A *Unique* Method, based on the Maximum Ordinality Principle, for Skipping *any* Exon in Duchenne Muscular Dystrophy (DMD)

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***Abstract* – The present article aims at showing the possibility of adopting a *Unique* Method for skipping any Exon, in Duchene Muscular Dystrophy (DMD), by means of the three following methodologies: first generation AONs, Morpholinos, second generation AONs (in short: f. g. AONs, Morpholinos, s. g. AONs). In each case, the Method leads to recognize the optimal Antisense and its corresponding Exon Skipping Efficiency for each methodology considered.**

This means that, in the case of three different Efficiencies, these will manifest a "hierarchy", which however is not always the same, because the "hierarchy" varies according to the specific Exon to be skipped. In addition, the same Method can be adopted in the case of multiple-drug Therapies.

Keywords- Exon-Skipping, Duchene Muscular Dystrophy (DMD), Maximum Ordinality Principle (MOP)

1. Introduction

The research for a *Unique Method* aimed at skipping *any* type of Exon, in the case of DMD, is strictly related to the fact that, at present, the research is usually focused on a *limited number* of Exons, generally corresponding to the most frequent cases of DMD, in the population of children affected by such a severe pathology.

This limitation is essentially due to: i) the expected *return* on investments on behalf of various Research Institutes and Pharmaceutical Companies; ii) the very rigid authorization procedures and associated conditions required by FDA and EMA, especially as far as the *wide number* of cases positively tested, as a solid base from a statistical point of view.

Both these reasons are evidently impossible to be satisfied in the case of very rare Exons, such as, for instance, Exon 39 (two sole cases in all Italy).

The research for a Unique Method, vice versa, could potentially overcome the two above-mentioned conditions.

2. The Rational of the Method

The Method consists in modeling both the considered Exon, the pertinent Antisense and the Final Compound as three *Self-Organizing Systems*, all of them described in the light of *the Maximum Ordinality Principle* (MOP), widely illustrated in [10].

In favor of the validity of the Method it is worth recalling that the latter is nothing but the transposition of the same method already adopted in the case of Protein-Protein Interaction (PPI) [1], where the process was analogously modeled in the light of the MOP.

Any interaction process, in fact, when modeled in mere "functional" terms, is always characterized by an intrinsic unsolvability in explicit terms, as a consequence of the famous "Three-body Problem" (H. Poincaré, 1889, more explicitly recalled in [1],[2]).

The same difficulties, of course, arise in the case of Exon-Antisense Interaction, because this is another process usually described in simple "functional" terms.

The MOP, vice versa, overcomes the limitations associated to the "Three-body Problem" and, consequently, when both Exon and Antisense are modeled as Self-Organizing Systems in the light of the MOP, the *explicit solution* to the interaction process can be obtained, in a fast and reliable way, as the *formal solution to an N-body interaction problem*.

The Method here presented is specifically referred to Exon 39, not only for the reasons previously anticipated, but precisely because such an Exon has some special characteristics that facilitate the transposition of the same Method to all the other Exons.

3. Reconfiguration of Exon 39

The modeling of the Exon Skipping process starts with the Ordinal Reconfiguration of Exon 39. Where the expression "Ordinal Reconfiguration" means that Exon 39 is modeled as a Self-Organizing System, characterized by its specific Generativity, which tends to structure the Exon according to the MOP (see Ref. [10]).

This means that the 138 Bases of Exon 39 are related to each other in terms of Ordinal Relationships, of *Generative Nature*, that, for their specific characteristics, can be termed as "Harmony Relationships" (ib.).

On the basis of such specific properties, the Ordinal Structure of Exon 39 can be obtained by simply defining the topology of an arbitrary couple of Bases, assumed as reference, together with some associated parameters which characterize the entire Structure of the Exon in the space.

Such a limited number of parameters, in fact, represent the input to a Simulator, termed as EQS (Emerging Quality Simulator), which is precisely based on the MOP and its associated Harmony Relationships.

The input to the Simulator is then represented by:

i) the *total number* of Bases of Exon 39 (*N1*);

ii) three topological parameters $(\Sigma_{12}, \Phi_{12}, \Theta_{12})_{N1}$ that define, in polar coordinates, the reciprocal positions of two *arbitrary* Bases (conventionally termed as "12"), understood as being *one sole* "*Ordinal" entity*. This is also the reason why the latter is topologically referred to its proper internal reference system;

iii) five additional parameters $(\varepsilon_1, \varepsilon_2, \lambda, \psi_1, \psi_2)_{N1}$ which, together with those previously mentioned, complete the definition of the so-called internal *Relation Space* (RS) of the Exon analyzed.

More specifically: ε_1 and ε_2 characterize the spatial orientation of the Exon (understood as a Whole), with respect to its internal reference axes; while $(\psi_1, \psi_2, \lambda)_{N1}$ define the periodicities (along the three basic axes) of the mathematical solutions which "emerge" from the MOP.

Such solutions are precisely those that give the positions of all the Bases with respect to the internal axes of the considered Exon. In this way, the afore-mentioned solutions characterize any considered Exon as a *unique*, *specific* and *irreducible* entity.

This also the reason why Exon 39, precisely because modeled as a "Self-Organizing" System of *Ordinal Nature* (see [10]), is also characterized by its own specific *self-organizing capacity*, whose *activity* can faithfully be represented by its associated "virtual work", defined (in polar coordinates) as

$$W = \sum_{j=2}^{N} \{(\rho_{1j}) + (\rho_{1j}\varphi_{1j}) + (\rho_{1j}\vartheta_{1j})\} \qquad (1),$$

where the subscripts 1j indicate the couples of Bases successively considered in the sum.

In the case of Exon 39, the total number of Bases equals 138 and, by assuming that:

$$(\Sigma_{12}, \Phi_{12}, \Theta_{12})_{N1} = (0.05,\ -0.15,\ 0.03) \qquad (2)$$

$$(\varepsilon_1, \varepsilon_2)_{N1} = (-100, -4000) \qquad (3)$$

and $$(\psi_1, \psi_2, \lambda)_{N1} = (1, 1.5, 2) \qquad (4)$$

we can obtain the Ordinal Reconfiguration of Exon 39, as represented in Fig. 1, which is characterized by a Virtual Work W1 = 53.96.

4. The research for the optimal f. g. AON that leads to the most efficient Exon Skipping process

The research for the most appropriate f. g. AON to skip Exon 39 can be articulated in two steps.

The first step consists in assuming as a *starting reference* AON characterized by an integer number of Bases (N2) that approximates the ratio N1/10.

In our case:

$$N2 = N1/10 = 138/10 = 13.8 \qquad (5)$$

which can consequently be rounded to N2 = 14.

At this stage, in order to maximize the reciprocal "electivity" between any selected AON and Exon 39 to be skipped and, at the same time, to minimize any possible future "mis-targeting", the input parameters to EQS, finalized to find the most appropriate AON, are assumed exactly equal to the corresponding parameters of Exon 39. In this way both Exon 39 and any considered AON will be characterized by the *same Relational Space* (RS).

The same assumption is also adopted to characterize the Relational Space of the Final Compound, this is because the latter is precisely that Self-Organizing System which emerges from the interaction process between Exon 39 and any selected AON.

The second step substantially consists in an iterative procedure finalized to choose the most appropriate f. g. AON.

In fact, by considering that the Final Compound is made up of $\{N_1 + N_2\}$ Bases, the same EQS directly shows on the screen of the computer the Topological Configuration of such a Final Compound, as the result of the Interaction process between Exon 39 and any arbitrary AON previously selected.

From the Topological Configuration of the Final Compound it is possible to recognize whether the latter represents an Exon Skipping process and, in particular, what is its correspondent Efficiency.

Such an evaluation can also be confirmed by means of some specific Indicators elaborated by EQS in an appropriate section. One fundamental Indicator, among others, is represented by the ratio

$$(\delta W)_r = \{W_3 - (W_1 + W_2)\}/(W_1 + W_2) \qquad (6),$$

that is: the excess of the Virtual Work of the final compound (W_3), with respect to the sum $(W_1 + W_2)$ between the Virtual Works of Exon 39 and that of the related AON adopted, when the previous difference is referred to the latter sum.

Indicator (6) is particularly meaningful because it accounts for two different aspects: the "deformation" (in length and/or in width) of the Final Compound and, at the same time, the specific typology of its "spatial re-conformation", characterized by a more or less degree of "chirality".

The optimal AON, in fact, is precisely that characterized by a number of Bases which leads to the minimum negative value of ratio (6). That is

$$\min \{ (\delta W)_r \} < 0 \qquad (7).$$

The optimal number of Bases can consequently be found by means a simple iterative procedure. In fact, through successive steps, by adopting as an input to EQS both a progressively increasing and decreasing number of Basis of the considered AON (in our case: 15, 16, 17, 18, etc., as well as 13, 12, 11, etc.), it is possible to find the appropriate value of N2 that satisfies condition (7).

In the case of Exon 39, the optimal value of N2 equals 18, because this is precisely the number of Bases that leads to the minimum value

$$(\delta W)_r = -0.056 \qquad (7.1).$$

This is because

$$(W_1, W_2, W_3) = (53.96,\ 11.94,\ 62.16) \qquad (7.2).$$

The corresponding Ordinal Configuration of such an AON, made up of 18 Bases is represented in Fig. 2, where the Bases 15, 16, 17 are explicitly pointed out because they will be reconsidered later on.

At the same time, Fig. 3 represents the Topological Configuration of the Final Compound, from which it is possible to recognize the corresponding wide "disarticulation" that indicates the Efficiency of the Exon Skipping process.

More precisely, Fig. 3 represents the "Evolutionary Efficiency" of the Exon Skipping process, which can be quantified on the basis of Eqs. (7.1) and (7.2). In fact, the corresponding values are equivalent to write

$$W_3 = 0.944 \cdot (W_1 + W_2) \qquad (7.3).$$

This means that the Final Compound, in spite of its extremely reduced level of *chirality*, it is able to manifest a wide "disarticulation". This result allows us to assume an Evolutionary Efficiency of the Exon Skipping process equal to 94.4 %.

This value, however, only refers to the Exon Skipping as an Evolution Process. In fact, it does not accounts for the "resistance" due to Introns that surround Exon 39.

The corresponding efficiency of such a "detaching process" can be a valuated, in analogy to Eq. (6), by considering the following ratio

$$(\delta W)_r^{*} = \{(W_1 + W_2) - W_I\} / (W_1 + W_2) \qquad (8)$$

where W_I represents the Virtual Work associated to the "resistant" Introns.

In this respect, if we assume $W_I = 0.5 \cdot W_1$, we have

$$(\delta W)_r^{*} = (53.96 + 11.94) - 0.5 \times 53.96 / (53.96 + 11.94) = 59.06\%. \qquad (8.1).$$

This means that the global Efficiency of the Exon Skipping process is given by the product of the two previous different efficiencies, that is

$$59{,}06\ \% \times 94.4\% = 55.75\% \qquad (9).$$

Such a result indicates that the "Evolutionary" Efficiency of 94.4 % is widely reduced by the efficiency of the "detaching process".

The value of the Global Efficiency (9) evidently depends on the extension of Introns and it can evidently vary according to the considered Exon analyzed.

In all cases, the values so obtained are always a little higher than the corresponding values of about 30% that can be found in Literature ([5]), especially because the latter do not usually refer to optimal efficiencies.

This also depends on the fact that the methodology of evaluation here considered, based on the MOP, accounts for *Quality* aspects that are characteristic of Self-Organizing Systems. Aspects that, vice versa, are usually neglected on behalf of any com-possible functional approach.

5. The reproduction in Laboratory of the f. g. AON previously obtained

Such a reproduction can obtained by starting from a f. g. AON already known in Literature, so as to introduce some modifications in order to get a sufficiently faithful reproduction of the Ordinal AON previously obtained.

In this process, however, it is worth remembering that any reproduction in Laboratory always represents a "*cardinal reduction*" of the reference Ordinal AON, because the latter was obtained in Ordinal terms. Consequently, the "reproduction" is always thought in terms of "forces", whereas the AON previously obtained is structured in terms of Ordinal Relationships.

As an example, it is possible to consider the AON H48AON2, made up of 21 Bases. So that, after having eliminated the three "C" as indicated in Fig. 4, and also having contextually introduced the rotations of the Bases 15, 16, 17 as indicated in the same Fig. 4, it is possible to get a "satisfactory representation" of the reference Ordinal AON.

Besides such an approach, another possibility consists in introducing a slight modification in the SR of the f. g. AON previously found, in order to start from a Reconfiguration which is easier to be reproduced in cardinal terms and, at the same time, it always presents a satisfactory value of the Global Efficiency of the Exon Skipping process.

This exit can be obtained by modifying the value of ψ_2 from 1.5 to 1.

In this way the corresponding Ordinal AON assumes the Configuration indicated in Fig. 5, which appears as being easier to be reproduced in Laboratory.

In this case the Exon Skipping process presents a value of $(\delta W)_r$ = -0.0797 and, correspondently, an Evolutionary Efficiency of a 92.03 %, which is a little lower than the previous case. Such a value, however, can be considered as being acceptable, in the light of the fact that such an approach facilitates the cardinal reproduction of the Ordinal AON.

Correspondently, the "detaching" Efficiency is equal to 53.56 %. This means that the global Efficiency of the Exon Skipping process based on this "modified" Ordinal AON equals 49.29%. A value that is a little lower than the case of the original Ordinal AON. However, such a "modified" configuration facilitates its reproduction in functional "cardinal terms", by starting from similar AONs already known in Literature.

In addition, it is worth considering that the above mentioned modification of the value of ψ_2 (from 1.5 to 1) does not affect very much the initial assumption about the equality of the Relational Spaces: SR2 = SR1.

6. The research for the optimal Morpholino that leads to the most efficient Exon Skipping process

The Method for such a type of research is perfectly analogous to the one previously shown. In fact:

- the Reconfiguration of Exon 39 is exactly the same as the one considered in the previous case
- the only difference consists in the fact that AONs are now replaced by Morpholinos.

In a perfect analogy with the previous case, the first step consists in assuming a reference Morpholino, whose Relational Space (RS) is characterized by the following parameters:

$$(\Sigma_{12}, \Phi_{12}, \Theta_{12})_{N2} = (0.05,\ 0.05,\ 0.01) \qquad (10)$$

$$(\varepsilon_1, \varepsilon_2)_{N2} = (-1500\ ,\ -2000\ ) \qquad (11)$$

$$(\psi_1, \psi_2, \lambda)_{N2} = (\ 1\ ,\ 1.2\ ,\ 1) \qquad (12).$$

Such values of the RS, however, can also be slightly modified, if necessary, in order to improve the reciprocal "electivity" with the Exon 39, so as to minimize any possible future "mis-targeting".

The second step consists in an analogous iterative procedure finalized to choose the Optimal Morpholino, characterized by the most appropriate number of Bases.

The number of Bases generally considered ranges in the interval from 25 to 34 pairs of Bases (N2).

At this stage, by considering that the Final Compound is made up of (N1+ N2) Bases, the same EQS Simulator directly shows, on the screen of the computer, through successive tests, the Topological Configurations of the Final Compound, as the result of the Interactions between Exon 39 and each Morpholino previously selected.

To this purpose, by considering that the Relational Space SR2 of the Morpholino differs from SR1, the Relational Space SR3 of the Final Compound will be defined on the basis of the mean values between SR1 and SR2.

In this way the Topological Reconfigurations of the Final Compound enable us to recognize when, in actual fact, there is an Exon Skipping and, in particular, what is its correspondent Efficiency.

Such an Efficiency can be obtained on the basis of the fundamental Indicator represented by the ratio (6), which enables us to recognize that the optimal Morpholino is the one characterized by 27 pairs of Bases. In fact, the corresponding value of the fundamental indicator is

$$(\delta W)_r = -0.117 \qquad (13),$$

which corresponds to an Efficiency of 88,3 %.

The spatial Configuration of this Morpholino is given in Fig. 6, while Fig. 7 represents the Topological Configuration of the Final Compound, which shows a corresponding "disarticulation" which indicates the Efficiency of the Exon Skipping process.

More precisely, Fig. 7 represents the "Evolutionary Efficiency" of the Exon Skipping process.

Such an Efficiency, however, as already seen in the case of the Optimal AON, does not account for the "resistance" do to the Introns that surround Exon 39.

The efficiency of such a "detaching process" can be then evaluated, in analogy with the case of the Optimal AON, always on the basis of Eq. (8). In this case, by considering the particularly high value of W2, it is possible to assume

$$W_I = 0.8 \cdot W_1 \qquad (14),$$

so that we have:

$$(\delta W)_r^* = (53.96 + 127.87) - 0.8 \times 53.96\ /\ (53.96+127.87) = 76{,}25\% \qquad (15).$$

This means that the global Efficiency of the Exon Skipping process is given by the product of the two efficiencies (13) and (15), that is

$$76.25\ \% \times 88.3\% = 67.32\% \qquad (16).$$

This result clearly shows that the "Evolutionary Efficiency" is widely reduced by the efficiency of the "detaching process". Nonetheless, the value obtained is always higher than the corresponding values that can be found in Literature (a little higher than 30%), [6], especially because the latter do not usually refer to optimal efficiencies.

This also depends on the fact that, as already anticipated, the evaluation methodology here considered, based on the MOP, always accounts for *Quality* aspects that are characteristic and specific of Self-Organizing Systems, which, however, are usually neglected on behalf of any com-possible functional approach.

7. The reproduction in Laboratory of the selected Morpholino

Such a reproduction, in analogy with the case of the Optimal AON, may start from considering a particular sequence of Bases typical of Morpholinos, as already available in Literature, and characterized by a similar number of Bases [6]. For example, the sequence:

UUU CCU CUC GCU UUC UCU CAU CUG UGA.

The appropriateness of such a choice can easily be confirmed on the basis of a direct comparison between the Ordinal Topology of the Morpholino (see Fig. 6) and the topological dimensions (and relative distances) of the sequence of Bases considered to reproduce the Morpholino in Laboratory.

Such a comparison is generally sufficient to confirm the preliminary choice. Even if, in some cases, the same comparison may suggest some modifications to the initially considered sequence.

In all cases, a more general procedure consists in modeling the Morpholino as an *Ordinal Matrix*, made up of 27 *Ordinal sub-Matrices*, all related between them in terms of Harmony Relationships, where each one represents a *couple of Bases* of the Morpholino.

Such an Ordinal Topological Configuration represents the best Reference Structure for a more appropriate comparison with the sequence of Bases chosen for the reproduction in Laboratory of the reference Ordinal Morpholino.

7. The research for the optimal second generation AON that leads to the most efficient Exon Skipping process

The Method adopted in this case is perfectly analogous to the previous ones. In fact:

- the Reconfiguration of Exon 39 is the same as the one considered in the two previous cases
- the only difference with the previous cases is that both first generation AONs and Morpholinos are now replaced by second generation AONs.

In a perfect analogy with the previous cases, the first step consists in assuming a starting reference s. g. AON whose Relational Space (RS) is characterized by the following parameters:

$$(\Sigma_{12}, \Phi_{12}, \Theta_{12})_{N2} = (0.05, 0.05, 0.01) \quad (17)$$

$$(\varepsilon_1, \varepsilon_2)_{N2} = (-300, -3000) \quad (18)$$

$$(\psi_1, \psi_2, \lambda)_{N2} = (1, 1.5, 2) \quad (19).$$

Such a set of parameters, however, can also be slightly modified, if necessary, in order to improve the reciprocal "electivity" with the Exon 39, so as to minimize any possible future "mis-targeting".

The second step consists in an analogous iterative procedure finalized to choose the Optimal s. g. AON, characterized by the most appropriate number of Bases.

The number of Bases generally considered ranges in the interval from 15 to 24 Bases (N2).

At this stage, the same EQS Simulator directly shows on the screen of the computer, through successive tests corresponding to the above mentioned different number of Bases, the Topological Configurations of the Final Compound, made up of N1+N2 Bases, as the result of the Interactions between Exon 39 and each s. g. AON previously selected.

Also in this case, however, by considering the differences between the Relational Spaces SR2 and SR1, the Relational Space SR3 of the Final Compound is usually defined on the basis of the mean values between SR1 and SR2.

In this way the Topological Reconfigurations of the Final Compound enable us to recognize when, in actual fact, there is an Exon Skipping and, in particular, what is its correspondent Efficiency.

Such an Efficiency can be obtained on the basis of the fundamental Indicator represented by the ratio (6), which enables us to recognize that the optimal s. g. AON is the one characterize by 18 Bases.

In fact, the corresponding value of such a fundamental indicator is

$$(\delta W)_r = -0.204 \quad (20),$$

which corresponds to an Evolutionary Efficiency of about 80%.

The spatial Ordinal Configuration of this s. g. AON is given in Fig. 8, while Fig. 9 represents the corresponding Topological Configuration of the Final Compound, from which it is possible to recognize its related "disarticulation" that indicates the degree of Efficiency of the Exon Skipping process.

More precisely, Fig. 9 represents the "Evolutionary Efficiency" of the Exon Skipping process.

Such an Efficiency, however, as already seen in the previous cases, does not account for the "resistance" due to the Introns that surround Exon 39.

The efficiency of such a "detaching process", in analogy with the previous cases, can be evaluated on the basis of Eq. (8).

By assuming that $W_I = 0.5 \cdot W_1$, we have

$$(\delta W)_r^* = (53.96 + 21.21) - 0.5 \times 53.96 / (53.96 + 21.21) = 64.10\%. \quad (21).$$

This means that the global Efficiency of the Exon Skipping process is given by the product of the two efficiencies (20) and (21), that is

$$64.10\% \times 80\% = 51.28\% \quad (22).$$

This result shows that the "Evolutionary Efficiency", as in the previous cases, is widely reduced by the efficiency of the "detaching process". Nonetheless, such a value is always higher than the corresponding values of 30-40% that can be found in literature ([7]).

This always depends on the same already mentioned aspect: the evaluation methodology here considered, based on the MOP, usually accounts for *Quality* aspects that are characteristic and specific of Self-Organizing Systems, which, vice versa, are usually neglected on behalf of any functional approach.

8. The reproduction in Laboratory of the selected s. g. AON

Such a reproduction, in analogy with the case of Morpholino, may start from considering a particular sequence of Bases typical of s. g. AONs, already available in Literature (e. g. [7]), and characterized by a similar number of Bases. For example, in the case of 18 Bases, we can consider the following sequence

UUU CCU CUC GCU UUC UCU.

Also in this case, the appropriateness of such a choice can be confirmed on the basis of a direct comparison between the Ordinal Topology of the s. g. AON and the topological dimensions (and relative distances) of the sequence of Bases considered in its reproduction in Laboratory.

Such a comparison is generally sufficient to confirm the preliminary choice. Even if, in some cases, the same comparison may suggest some modifications to the initially considered sequence.

However, as in the case of the Morpholino, a more general procedure consists in modeling the Ordinal s. g. AON as an *Ordinal Matrix*, made up of 18 *Ordinal sub-Matrices*, all of them related in terms of Harmony Relationships, where each one represents a single Base of the Ordinal s. g. AON.

At this stage, it is worth considering some further important properties of the Method here proposed.

9. Informatics Advantages

The informatics advantages of the present Method are directly referable to the *formal properties* that are intrinsic to the mathematical models adopted. In fact, any system, when modeled on the basis of the MOP, always presents *explicit solutions* in terms of *Incipient Differential Calculus* (see [3] and [4]).

This means that the Method here proposed has the *capacity* of *predicting, in explicit formal terms, the 3D structure of the resulting compound* essentially because the latter is understood as a Self-Organizing System of *ordinal nature*. [8],[9].

This correlatively means: i) *a reduced number of computations*; ii) *a total absence* of *High Performance Computing* (HPC); iii) *a reduced incidence* of *special numerical methods* to be adopted in order to get the corresponding solution (ib.).

What's more, the explicit solutions so obtained can also be termed as "*emerging solutions*", because *they always show an ordinal information content which is much higher than the corresponding content of the initial formulation of the problem*. (ib.).

This is because the MOP is specifically finalized to describe "Self-Organizing" Systems according to a *holistic approach*, in which, as is well-known, "*the whole is much more than the sum of its parts*".

In such a perspective, all the biological processes can always be modeled as being "Self-Organizing Systems" in the light of the MOP [10].

10. General Applicability of the Method to skip all the Exons in DMD

As a consequence of the previous considerations, the Method illustrated in the case of Exon 39 can be considered as having a general applicability *to all the other Exons of Dystrophyn*.

In other words, it can be adopted to skip *any chosen Exon* in the case of DMD.

The Method, in fact, is able to show that:
any Exon, by starting from its initial conditions (that is between its flanking Introns), when interacts with an appropriate Antisense (specifically selected according to the Method previously illustrated), may present a *dominating evolutionary aspect* able to induce a correlative Exon-Skipping process. Such a dominating aspect, in fact, is due to *the internal topological reconfiguration of the Exon, as a consequence of the*

wide variation of its chirality due to the interacting process with the selected Antisense (cfr. [11]).

This evolving process, in fact, ends up by radically "modifying" any considered Exon and, consequently, after having disarticulated the Exon from its flanking Introns, it "guides" the related successive evolution toward the Exon Skipping,

In addition to the previous considerations, it is worth recalling that the Method, although shown with reference to Exon 39, can be considered as being valid *for any other Exon* because of the following reasons:

i) The Method has a very general validity because it is based on the MOP, whose validity is even more general than some well-known Principles considered in the Traditional Approach. Such as, for instance, the "Energy Conservation Principle";

ii) This represents the fundamental reason why the Method proposed is precisely the same for analyzing the Exon Skipping of *any* Exon. In fact:

- it starts from the Ordinal Reconfiguration of the considered Exon which is always obtained in the light of the MOP;
- the same happens with reference to the Ordinal Reconfiguration of the Optimal Antisense, characterized by its specific SR;
- the same MOP is correlatively adopted to find the Ordinal Reconfiguration of the Final Compound;
- at the same time, the Method adopts the same Exon Skipping criterion, based on the concept of Virtual Works, that is $dLr < 0$ (Eq. (6)), which is also confirmed by the graphical Topological Reconfiguration of the Final Compound.

11. General validity of the Method

The Method previously illustrated, has already been adopted in the case of Protein-Protein Interaction (PPI) [1]. Thus it can be considered as being applicable to the majority of biological problems usually dealt with through informatics methods.

The same method, in fact, can also be adopted in the case of Molecular Docking and Drug Design (ib.),[2]. This is because the Method enables us to choose the *optimal ligand*, that is the one characterized by the most appropriate "electivity", as already shown in the case of Exon Skipping in DMD.

Consequently, the general validity of the Method here proposed appears much more evident when it is considered in its most appropriate general context, that is, *The Maximum Ordinality Principle*.

In fact, by adopting the MOP as the basic reference criterion, it is possible to realize mathematical models of *all the biological Systems*, with very significant related advantages [9],[10].

Such considerations can also represent, at the same time, the most valid introduction to the next Section.

12. General Conclusions

The Method here proposed seems to be able to give a significant contribution to DMD therapy. This is precisely because the results previously shown indicate that, apart from some other *innovative* aspects, the Method is able to overcome the two "limiting" initial conditions. In fact:

i) the extremely simple procedure to select the most appropriate Antisense for each Exon to be skipped reduces the theoretical and experimental research costs usually finalized to this aim;

ii) in particular, the experimental costs are always reduced precisely because the experimental confirmation tests can always be focused on *one sole* Antisense per *each* Exon. Additional tests, in fact, concerning different Antisenses (although always possible) are not strictly necessary;

iii) in addition, after having shown the Efficiency of the various Antisenses selected with respect to the most frequent cases of DMD, and after having also obtained the pertinent authorization from FDA and EMA, it should result as being much easier to obtain the same authorization also for the case of extremely rare Exons;

iv) This is because the new Antisenses, corresponding to such extremely rare Exons, are always selected on the basis of the *same* and *unique* method, already shown as being valid for the most frequent cases of DMD;

v) without mentioning that the respect of the condition $SR2 = SR1$, which concerns the reciprocal "electivity" between such rare Exons and the corresponding selected Antisenses, reduces a possible "mis-targeting" in each operative Exon-Skipping Process;

vi) by always remembering, above all, that the *Unique* Method here proposed, adopted to describe the dynamic evolutions of any Exon-Skipping process, has a very general validity. This precisely because, as previously said, it is based on the *Maximum Ordinality Principle*, whose validity is not limited to the sole case of the Exon-Skipping process (see [12]);

vii) without forgetting, in addition, that when the Exon-Skipping process is modeled by means an appropriate simulator based on the MOP, such as, for instance, EQS Simulator, that model can be run on a simple personal computer, with a computation time of a few minutes;

viii) this also means that, by adopting the afore-mentioned Method, any researcher would be able to analyze the dynamic behavior of any selected Exon-Skipping process by means of his/her own PC, by simply sitting at his/her own desk;

ix) Such a systematic research can usually lead to a "hierarchy" between the three Antisenses corresponding to the three different methodologies here considered (f. g. AONs, Morpholinos, s. g. AONs). A "hierarchy" which is evidently based on the global Efficiency corresponding to each methodology adopted. Such a "hierarchy", however, as already said, is not "defined" once and far all. In fact, it usually varies according to the specific Exon analyzed;

x) This represents an additional important aspect (among others, of course), that reduces the correlative research investments in order to find the best Antisense to be adopted for each Exon to be skipped.

As a general conclusion we may thus assert that, apart from some always possible improvements of the Method, the present work seems already apt to show the plausibility of a *Unique* Method for the Exon-Skipping process of any Exon in DMD.

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