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Comparison of U7snRNA-mediated exon skipping as a powerful therapeutic tool for the treatment of DMD

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Duchenne muscular dystrophy (DMD) is a progressive X-linked muscle disease caused by mutations that disrupt the open reading frame (ORF) of the DMD gene, resulting in the absence of dystrophin, a muscle-specific structural protein crucial for muscle cell integrity. The gene corrective therapy using U7 small nuclear RNA (U7 snRNA) resulting in a selective exclusion of exon 21 in DMD patients who carry the DMD mutation is an approach that is already in clinical trial. Here, we developed a viral-based exon-skipping platform utilizing rAAV to encapsidate a single copy of U7snRNA targeting the splice acceptor (SAS) donor (SDS) or enhancer (ESE) sites of the following DMD exons: 12, 17, 18, 19, 21 and 46. All sequences were designed using Human Splicing Finder database. Infection and assessment of exon skipping was performed in Immortalized test-inducible-MDx0 fibroblasts (FM) cells derived from healthy or DMD patient primary fibroblasts. Treatment with desoxycycline allows FM cells to be transdifferentiated into myoblasts, and then myotubes, allowing characterization of DMD mRNA splicing and dystrophin expression. In this study, we used FM cells with exon deletions and deletions amenable to adjacent exon skipping. The FM cell lines were treated with various rAAV-U7snRNA doses and then they were differentiated for 7-14 days. The untreated FM cells from healthy patient were used as a control. To detect the exons skipping level, RT-PCR was used utilizing U7 snRNA or wild-type primers. The data revealed that most sequences targeting individual DMD exons showed a dose-dependent exon skipping in the tested cells. This skipping results in WT or in-