Development of novel treatments for myotonic dystrophy: in vivo drug discovery

Dr Rubén Darío Artero Allepuz
Faculty of Biological Sciences, University of Valencia

Dr. José Ignacio Borrell Bilbao
Universitat Ramon Llull. Escola Tècnica Superior IQS

Dr. Josep Castells Boliart
Institut Universitari Ciència i Tecnologia Mollet

1. Summary of the project

Background/main objective: We proposed the identification and design of new molecules as future therapeutic agents for myotonic dystrophy type 1 (DM1; OMIM 160900; world prevalence 1/8,000), because there is currently no effective therapy. DM1 is a neuromuscular disease the symptoms of which are typically myotonia, muscle weakness, muscle degeneration and pain. The disease is characteristically multisystemic including cardiovascular and digestive impairment, hyperinsulinemia and cataracts, among others.

DM1 is a genetic disease associated with the abnormal expansion (>50) of the CTG trinucleotide repeat in a noncoding region of the *DMPK* gene. When the gene is expressed, it generates RNA molecules in which the CUG expansions fold into a hairpin secondary structure that sequesters nuclear proteins, in particular MBNL1, whereas the expression of the *DMPK* does not seem to be compromised.

General methodology: We proposed three complementary approaches: (1) screen of large collections of drug-like compounds; computational design, synthesis and determination of the biological activity of potential inhibitors of (2) MBNL1 binding to the CUG repeat expansions and (3) of potential inhibitors of the folding of the expansion hairpins. The molecules were tested in disease models previously established in *Drosophila*. Positives were validated in a cell model of the disease. This approach is possible because of the multidisciplinary expertise of the group members, the tools already developed, and the previous results in similar large in vivo screens.

The specific aims (SA) of the overall project were the following:

SA1. Generation of the chemical diversity following three complementary strategies
SA2. In vivo screen of chemical suppressors in *Drosophila* models of the disease

SA3. Validation of activity in *Drosophila*

SA4. Validation of activity in mammal models of the disease

SA5. Mechanism of action of the promising compounds (leads)

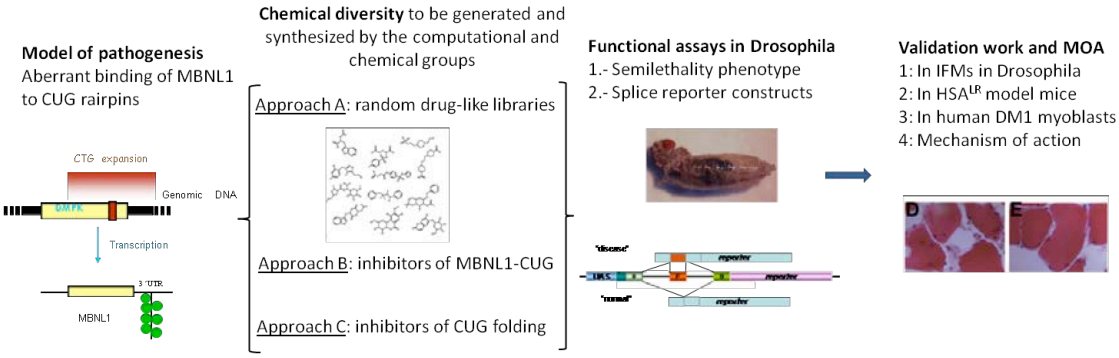

Figure 1. Outline of the general work plan. The first objective was aimed at generating chemical diversity via three complementary approaches. The chemical diversity generated by the computational group will be synthesised by the chemical group of the consortium and tested in vivo in Drosophila models. The initial activities will be validated in vivo in Drosophila and in mammalian models and the mechanism of action of the promising compounds will be explored.

2. Results

The main results achieved were:

1. A computational model of the CUG hairpins was developed by means of molecular dynamics simulations, which made it possible to conclude that the number of CUG repeats directly influences the conformation of the non-canonical U-U pairs.

2. Of a total of 779 compounds tested in the spliceosensor-based *Drosophila* myotonic dystrophy (DM) model (minigen:luc flies; as described in Garcia-Alcover et al. 2014), we have identified three hits (IUCT-309, IUCT-290 and IUCT-169) as interesting for subsequent chemical development into DM leads. The rate of success, approximately 4 hits per 1000 compounds tested, is in agreement with

what was observed independently in the biopharmaceutical laboratory Valentia BioPharma in similar in vivo screens.

3. We have generated experimental evidence that molecules identified in silico in the virtual screening procedure, developed by the IQS partner, actually bind to CUG repeats, thus contributing to defining the LR, RP and TFL series. These data are derived from in vitro fluorescence polarization experiments. The data correlate well with the biophysical predictions and establish a threshold of approximately 100 kcal/mol to score a CUG hairpin binding prediction as likely real.

4. We have generated experimental evidence, for all the hits selected, of activity in a cellular model of the disease derived from skin fibroblasts transdifferentiated to myoblasts. We found significant improvement in disease-associated phenotypes such as number of foci per cell and/or number of cells without foci, and response to the dose of the hit compounds. Additional data generated are toxicity in human myoblasts (IC_{50}) and solubility in water.

5. In the particular cases of LR08 and RP33 we have generated preliminary evidence in human myoblasts suggesting that these molecules can release MBNL1 from its sequestration by CUG repeat expansions.

6. Our preliminary evidence suggests that the hits identified use at least two mechanisms of action. Whereas the compounds IUCT-309, IUCT-290 and IUCT-169 do not bind to CUG repeats and seem to boost the endogenous transcription of *muscleblind* (data not shown), the remaining hits could compete with MBNL1 for its binding to repeats and/or stabilize the repeats in a conformation that inhibits such interaction.

3. Relevance and potential implications

In this project we proposed a search for drugs that could potentially give rise to pharmacological lead compounds for the treatment of myotonic dystrophy. The relevance and clinical implications of the **global** results obtained can be summarized in the following points:

1. We have developed a computational model of the CUG hairpins, which served not only for hit identification (series LR, RP and TFL) but also as a tool to keep on working on the chemical optimization of them. We have optimized several methods for the efficient evaluation of biological activity, in particular the IN Cell Analyzer system, which allows automatic quantification of foci in the cell model of the disease.

2. The in vivo and in silico screens identified six initial hits: IUCT-309, IUCT-290, and IUCT-169 and the families LR, RP and TFL, which will provide the foundations for additional projects aiming at their development as pharmacological leads.

3. As a follow-up of the project, we have been awarded an “Acción Especial en Salud del Instituto de Salud Carlos III (ref. PI13/00386)”, in which we propose to improve parameters of activity, solubility and toxicity of the LR, RP and TFL hits. Under the scientific direction of Prof. Manuel Pérez Alonso, member of the research team, this project teams up the academic partners IQS (Universidad Ramon Llull) and Translational Genomics (Instituto de Investigación Sanitaria INCLIVA), as well as the biopharmaceutical laboratories IUCT (from the InKemia IUCT Group) and Valentia BioPharma.

4. It was possible to file European patent applications with reference numbers EP14382450 (covering the IUCT309 hit) and EP14382449 (covering the TFL family of hits), with priority date 14/11/14.

5. We are currently assessing the convenience of new patent applications to protect the pharmacological development of additional hits (IUCT290 y IUCT-169) as novel drugs for myotonic dystrophy.

6. As a result of these promising results, there is a biotechnology company interested in developing and producing these compounds.

In summary, and beyond the specific results stated above, this project served to establish a long-standing public-private cooperation around the development of drugs for myotonic dystrophy. As a result of this collaboration it will be possible for the results to reach society in the short to medium term, in the form of products that could benefit people afflicted by myotonic dystrophy.

4. Literature generated

Given the biomedical implications of the research carried out, in the short term we have prioritized the protection of commercial rights (patents) over publications. This allows its transfer to the biotechnology sector and also the chemical optimization of the hits identified in collaboration with biopharmaceutical companies. Nonetheless, we are currently putting together a manuscript that will describe the computational model generated, its use in the discovery of drug hits and their validation in in vivo assays, including in human cell lines. Two communications have been presented at meetings:

A. López-González. R. Estrada-Tejedor, J. Borrell, J. Teixidó, Biomolecules modeling implied in myotonic dystrophy type 1 for structure based drug design, RICT 2013 - Drug Discovery and Selection, 3-5 Jul 2013, Nice.

A. López-González. R. Estrada-Tejedor, J. Borrell, J. Teixidó, Biomolecules modeling implied in myotonic dystrophy type 1 for structure based drug design,

International myotonic dystrophy consortium meeting, IDMC-9, 16-19 Oct 2013, San Sebastián.