miRNA-Based Therapeutics in DM

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Myotonic dystrophy involves two types of chronically debilitating rare neuromuscular diseases: type 1 (DM1) and type 2 (DM2). It is well documented that key clinical symptoms in DM are associated with a strong mis-regulation of RNA metabolism observed in patient's cells. This mis-regulation is triggered by two leading DM-linked events: the sequestration of Muscleblind-like proteins (MBNL) and the mis-regulation of the CUGBP RNA-Binding Protein Elav-Like Family Member 1 (CELF1) that cause significant alterations to their important functions in RNA processing. Recently, it has been identified that specific microRNA (miRNA) molecules display roles in endogenous modulation of the expression of MBNL and CELF1 proteins pointing to them as useful targets for the development of innovating therapeutic disease approaches by restoring normal MBNL or CELF1 function. Additional miRNAs have also been identified with potential use as therapeutic tools, through miRNA-based and miRNA-targeting drug development strategies, or as promising biomarker targets in DM.

myotonic dystrophy rare disease microRNA oligonucleotides therapy development

The successful achievement of a valid miRNA-based therapeutic approach for DM patients, still in very premature phases, will need further research and progression from two different avenues. One is in connection with typical challenges faced in every drug development process. The second will require new and innovative approaches to combat the disease. In this case, we define them in more depth.

1. MiRNA-Based and miRNA-Targeting Drug Development Challenges

Despite its potential, development of miRNA-based and miRNA-targeting drugs still needs time and technical breakthroughs, especially with regard to stability and delivery issues that complicate the development of miRNA therapeutics and arrival of validated drugs in the market. For this reason, most of the technologies are still in preclinical phases, with only a few molecules undergoing clinical evaluation in fields like cancer or hepatitis with a strong knowledge about the disease[1]. Regarding DM, miRNA-based and miRNA-targeting drug development is even farther behind when compared with these other diseases. This situation may be linked to DM´s rare disease status, where the development of therapeutics was neglected for a long time, as well as other issues such as the high level of disease complexity, with no clear endpoints for drug evaluation. Another issue is the recent understanding of the genetic causes of the disease as well as the identification of a highly novel pathogenic mechanism with potential disease targets linked to sequestration by a toxic RNA. First proof-of-concept results achieved in the field of DM are the real starting point for achieving a safe and effective treatment for DM patients[2]. Even so, the final use of miRNA-based drugs will pass through the challenge of identifying the most efficacious

therapeutic candidates and the evaluation of new antisense oligonucleotide technologies with different chemistry and delivery options.

Currently, examples of the most developed approaches in other disease fields include antagomiRs [3] and miRNA mimics [4], for which there is also proof-of-concept in DM [2] [5] [6] [7] [8] [9] [10] [11]. However, the use of blockmiRs (or miRNA masking approach) may be a very interesting alternative therapeutic option for target upregulation. Since each miRNA is able to regulate hundreds of genes, the action of antagomiRs is recognized as “sequence-specific,” which is a feature that causes off-target side effects and unwanted toxicity. However, a blockmiR is a “gene-specific” option to achieve specific mRNA target upregulation with exquisite specificity and low undesirable off-target effects.

The initial in vivo studies with miRNA-targeting products frequently resulted in little success, which displays weak therapeutic outcomes [12] . These studies require repeated administration to achieve persistent miRNA inhibition, have high production costs, and/or show low efficiency in some tissues and cell types. These effects may be a consequence of high levels of molecule degradation in the bloodstream and/or poor delivery to the final target site. Therefore, the correct delivery of the oligonucleotide product to the targeted organs in order to maintain adequate treatment specificity can require passive or active strategies. Targeting of organs like the liver, spleen, and lymph nodes take advantage of their tendency to internalize accumulated particles (nanoparticles or liposome-like particles) that incorporate the oligonucleotide molecule. However, different organs or systems, such as skeletal muscle heavily affected in DM, will need the use of specific binding molecules to activate the endocytosis in the cells of interest. One of the major issues for all RNA-based therapeutics is that these molecules are very unstable and prone to degradation by RNases because of their 2´-OH chemical group. Recent strategies for increasing stability of antimiRs, as well as improving cell intake and tissue distribution, have introduced different types of chemical modifications previously developed for ASOs, such as 2′-O-methoxyethyl (2′-MOE), 2′-fluoro, phosphorothioate (PS), or locked nucleic acid (LNA), among others. Modified antimiRs currently under evaluation display, in some cases, improved target modulation compared with unmodified antimiRs with promising results in in vivo models of cancer, cardiac disease, and diabetes, and in non-human primates [12]. Similarly, different chemical modifications could provide interesting improvement opportunities for the already promising miRNA-based and miRNA-targeting anti-DM molecules identified for the therapeutic intervention of miR-1, miR-206, miR-23b, or miR-218 levels [2] [5] [6]. Otherwise, beyond the practice of basic in vitro transfection methodology options for the development of “miRNA-gain-of function,” in vivo treatments include the use of viral transduction of pri-, pre-, or mature miRNAs. Different studies have demonstrated the in vivo validity of this strategy by reintroducing specific miRNAs that achieve a fine-tune miRNA product expression to block lung and liver cancer processes [13] [14] [15]. Depending on the viral vector type and the proliferation status of the target cells, DNA-encoded approaches may be continuously expressed, facilitating a prolonged miRNA increase, as well as a suppressive response. However, safety issues still need to be resolved for this technology [15]. This last limitation has led to the development of encapsulating nanoparticle approaches following the knowledge gained from the development of siRNA delivery methods (very similar in structure and functions) to increase the efficacy of in vivo delivery of miRNA-based drugs [12]. Thus, the use of poly(lactide-co-glycolide) (PLGA), TargomiRs, N-acetyl-D-galactosamine (GalNac), or

synthetic polyethyleneimine polymer particles, some already in clinical trial evaluation for cancer or diabetes disease, is also suggested for anti-DM miRNA-based and miRNA-targeting drug developments.

2. DM Drug Development Opportunities for miRNA-Based and miRNA-Targeting Products

The different attempts for developing a treatment in DM have shown how intricate it can be due to the high complexity of the disease. This is why the incorporation of additional and innovative strategies is desired. Only recently have proof-of-concept miRNA-based therapeutic strategies been tested, like blocking endogenous miRNAs [2] or delivering exogenous miRNAs by mimetic or miRNA-encoding expression vectors [5] [6]. However, they were usually presented as short experiments in the context of larger basic DM-linked research approaches. Tangible advances in rational drug developments are a prerequisite for further therapeutic success, which is already ongoing in diseases like cancer or diabetes with miRNA-based and miRNA–targeting products already in clinical evaluation. Based on this, the consistent identification of myomiRs as mis-regulated in DM [16] [17][18] [19] is offering an exciting starting point for miRNA intervention approaches, be they individually modulated or in combination. After muscle injury, the treatment of mice with a combination of three myomiRs, but also with other miRNAs, led to enhanced muscle regeneration and prevented fibrosis of regenerating muscle [20].

Basic but innovative DM research strategies involving miRNAs are promising. One example is the transfection with one artificial mirtron. Mirtrons are introns that form pre-miRNA hairpins after splicing to produce RNA interference effectors distinct from Drosha-dependent intronic miRNAs. After transfection, the mirtron causes a successful functional knockdown of the mutated DMPK gene in a murine myoblast line containing a pathogenic copy of the gene with more than 500 CUG repeats. Correction of Serca-1 mRNA DM1-associated splicing abnormality was detected, which demonstrated the therapeutic potential of this RNAi strategy [21]. Roles for miR-15b, miR-16, and miR-214 are also described when proposing that the CUGexp may serve as a target for concerted regulation by miRNAs and may also act as molecular sponges for natural miRNAs with CAG repeats in their seed regions, which affects their physiological functions [9].

Until recently, the use of small molecules for the direct targeting and modulation of specific miRNAs was not possible. However, this avenue has been opened by first identifying azobenzene as a specific and efficient inhibitor of the biogenesis of miR-21 [22] that have mechanism of action linked to the direct binding of the pre-mir-21 hairpin and interference with further Dicer processing. One interesting subject would be to study if some of the several small molecules in development are able to modulate miRNA profiles implicated in DM. This could identify unique tools for investigating miRNA roles in DM as well as promising reagents to further boost patient response to ongoing treatment developments.

In addition, given that DM1 and DM2 are multisystemic disorders, investigating tissues other than skeletal muscles or heart would also be interesting to elucidate miRNAs' contributions to other DM clinical symptoms. Some pieces of data have recently become available from DM-linked cancer and cataracts. Fernández-Torrón et al. (2016) described that miR-200/141 downregulation was linked to higher cancer risk observed in DM1 patients as the first

route of explanation for this unexpected feature [23] . Likewise, a study regarding cataracts, frequently found in DM patients, was published presenting a prediction of miRNA networks in DM1 and DM2 for the genes differentially expressed in lens epithelial samples from patients [24] . Notably, only the predicted miRNAs miR-197 and miR-29c were shared between both pathologies at this level. Furthermore, miR-29 and miR-133, predicted in the eye in this study, were previously found as mis-regulated in other DM tissues, which makes these miRNAs very promising for overall disease observation and intervention. In addition, being that the majority of (therapeutic) studies in DM currently focus on type 1, it would be advantageous to give more attention to type 2. Because they are so similar in nature, some of the miRNA-based approaches could be of common benefit to both types of DM. This rationale could also be applied to other rare diseases with the same mutation starting point (a CTG expansion) and/or with similar MBNL loss of function such as in spinocerebellar ataxia type 8 (SCA8) [25] and Fuch's dystrophy [26] . There is recent evidence that miRNA dysregulation can potentially contribute to disease pathogenesis in other repeat expansions disorders (in addition to CTG sequence) like Huntington's disease (HD), Fragile-X syndrome (FXS), and spinocerebellar ataxias (SCAs) (reviewed in [27]). These diseases also lack conventional therapies. It is still too early to tell if miRNA dysregulation is common for repeat expansion diseases. Therefore, a better understanding of miRNA function and biogenesis may lead, as expected in DM, to the development of new therapeutic strategies for preventing or delaying the disease.

Since MBNL1 and 2 and CELF1 are the first line of therapeutic targets in DM disease through fine-tuning regulatory miRNA expression levels, renewed efforts are needed to identify additional miRNA candidates and further develop those already identified.

References

1. Bonneau, E.; Neveu, B.; Kostantin, E.; Tsongalis, G.J.; De Guire, V; How close are miRNAs from clinical practice? A perspective on the diagnostic and therapeutic market.. *EJIFCC* **2019**, *30*, 114-127, -.
2. Estefania Cerro-Herreros; María Sabater-Arcis; Juan M. Fernandez-Costa; Nerea Moreno; Manuel Perez-Alonso; Beatriz Llamusí; Ruben Artero; miR-23b and miR-218 silencing increase Muscleblind-like expression and alleviate myotonic dystrophy phenotypes in mammalian models.. *Nature Communications* **2018**, *9*, 2482, 10.1038/s41467-018-04892-4.
3. Harry L A Janssen; Sakari Kauppinen; Michael R Hodges; Maria D Sanchez-Niño; Alberto Ortiz; HCV infection and miravirsen.. *New England Journal of Medicine* **2013**, *369*, 878-8, 10.1056/NEJMc1307787.
4. Corrie L. Gallant-Behm; Joseph Piper; Joshua M. Lynch; Anita G. Seto; Seok Jong Hong; Thomas A. Mustoe; Catherine Maari; Linda A. Pestano; Christina M. Dalby; Aimee L. Jackson; et al.Paul RubinWilliam S. Marshall A MicroRNA-29 Mimic (Remlarsen) Represses Extracellular Matrix

Expression and Fibroplasia in the Skin. *Journal of Investigative Dermatology* **2019**, *139*, 1073-1081, 10.1016/j.jid.2018.11.007.

5. D. Koutalianos; A. Koutsoulidou; N. P. Mastroyiannopoulos; Denis Furling; L. A. Phylactou; MyoD transcription factor induces myogenesis by inhibiting Twist-1 through miR-206. *Journal of Cell Science* **2015**, *128*, 3631-3645, 10.1242/jcs.172288.
6. Frédérique Rau; Fernande Freyermuth; Charlotte Fugier; Jean-Philippe Villemin; Marie-Christine Fischer; Bernard Jost; Doulaye Dembele; Geneviève Gourdon; Annie Nicole; Denis Duboc; et al.Karim WahbiJohn W DayHarutoshi FujimuraMasanori P TakahashiDidier AuboeufNatacha DreumontDenis FurlingNicolas Charlet-Berguerand Misregulation of miR-1 processing is associated with heart defects in myotonic dystrophy. *Nature Structural & Molecular Biology* **2011**, *18*, 840-845, 10.1038/nsmb.2067.
7. Auinash Kalsotra; Kun Wang; Pei-Feng Li; Thomas A. Cooper; MicroRNAs coordinate an alternative splicing network during mouse postnatal heart development. *Genes & Development* **2010**, *24*, 653-658, 10.1101/gad.1894310.
8. Xiaopeng Shen; Benjamin Soibam; Ashley Benham; Xueping Xu; Mani Chopra; Xiaoping Peng; Wei Yu; Wenjing Bao; Rui Liang; Alon Azares; et al.Peijun LiuPreethi H. GunaratneMark MercolaAustin J. CooneyRobert J. SchwartzYu Liu miR-322/-503 cluster is expressed in the earliest cardiac progenitor cells and drives cardiomyocyte specification.. *Proceedings of the National Academy of Sciences* **2016**, *113*, 9551-6, 10.1073/pnas.1608256113.
9. Edyta Koscianska; Tomasz M. Witkos; Emilia Kozlowska; Marzena Wojciechowska; Wlodzimierz J. Krzyzosiak; Cooperation meets competition in microRNA-mediated DMPK transcript regulation.. *Nucleic Acids Research* **2015**, *43*, 9500-18, 10.1093/nar/gkv849.
10. Wei Dong; Xuanying Chen; Menghong Wang; Zeqi Zheng; Xing Zhang; Qunlin Xiao; Xiaoping Peng; Mir-206 partially rescues myogenesis deficiency by inhibiting CUGBP1 accumulation in the cell models of myotonic dystrophy. *Neurological Research* **2018**, *41*, 9-18, 10.1080/01616412.2018.1493963.
11. Bo-Wen Zhang; Han-Fang Cai; Xue-Feng Wei; Jia-Jie Sun; Xian-Yong Lan; Chu-Zhao Lei; Feng-Peng Lin; Xing-Lei Qi; Martin Plath; Hong Chen; et al. miR-30-5p Regulates Muscle Differentiation and Alternative Splicing of Muscle-Related Genes by Targeting MBNL. *International Journal of Molecular Sciences* **2016**, *17*, 182, 10.3390/ijms17020182.
12. Rajesha Rupaimoole; Frank J. Slack; MicroRNA therapeutics: towards a new era for the management of cancer and other diseases. *Nature Reviews Drug Discovery* **2017**, *16*, 203-222, 10.1038/nrd.2016.246.
13. Phong Trang; Pedro P. Medina; Jason F. Wiggins; Lynnsie Ruffino; Kevin Kelnar; Michael Omotola; Robert Homer; David Brown; Andreas G. Bader; Joanne B. Weidhaas; et al.Frank J.

Slack Regression of murine lung tumors by the let-7 microRNA.. *Oncogene* **2009**, *29*, 1580-7, 10.1038/onc.2009.445.

14. Janaiah Kota; Raghu R. Chivukula; Kathryn A. O'donnell; Erik A. Wentzel; Chrystal L. Montgomery; Hun-Way Hwang; Tsung-Cheng Chang; Perumal Vivekanandan; Michael Torbenson; K. Reed Clark; et al.Jerry R. MendellJoshua T. Mendell Therapeutic microRNA delivery suppresses tumorigenesis in a murine liver cancer model.. *Cell* **2009**, *137*, 1005-17, 10.1016/j.cell.2009.04.021.
15. Dacheng Peng; Cheng Qian; Yonglian Sun; Miguel A Barajas; Jesus Prieto; Transduction of hepatocellular carcinoma (HCC) using recombinant adeno-associated virus (rAAV): in vitro and in vivo effects of genotoxic agents.. *Journal of Hepatology* **2000**, *32*, 975-985, 10.1016/s0168-8278(00)80102-6.
16. Stefano Gambardella; Fabrizio Rinaldi; Saverio M Lepore; Antonella Viola; Emanuele Loro; Corrado Angelini; Lodovica Vergani; Giuseppe Novelli; Annalisa Botta; Overexpression of microRNA-206 in the skeletal muscle from myotonic dystrophy type 1 patients. *Journal of Translational Medicine* **2010**, *8*, 48-48, 10.1186/1479-5876-8-48.
17. Riccardo Perbellini; Simona Greco; Gianluca Sarra-Ferraris; Rosanna Cardani; Maurizio C. Capogrossi; Giovanni Meola; Fabio Martelli; Dysregulation and cellular mislocalization of specific miRNAs in myotonic dystrophy type 1. *Neuromuscular Disorders* **2011**, *21*, 81-88, 10.1016/j.nmd.2010.11.012.
18. Alessandra Perfetti; S. Greco; R. Cardani; B. Fossati; G. Cuomo; R. Valaperta; F. Ambrogi; A. Cortese; A. Botta; A. Mignarri; et al.M. SantoroC. GaetanoElena CostaM. T. DottiG. SilvestriR. MassaG. MeolaF. Martelli Validation of plasma microRNAs as biomarkers for myotonic dystrophy type 1. *Scientific Reports* **2016**, *6*, 38174, 10.1038/srep38174.
19. KathlinK Ambrose; Taufik Ishak; Lay-Hoong Lian; Khean-Jin Goh; Kum-Thong Wong; Azlina Ahmad-Annuar; Meow-Keong Thong; Deregulation of microRNAs in blood and skeletal muscles of myotonic dystrophy type 1 patients. *Neurology India* **2017**, *65*, 512, 10.4103/neuroindia.NI_237_16.
20. Tomoyuki Nakasa; Masakazu Ishikawa; Ming Shi; Hayatoshi Shibuya; Nobuo Adachi; Mitsuo Ochi; Acceleration of muscle regeneration by local injection of muscle-specific microRNAs in rat skeletal muscle injury model. *Journal of Cellular and Molecular Medicine* **2009**, *14*, 2495-2505, 10.1111/j.1582-4934.2009.00898.x.
21. Yiqi Seow; Christopher R. Sibley; Matthew J.A. Wood; Artificial mirtron-mediated gene knockdown: Functional DMPK silencing in mammalian cells. *Structure of the 30S ribosomal decoding complex at ambient temperature* **2012**, *18*, 1328-1337, 10.1261/rna.030601.111.
22. Kiranmai Gumireddy; Douglas D. Young; Xin Xiong; John B. HogenEsch; Qihong Huang; Alexander Deiters; Small-molecule inhibitors of microrna miR-21 function.. *Angewandte Chemie*

International Edition **2008**, *47*, 7482-4, 10.1002/anie.200801555.

23. Roberto Fernández-Torrón; José-Ignacio Emparanza; Miren Maneiro; Juan-José Poza; Juan-Bautista Espinal; Miren Zulaica; Irune Ruiz; Loreto Martorell; David Otaegui; Ander Matheu; et al.Mikel García-PugaAna-María CoboAdolfo López De Munain Cancer risk in DM1 is sex-related and linked to miRNA-200/141 downregulation. *Neurology* **2016**, *87*, 1250-1257, 10.1212/wnl.0000000000003124.
24. Dewang Shao; Xiaoquan Zhu; Wei Sun; Lu Huo; Wei Chen; Hua Wang; Bing Liu; Peng Pan; Investigation of the molecular mechanisms underlying myotonic dystrophy types 1 and 2 cataracts using microRNA-target gene networks.. *Molecular Medicine Reports* **2017**, *16*, 3737-3744, 10.3892/mmr.2017.7059.
25. Randy S. Daughters; Daniel L. Tuttle; Wangcai Gao; Yoshio Ikeda; Melinda L. Moseley; Timothy J. Ebner; Maurice S. Swanson; Laura P. W. Ranum; RNA Gain-of-Function in Spinocerebellar Ataxia Type 8. *PLOS Genetics* **2009**, *5*, e1000600, 10.1371/journal.pgen.1000600.
26. Jintang Du; Ross A. Aleff; Elisabetta Soragni; Krishna Kalari; Jinfu Nie; Xiaojia Tang; Jaime Davila; Jean-Pierre Kocher; Sanjay V. Patel; Joel M. Gottesfeld; et al.Keith H. BaratzEric D. Wieben RNA toxicity and missplicing in the common eye disease fuchs endothelial corneal dystrophy.. *Journal of Biological Chemistry* **2015**, *290*, 5979-90, 10.1074/jbc.M114.621607.
27. Koscianska, E.; Kozlowska, E.; Jaworska, E.; Krzyzosiak,W.J.; MicroRNA deregulation in trinucleotide repeat expansion disorders. *Applied RNAi. From Fundamental Research to Therapeutic Applications* **2014**, -, 227–245, -.