GMR
The Original

THERAPEUTIC STRATEGIES FOR THE CORRECTION OF SPLICING IN HEREDITARY DISEASES: METHODS AND CLINICAL TRIALS

Maxim Murazovich Davresh[1], Sofia Arsenovna Gasparyan[2], Anastasia Stanislavovna Khorina[3], Anatoliy Anatolyevich Dragnev[4], Faina Fikretovna Emirova[5], Seidfatima Seid-Islamovna Seidova[6], Jasurbek Kakhramonjon Ugli Uktamov[7], Magomed Shakhbanovich Ramazanov[8], Bashir Islamovich Khanov[9]

[1]Saratov State Medical University, 112 Bolshaya Kazachia Street, 410012, 0009-0004-4096-8507, Davreshyan02@Mail.Ru
[2]Federal State Budgetary Educational Institution Of Higher Education "Russian University Of Medicine" Of The Ministry Of Health Of The Russian Federation., Moscow, 4 Dolgorukovskaya St., 127006, 0009-0008-7636-3955, Gasparyan-Sofiya@Bk.Ru
[3]Moscow State University Of Medicine And Dentistry , Nastya.Horina03@Mail.Ru ,0009-0002-1714-5372
[4]Tver State Medical University, 4 Soviet St., 170100, Dragnev2022@Mail.Ru, 0009-0008-1862-9997
[5]Astrakhan State Medical University,Bakinskaya 121, 414000, Femirova@Internet.Ru, 0009-0000-8671-9232
[6]Astrakhan State Medical University Bakinskaya 121, 414000, Seidova.080303@Mail.Ru, 0009-0002-1312-2229
[7]Oktamov02@Inbox.Ru, Astrakhan State Medical University , Bakinskaya 121, 414000, 0009-0004-8429-5834
[8]Astrakhan State Medical University , Bakinskaya 121 414000, Mgadzhiramaz@Mail.Ru, 0009-0005-5326-8769
[9]Astrakhan State Medical Academy,Bakinskaya 121 Street, Astrakhan, 414000, 0009-0008-1791-1704, khanov.bashir@mail.ru

Abstract

Disorders of pre-mRNA splicing play a key role in the pathogenesis of a significant part of monogenic diseases, as they alter the composition of mature mRNA, reduce the synthesis of functional protein, and form clinical heterogeneity of the phenotype. In recent years, splicing correction has evolved from an experimental approach to a clinically applied strategy, primarily for spinal muscular atrophy and progressive Duchenne muscular dystrophy.

The purpose of this article is to analyze modern therapeutic approaches to splicing modification, their molecular mechanisms, limitations, and clinical research results.

The paper provides a review of Russian publications reflecting the development of antisense oligonucleotides, small splicing modifier molecules, and exon-skipping technologies.

It has been shown that the drugs providing the inclusion of exon 7 of the SMN2 gene in spinal muscular atrophy and the omission of individual exons of the DMD gene in Duchenne muscular dystrophy have the greatest evidence base. At the same time, there are problems of mutation specificity, the need for early initiation of therapy, uneven tissue delivery, and the high cost of long-term treatment.

It is concluded that the further development of the direction is associated with the personalization of the design of molecules, the standardization of clinical endpoints and the expansion of national patient registries.

Keywords: splicing, hereditary diseases, antisense oligonucleotides, exon skipping, spinal muscular atrophy, Duchenne myodystrophy, nussinersen, risdiplam, clinical trials.

INTRODUCTION

Post-transcriptional processing of pre-mRNA determines the final composition of the coding sequence and, consequently, the spectrum of synthesized protein isoforms. Even single changes in donor or acceptor sites, in splicing enhancers and silencers, or in spliceosome proteins can shift the balance between inclusion and exclusion of exons, cause a shift in the reading frame and premature termination of translation. In clinical genetics, this makes splicing disorders one of the most significant pathogenetic mechanisms of monogenic diseases, and in therapy it is an attractive point of intervention, since RNA modification allows affecting the primary defect without constant genome editing [4, 8, 11].

Currently, the concept of splicing correction has changed significantly: from the idea of local intervention at the transcript level to the platform direction of precision medicine. Two models have become the most developed. The first is based on enhanced incorporation of a functionally significant exon into mature mRNA, as implemented in 5q-associated spinal muscular atrophy by modulating SMN2 splicing. The second is aimed at programmatically skipping a specific exon to restore the reading frame, which is especially important in Duchenne muscular dystrophy. In these models, clinically applicable classes of drugs were formed - antisense oligonucleotides and small splicing modifier molecules. [3, 6, 8, 10-12].

Russian publications in recent years confirm that the largest amount of clinical and organizational data has been accumulated specifically on spinal muscular atrophy and Duchenne muscular dystrophy. The Russian literature presents the results of the extended access to nussinersen program, reviews of pathogenetic therapy in children and newborns, data from the Russian registry of patients with SMA, as well as work on the applicability and experience of exon-skipping in dystrophinopathies [1-3, 5-10]. These materials allow us to move from a description of the molecular mechanism to an assessment of the actual clinical benefits, limitations of patient routing, and factors determining the effectiveness of treatment.

Despite significant progress, the correction of splicing is not yet a universal solution for all hereditary diseases. The effect depends on the type of mutation, on the preservation of the target transcript, on the possibility of delivering the drug to critical tissues, and on the time of initiation of treatment. Therefore, the evaluation of modern strategies should take into account not only biological rationality, but also the design of clinical trials, the characteristics of the included cohorts, the functional scales used, and the results of long-term follow-up [12].

The purpose of the article is to systematize modern therapeutic strategies for correcting splicing in hereditary diseases, characterize their molecular foundations, and analyze data from clinical trials and Russian real-world practice.

MATERIALS AND METHODS OF RESEARCH

Methodologically, the methods of comparative analysis, thematic classification and meaningful interpretation of the results were used. The publications were compared according to several parameters: the type of therapeutic platform, the molecular target, the nosological form, the stage of clinical implementation, the nature of endpoints and application limitations. Special attention was paid to the works in which the data of real clinical practice in Russia were presented, since they make it possible to assess the reproducibility of the effects obtained in registration and post-registration studies.

RESULTS AND DISCUSSIONS

Splicing correction is fundamentally different from classical substitution therapy in that it changes the fate of a cell's own pre-mRNA. With this approach, the clinical effect is achieved either by enhancing the inclusion of an exon necessary for the synthesis of a full-size protein, or by its controlled exclusion if skipping a specific site restores the reading frame.

Theoretically, this makes the technology especially valuable for diseases in which the pathogenic variant does not destroy the transcript completely, but shifts the balance of alternative splicing. Russian and foreign

reviews emphasize that the most clinically mature areas today are associated with SMN2-associated SMA therapy and exon skipping in dystrophinopathies [11].
In 5q-CMA, the therapeutic logic is based around the SMN2 gene, which differs from SMN1 by a critical substitution that reduces the inclusion of exon 7. As a result, a predominantly shortened and functionally defective protein is formed. Nussinersen is an antisense oligonucleotide that binds to the inhibitory region of SMN2 pre-mRNA and increases the proportion of the full-length transcript. Risdiplam acts differently: as a small molecule, it modifies SMN2 splicing systemically and allows for increased protein production when taken orally. From a clinical point of view, these are two different but conceptually similar options for correcting the same splicing defect. [2, 3, 8, 10, 12].

Table 1 - The main strategies for therapeutic correction of splicing

Strategy	**The molecular principle**	**Examples**	**Clinical significance**
Antisense oligonucleotides for exon inclusion	They block inhibitory regions of pre-mRNA and increase the incorporation of a therapeutically significant exon into the mature transcript	Nussinersen at 5q-SMA	The most proven model of splicing correction in neurology; requires early initiation of treatment [2, 3, 8]
Small molecules - splicing modifiers	They change the interaction of splicing elements with pre-mRNA and increase the production of full-length mRNA	Risdiplam at 5q-SMA	Systemic action and oral route of administration, which simplifies long-term therapy [3, 10, 12]
Exon skipping in dystrophinopathies	The skipping of a certain exon is induced to restore the reading frame	Approaches to exon 45, viltolarsen, golodirsen	It allows to obtain a shortened but functionally significant dystrophin; effective only in genetically selected groups [1, 6, 9, 11]
Personalized RNA platforms of a promising level	Targeted transcript processing, combining ASO with other molecular interventions	Customized designs for rare variants	They are promising for ultra-rare forms, but so far they are limited by the evidence base and the complexity of clinical validation [8, 11]

Domestic data on nussinersen obtained under the extended access program show that even in a cohort that is heterogeneous in age and severity, it is possible to achieve clinically significant improvements in motor function and stabilization with an acceptable safety profile [2]. The review on pathogenetic therapy in children and newborns emphasizes that the maximum benefit is observed at the earliest start of treatment,

even before the development of irreversible loss of motor neurons [3]. This position is consistent with international risdiplam data, where an increase in SMN protein levels was accompanied by an improvement in motor skills and respiratory parameters in infants with type 1 SMA [12]. Thus, for SMA, splicing correction has already ceased to be an exclusively experimental solution and has become the standard of pathogenetic treatment, especially with timely diagnosis.

However, the results of SMA therapy cannot be evaluated in isolation from the organizational context. An analysis of the Russian SMArt Retro registry has shown that even with the increased availability of pathogenetic drugs, the timing of diagnosis verification and patient routing remains essential [5]. Studies on the functional classification of 5q-SMA patients demonstrate the need for unified and sensitive monitoring tools, since the choice of scales directly affects the interpretation of the therapeutic response [7]. Consequently, the success of splicing correction therapy in real practice is determined not only by the properties of the molecule, but also by the quality of screening, logistics, interdisciplinary monitoring, and standardization of endpoints [5, 7, 8].

include the missing exon, but to programmatically skip it to restore the open reading frame and synthesize a shortened but partially functional dystrophin. This approach conceptually brings severe dystrophinopathy closer to the milder phenotype of Becker muscular dystrophy. Its main advantage lies in its targeting, while the main limitation is mutational specificity: each drug is effective only in a subgroup of patients with a certain type of DMD gene rearrangement. [1, 6, 9, 11].

Russian research confirms the practical importance of this area. In the work devoted to the applicability of exon skipping in the Russian population of patients with Duchenne myodystrophy, it was shown that the potential suitability for such therapy depends on the structure of the deletion and requires careful genetic mapping at the stage of primary diagnosis [6]. The experience of using exon 45 skip therapy indicates the possibility of personalized use of this technology in selected patients [1]. A review of wiltolarsen clinical trials shows that an increase in dystrophin levels and a slowdown in functional decline are considered key arguments in favor of exon skipping, however, a final assessment of the long-term clinical benefit requires longer follow-up and comparable control groups [9]. A foreign analysis of golodirsen approval also highlights that for dystrophinopathies, splicing correction therapy remains highly personalized and does not eliminate all the phenotypic variability of the disease [11].

Table 2 - Clinically significant data on splicing correction

Nosology and approach	Design or source	Main results	Key constraints
5q-SMA, nussinersen	Extended Access Program in Russia [2]	Improvement or stabilization of motor functions and an acceptable safety profile in real practice are noted.	Intrathecal administration, dependence of the effect on the time of initiation of therapy
5q-SMA, Risdiplam	FIREFISH, a foreign clinical trial [12]	Increased levels of functional SMN protein, improved motor and respiratory parameters in infants	The need for long-term observation and comparison with historical and real cohorts
5q-SMA, routing and monitoring	SMArt Retro register and work on functional classes [5, 7]	The importance of early diagnosis verification and standardization of response assessment scales is shown.	Heterogeneity of cohorts and organizational differences between centers

Duchenne myodystrophy, missing exon 45	Domestic clinical experience [1]	The feasibility of personalized exon skipping in genetically selected patients has been confirmed	Narrow mutational applicability and the need for precise molecular typing
Duchenne myodystrophy, viltolarsen and golodirsen	Review of clinical trials and foreign analysis [6, 9, 11]	There are signs of dystrophin recovery and slowing of functional decline.	Long-term clinical benefit requires further confirmation

A comparison of the two main clinical models - SMA and Duchenne muscular dystrophy - allows us to identify several common patterns. Firstly, the therapy is more effective the earlier the intervention is initiated and the greater the cellular reserve of the target organ. Secondly, even a convincing molecular effect is not always automatically transformed into an equally pronounced functional benefit, since the clinical outcome is influenced by age, stage of the disease, initial neurological deficit and concomitant supportive therapy. [1-3, 5, 7, 9, 12]. Thirdly, the transition from registration studies to real practice reveals new challenges: the need for regular reassessment of the functional status, economic sustainability of treatment, and long-term safety monitoring.

The issue of delivery deserves a separate discussion. Intrathecal administration of nussinersen provides access to the central nervous system, but is associated with invasiveness, which is especially important in children with scoliosis and severe orthopedic deformities [2, 3, 8]. Oral risdiplam is more convenient in terms of adherence, but the choice between drugs is determined not only by the route of administration, but also by the patient's age and clinical task and the already formed level of loss of function. For exon skipping in dystrophinopathies, the barrier is not so much the route of administration as the heterogeneity of the mutation spectrum, the limited applicability of individual molecules, and the need for strict genetic stratification. [1, 6, 9, 11].

One biochemical marker is not enough to evaluate the effectiveness of splicing-correcting agents. In SMA, an increase in SMN protein levels and stabilization of neuromuscular function should correlate with the achievement of age-related motor skills, the duration of independent sitting, the need for respiratory support, and the dynamics of standardized scales. In dystrophinopathies, quantitative assessment of dystrophin, remote and outpatient tests, as well as the rate of walking loss come to the fore. That is why Russian works on functional stratification and registry monitoring are of no less practical importance than reviews of medicinal molecules: they set a coordinate system in which the molecular effect and clinical benefit can be compared. [2, 5, 7, 9, 12].

The safety profile of splicing corrective drugs should also be analyzed differentially. For nussinersen, not only pharmacological, but also procedural risks associated with repeated intrathecal injections are important. Long-term systemic monitoring is of key importance for risdiplam, since the treatment is designed for long-term use. In the case of exon skipping in Duchenne myodystrophy, the problem of interpreting intermediate biomarkers remains significant: an increase in the amount of dystrophin is not always immediately translated into a proportional slowdown in clinical deterioration, which requires caution when extrapolating early results to a long-term prognosis [2, 9, 12].

The prospects of the direction are related to further personalization of antisense molecule design, improvement of tissue delivery, development of combined approaches and the use of national registries as a tool for patient selection and post-registration effectiveness assessment. Russian review papers also point to the need to expand neonatal SMA detection and unify clinical routes, since it is the early verification of diagnosis that provides the maximum window for realizing the potential of splicing corrective therapy. In the broader context of hereditary diseases, this area represents a model for the transition from general symptomatic care to targeted effects on the biosynthesis of pathologically altered RNA.

At the same time, it should be borne in mind that not every splicing anomaly is equally suitable for drug correction. Validated targets, standardized biomarkers, and sufficiently large clinical samples are still missing for a number of rare nosologies. Therefore, further development of technologies will require a combination of high-resolution molecular diagnostics, clinical stratification of patients, and adaptive research designs that allow evaluating the therapeutic effect in small cohorts without loss of evidence [4, 8, 11].

The translation of experimental RNA platforms into a routine clinic is complicated not only by biological, but also by regulatory factors. For ultra-rare variants and individualized designs, it is especially difficult to form comparable control groups and select endpoints that simultaneously reflect the molecular response and have clinical significance. In this regard, the role of adaptive designs, consistent verification of biomarkers, and long-term post-marketing surveillance is increasing. Foreign experience in the development of oligonucleotide drugs shows that the transition from experimental evidence of feasibility to sustainable clinical practice requires standardization of production, reproducible quality control, and transparent patient selection criteria [8, 11].

For Russian practice, this means the need to further centralize molecular diagnostics and strengthen the link between the laboratory conclusion, the clinical center and the patient registry. In spinal muscular atrophy, such integration is especially important, since the decision to choose nussinersen or risdiplam should be made taking into account age, functional class, rate of progression, and therapy already implemented. In Duchenne myodystrophy, a detailed interpretation of the mutational variant becomes a key condition, on which the very fundamental possibility of exon skipping depends. Consequently, the clinical value of splicing correction increases as diagnostic routing improves and registry data on long-term outcomes accumulates. [1, 5, 6, 10].

Another important area is the unification of follow-up after the start of therapy. Splicing-corrective drugs rarely allow you to evaluate the final result in a short time interval, so the doctor needs to monitor not only the early dynamics, but also the trajectory of the functional status over the months and years. This requires the consistent use of motor scales, respiratory indicators, genetic data, and information on treatment tolerance. Without such standardization, the comparison of results from different centers and different therapeutic platforms will be limited, and conclusions about the clinical benefit will be less stable [5, 7, 8].

Finally, the economic and organizational dimension of splicing correction therapy goes beyond pharmacology. The high cost of long-term courses, the need for multidisciplinary management, repeated functional tests and the creation of registry infrastructure form a new model of care for patients with rare hereditary diseases. Therefore, the clinical effectiveness of these technologies should be evaluated together with indicators of accessibility, commitment and sustainability of the healthcare system to long-term care for such patients.

CONCLUSION

Splicing correction is currently one of the most mature forms of molecular-based therapy for hereditary diseases. The greatest clinical progress has been achieved in 5q-associated spinal muscular atrophy and Duchenne myodystrophy, where drugs have been developed and implemented that either enhance the inclusion of a functional exon or provide programmable skipping of a transcript section to restore the reading frame. For SMA, the results of therapies affecting SMN2 are particularly convincing, whereas in dystrophinopathies, the promise of exon skipping is combined with pronounced mutational selectivity.

The analysis shows that the further development of the field will be determined by early diagnosis, standardization of response criteria, accumulation of real-world practice data and personalized design of molecules for specific genetic variants. Thus, splicing-correcting technologies form the most important vector of modern medical genetics, combining fundamental concepts of RNA processing with clinical results and a real opportunity to change the course of previously incurable hereditary diseases.

LIST OF REFERENCES

1. Artemyeva S. B., Belousova E. D., Vlodavets D. V. Experience of using exon 45 skipping therapy in patients with progressive Duchenne muscular dystrophy // Medical genetics. - 2024. - Vol. 23, No. 1. - pp. 19-25. - DOI: 10.25557/2073-7998.2024.01.19-25.
2. Artemyeva S. B., Belousova E. D., Vlodavets D. V. Efficacy and safety of nusinersen drug in the framework of the extended access program in Russia // Neuromuscular diseases. - 2020. - Vol. 10, No. 3. - pp. 35-41. - DOI: 10.17650/2222-8721-2020-10-3-35-41.
3. Artemyeva S. B., Shidlovskaya O. A., Papina Yu. O. Approaches to pathogenetic therapy of spinal muscular atrophy in children and newborns // Neuromuscular diseases. - 2024. - Vol. 14, No. 1. - pp. 86-92. - DOI: 10.17650/2222-8721-2024-14-1-86-92.
4. Vlasenko A. I., Nazarov V. D., Lapin S. V. Molecular mechanisms of neurodegeneration in spinal muscular atrophy // Neuromuscular diseases. - 2024. - Vol. 14, No. 3. - pp. 72-80. - DOI: 10.17650/2222-8721-2024-14-3-72-80.
5. Vlodavets D. V., Shchagina O. A., Polyakov A.V., Kutsev S. I. SMArt Retro study: a retrospective analysis of data from the Russian registry of patients with spinal muscular atrophy // Neuromuscular diseases. - 2024. Vol. 14, No. 3. pp. 54-71.
6. Zinina E. V., Bulakh M. V., Ryzhkova O. P. The possibility of therapy aimed at skipping exons in Russian patients with Duchenne myodystrophy: present and future // Neuromuscular diseases. - 2024. - Vol. 14, No. 3. - pp. 12-23. - DOI: 10.17650/2222-8721-2024-14-3-12-23.
7. Papina Yu. O., Melnik E. A., Belousova E. D. Determination of functional class criteria in patients with spinal muscular atrophy 5q // Neuromuscular diseases. - 2024. - Vol. 14, No. 4. - pp. 58-70. - DOI: 10.17650/2222-8721-2024-14-4-58-70.
8. Rodenkov E. M., Kozhemyakina N. V., Zonis Yu. A. Modern approaches to therapy of spinal muscular atrophy // Genes and cells. - 2023. - Vol. 18, No. 3. - S. 189-204. - DOI: 10.23868/gc437467.
9. Suslov V. M., Rudenko D. I. Efficacy and safety of Viltepso® in Duchenne myodystrophy: a review of clinical trials // Russian Journal of Pediatric Neurology. - 2024. - Vol. 19, No. 3. - pp. 38-50. - DOI: 10.17650/2073-8803-2024-19-3-38-50.
10. Toropova K. A., Kurskova A. I., Korotkov M. Yu. Therapy of spinal muscular atrophy at the present stage: a review // Comorbid neurology. - 2025. - Vol. 2, No. 3. - pp. 51-59. - DOI: 10.62505/3034-185X-2025-2-3-51-59.
11. Aartsma-Rus A., Corey D. R. The 10th Oligonucleotide Therapy Approved: Golodirsen for Duchenne Muscular Dystrophy // Nucleic Acid Therapeutics. - 2020. - Vol. 30, No. 2. - P. 67–70.
12. Baranello G., Darras B. T., Day J. W. Risdiplam in Type 1 Spinal Muscular Atrophy // The New England Journal of Medicine. - 2021. - Vol. 384. - P. 915–923. - DOI: 10.1056/NEJMoa2009965.