Molecular Therapy
Nucleic Acids
Commentary

All roads lead to cure: Diversity of oligonucleotides in DM1 therapy

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Antisense oligonucleotides (ASOs) are powerful tools in therapeutic approaches for numerous genetic diseases as they modulate the processing of RNA by stimulating or inhibiting its interactions. Typically, ASOs target mRNAs, but they can be designed to target microRNAs (miRNAs) or long noncoding RNAs (lncRNAs). In the case of mRNAs, the major determinants of ASO activity are the specific targeted site of the transcript and the chemical modification pattern used. The gapmers are used to induce RNaseH enzymatic activity, which requires recognition of the DNA-RNA duplex and activates RNA degradation. Other ASOs are designed to bind strongly to a specific region of mRNA; they can be oligomers rather than oligonucleotides, and they alter the processing and/or the interactions of the targeted transcript. In a recent study, Boujnouni et al.[1] compared different types of ASOs targeting the *DMPK* transcript, a key therapeutic target for myotonic dystrophy type 1 (DM1), a neuromuscular disease. ASOs, differing in sequence, mechanism of action, and chemical modification pattern, were directly compared for induced on- and off-target effects. Further indications for DM1 therapy were provided based on the repertoire of activated changes in DM1 cells.

The gene and the mutation implicated in DM1 were described more than 30 years ago. The genetics of DM1 is an intriguing example, firstly because of the mutation, which is a large expansion of the trinucleotide motif in *DMPK* gene, and secondly due to the location of the mutation in the 3′ UTR. The expansion in DM1 reaches thousands of CTG repeats in the mutant *DMPK* gene and is transcribed into the main pathogenic molecule, i.e., mutant *DMPK* mRNA with the expanded tract of CUG repeats. There are multiple mechanisms of RNA toxicity in DM1, the most prominent of which is the sequestration of splicing regulators such as MBNL1. Consequently, *DMPK* mRNA is a promising therapeutic target, but it can be targeted in a variety of ways.[2] ASOs are typically designed to block or degrade the *DMPK* transcript. In clinical trials, among oligonucleotides and oligonucleotide-like molecules, gapmers targeting specific regions of *DMPK* mRNA are currently the most advanced.

Boujnouni et al.[1] tested (in DM1 myoblasts) two types of ASOs, blockers and gapmers, in two approaches: targeting a specific *DMPK* mRNA sequence and the CUG repeat tract. *DMPK* blockers were inefficient, while repeat gapmers showed limited activity in a therapeutic context and also induced the most substantial deregulation of other CUG-containing transcripts. Two other ASOs, *DMPK* gapmers and repeat blockers, provided key readouts in DM1-related molecular events: RNA foci reduction and splicing correction (Figure 1). Both of these ASOs also showed no significant off-target effects, as assessed by RNA sequencing (RNA-seq). In this study, emphasis was placed on direct comparison of molecular readouts resulting from ASO activity. Interestingly, correlations between various readouts were analyzed to obtain a full view of DM1 molecular phenotype correction. No clear correlation was found between *DMPK* mRNA downregulation and splicing correction events, suggesting that such molecular effects are not fully clear to be assessed separately in the therapeutic context.

An intriguing point in the case of potential ASO-based therapy for DM1 is the direct targeting of the mutation, i.e., the expanded tract of CUG repeats. For several repeat expansion diseases, it has already been shown in preclinical studies that a repeat-targeting approach can result in improved behavioral and motor phenotypes. A variety of repeat-targeting oligonucleotide-based tools were used in these studies in mouse models, e.g., blocking ASOs in fragile X-associated tremor/ataxia syndrome (FXTAS; CGG repeat expansion),[3] artificial miRNAs (amiRNAs) in Huntington's disease (HD; CAG repeat expansion),[4] and morpholino oligomers in DM1.[5] An important advantage of such strategies is a preferential targeting of mutant alleles, which seems to be even easier to achieve in DM1 due to large expansions. The mutant CUG tract in *DMPK* mRNA is a site for protein sequestration and activation of other mechanisms such as repeat-associated non-AUG (RAN) translation, and therefore CUG targeting is expected to provide corrections for many disruptions initiated by mutant RNA. An additional advantage of mutation targeting with ASOs in repeat expansion diseases is the potential diminishing of somatic repeat instability,[6] a key mechanism in disease progression.

A large effort is still needed to address issues related to ASO cellular activity, its localization, and interactions that overall impact both on-target and off-target effects. For DM1, the proposed mechanisms of ASO action are crucial to occur in the nucleus. Otherwise, RNA foci formation and protein sequestration would not be avoided. Still, we do not understand many aspects of mutant DMPK transcript functioning, and an insight here should enable the design of more efficient and specific approaches to target these mRNAs.

It is worth noting that other types of drug candidates are advanced in testing for DM1.[7] It is possible that combination

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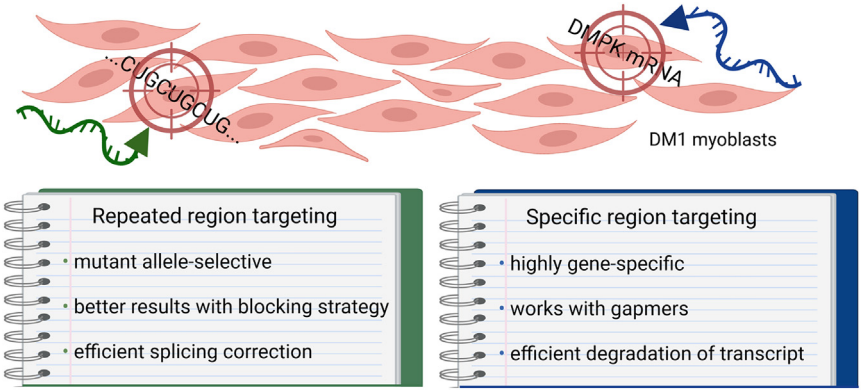

Figure 1. Summary of the comparison of repeat expansion and specific sequence targeting in *DMPK* mRNA

See the main text for more details. Created with BioRender.com.

therapy will be most beneficial in addressing all major disease symptoms. In DM1, muscles are major sites of disease pathology, but alleviation of myotonia is not the only expected benefit from therapy. Deficits related to *DMPK* mRNA dysfunction are also important to be diminished in the brain. Other tissues and organs, like the heart, are also affected. Therefore, ASO delivery issues in the context of DM1 are very demanding.

The title of this commentary is a reference to the proverb "all roads lead to Rome." One can cite another referring to this city, "Rome was not built in a day," to describe numerous challenges of bringing oligonucleotides to the clinic. Recent findings from clinical testing of ASOs for DM1 therapy showed the complexity of ASO-based approaches in humans.[8] Many options need to be tested, as potential therapeutics may fall short of the required clinical efficacy and/or safety criteria at various stages. The results of testing one type of ASO may be important in a certain context (pharmacokinetics, therapeutic readout, etc.) for the development of other types of ASOs; therefore, many of these tools should continue to be developed and comprehensively tested for DM1.

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AUTHOR CONTRIBUTIONS

A.F. has conceived and written this commentary.

DECLARATION OF INTERESTS

The author is a coinventor of patents (US9970004B2 and US10329566B2) concerning the application of the RNAi approach in the treatment of diseases caused by the expansion of CAG trinucleotide repeats.

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