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# Oligonucleotides



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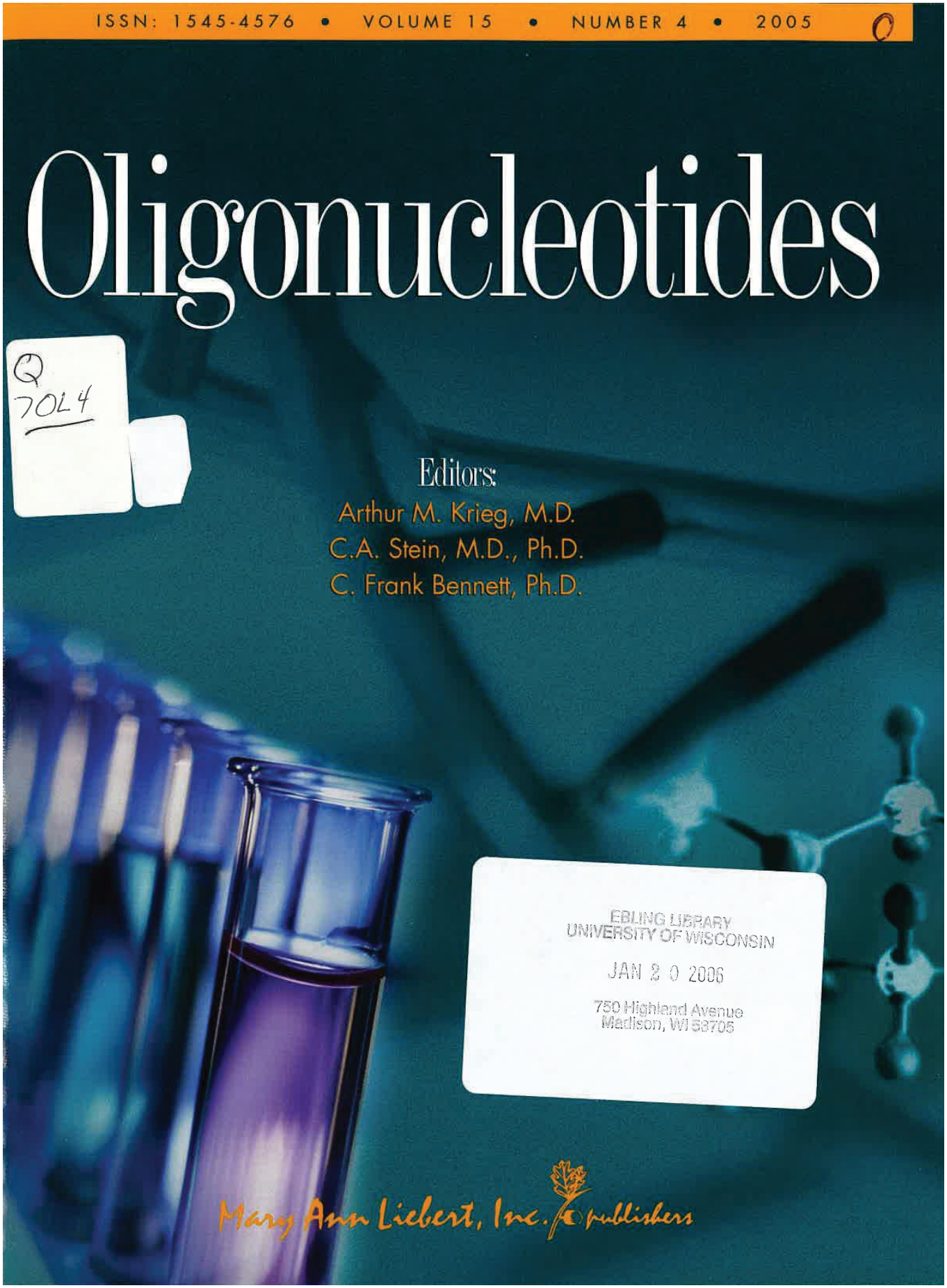



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# Functional Analysis of 114 Exon-Internal AONs for Targeted DMD Exon Skipping: Indication for Steric Hindrance of SR Protein Binding Sites

ANNEMIEKE AARTSMA-RUS, CHRISTA L. DE WINTER, ANNEKE A.M. JANSON, WENDY E. KAMAN, GERT-JAN B. VAN OMMEN, JOHAN T. DEN DUNNEN, and JUDITH C.T. VAN DEUTEKOM

## ABSTRACT

**As small molecule drugs for Duchenne muscular dystrophy (DMD), antisense oligonucleotides (AONs) have been shown to restore the disrupted reading frame of DMD transcripts by inducing specific exon skipping. This allows the synthesis of largely functional dystrophin proteins and potential conversion of severe DMD into milder Becker muscular dystrophy (BMD) phenotypes. We have previously described 37 exon-internal AONs that induce skipping of 14 DMD exons in human control myotube cultures. Here, we report 77 new AONs, effectively targeting an additional 21 exons. Of the 114 AONs thus far tested, 72 (67%) were effective. AON design initially was based on a partial overlap with predicted open secondary structures in the target RNA. We have analyzed various AON and target exon parameters in retrospect. Interestingly, we observed significantly higher SF2/ASF, SC35, and SRp40 values (as predicted by ESEfinder) for effective AONs when compared with ineffective AONs. In addition, the distance to the 3′ splice site was significantly smaller for effective AONs. No other significant correlations were observed. Our results suggest that effective exon-internal AONs primarily act by blocking SR binding sites (which often correspond to open structures) and that ESEfinder may be used to refine AON design for DMD and other genes.**

## INTRODUCTION

DUCHENNE MUSCULAR DYSTROPHY (DMD) is a severe disease that is generally caused by frame-disrupting mutations (over 60% deletions) in the *DMD* gene, which result in nonfunctional dystrophin proteins (Hoffman et al., 1987). Mutations that keep the reading frame intact give rise to internally deleted, semifunctional dystrophins and are associated with the milder Becker muscular dystrophy (BMD) (Hoffman et al., 1988; Monaco et al., 1988). This phenomenon underlies a new therapeutic approach for DMD, which is based on enlarging an out-of-frame DMD mutation into its nearest in-frame BMD counterpart. This can be achieved with antisense oligoribonucleotides (AONs) that induce specific exon skipping during pre-mRNA splicing (Takeshima et al., 2001; van Deutekom et al., 2001; Wilton et al., 1999).

As splicing requires the correct identification of the branch point, the 3′ and the 5′ splice sites of exons by the spliceosome, these sites initially seem obvious targets for AONs to induce exon skipping. Indeed, skipping of the in-frame exon 23 that is mutated in the *mdx* mouse model was successfully induced by AONs targeting these sites (Goyenvalle et al., 2004; Lu et al., 2003, 2005; Mann et al., 2002). In addition to the consensus splice site sequences, many exons contain splicing regulatory sequences, such as exonic splicing enhancer (ESE) sequences, to facilitate the inclusion of genuine exons by the spliceosome (Cartegni et al., 2002). A subgroup of splicing factors, called the SR proteins, can bind to these

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