needed in order to achieve more specificity and strength in treating genetic disorders originated due to alternative splicing switches.

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