Enzyme Enhancement Therapy through non-competitive pharmacological chaperones

Juan Aymami [1,2,*], Xavier Barril [3,6], Aida Delgado [3], Marc Revés [4], Rodolfo Lavilla [4], Katsumi Higaki [5], Ana María García-Collazo [1], Laura Rodríguez-Pascau [1], Elena Cubero[1], Pilar Pizcueta [1,] Marc Martinell [1].

1 Minoryx Therapeutics S.L., TecnoCampus Mataró-Maresme. Av. Ernest Lluch 32, TCM2. 08302 Mataró. Barcelona. Spain. Tel. +34 93 702 19 75. Fax- +34 93 702 19 76
Jaymami@minoryx.com
2 Universitat Politecnica Catalunya, Departament d'Enginyeria Quimica. Diagonal 647. 08028 Barcelona. Spain.
3 Universitat de Barcelona, Facultat Farmacia, Dept. Fisico Química. Av. Diagonal 643, 08028 Barcelona. Spain.
4 Universitat de Barcelona, Facultat Farmacia, Dept. Farmacología i Química-Farmacèutica. Av. Diagonal 643, 08028 Barcelona. Spain.
5 Tottori University, Faculty of Medicine, Division of Functional Genomics, Research Center for Bioscience & Technology. Yonago, 683-8503. Japan
6 Catalan Institution for Research and Advanced Studies (ICREA), Passeig Lluís Companys 23, 08010 Barcelona, Spain

Abstract. Most Pharmacological chaperones (PC's) described until now are substrate analogues which bind to the active site of the target protein. Consequently, such PC's also inhibit the target protein at higher concentrations thus rendering a narrow therapeutic window and have poor drug-like properties. Through our proprietary technology platform SEE-Tx™, we identify a new generation of non-substrate competitive pharmacological chaperones which potentially offer a much broader therapeutic window. What's more, such compounds are not substrate analogues, thus presenting much better drug-like properties, particularly for indications with CNS involvement. Here we present our methodology to identify non-competitive pharmacological chaperones applied to the enzyme beta-galactosidase, whose deficiency is related with GM1 Gangliosidosis and Morquio B.

Keywords: Pharmacological chaperones, Chemical chaperones, Enzyme enhancement therapy, GM1, Gangliosidosis, Morqui B, Lysosomal Storage Disease, Lysosomal Storage Disorders.

1 Introduction.

Many monogenic diseases are characterized by the presence of missense mutations which affect the folding and stability of a key enzyme, which is then degraded by the quality control machinery of the cell. This deficiency of enzymatic activity is what

originates the diseases. Pharmacological chaperones are a new class of small molecule drugs, which showed great potential for the treatment of such genetic diseases. They prevent the degradation of unstable enzymes with missense mutations, thus producing an enzyme enhancement effect normalizing enzymatic activity, which reverses and/or prevents disease progression.

There are several examples where pharmacological chaperones showed excellent efficacy in preclinical models. Nonetheless, success in clinical development has been somehow modest. Such pharmacological chaperones usually were substrate-like compounds which competed with such substrate, thus they inhibited the enzyme at higher concentrations and tend to presented poor drug like properties.

Pharmacological chaperone therapy has been largely experimented on the lysosomal storage diseases [1,2], starting with the pioneer work on Fabry disease in 1999[3].

2 Methodology.

Using the three-dimensional structure of proteins and proprietary computational technology, we identify and exploit previously uncharacterized druggable binding sites. In a first step, fast methods are used to identify putatively druggable sites. This is done with fpocket an open source program that automatically identifies cavities and assigns a druggability score[4,5]. This can be supplemented with visual inspection. Interesting systems are then subjected to molecular dynamics simulations with aqueous/organic solvent mixtures (MDmix). Analysis of solvation propensities reveals the presence of binding hot spots, which are clustered to identify druggable binding sites[6]. It is worth noting that the catalytic site of enzymes involved in lysosomal storage diseases are often considered not druggable because they are too small and polar, as expected for sugar-binding sites. However, a significant portion of the proteins investigated (~30%) present allosteric cavities that are predicted as druggable.

A range of organic solvents are investigated with MDmix simulations, and the observed densities for each atom type are converted to binding free energies with a proprietary method, which can be used to predict the binding free energy of a putative ligand[7]. This information is used to supplement a molecular docking program, which evaluates the chemical and shape complementarity of virtual compounds. A unified catalogue containing millions of compounds from a list of preferred vendors can be filtered in this manner. The most promising virtual hits are then purchased and subjected to experimental assays which evaluate effect on protein stability and enzyme enhancement activity (Fig.1).

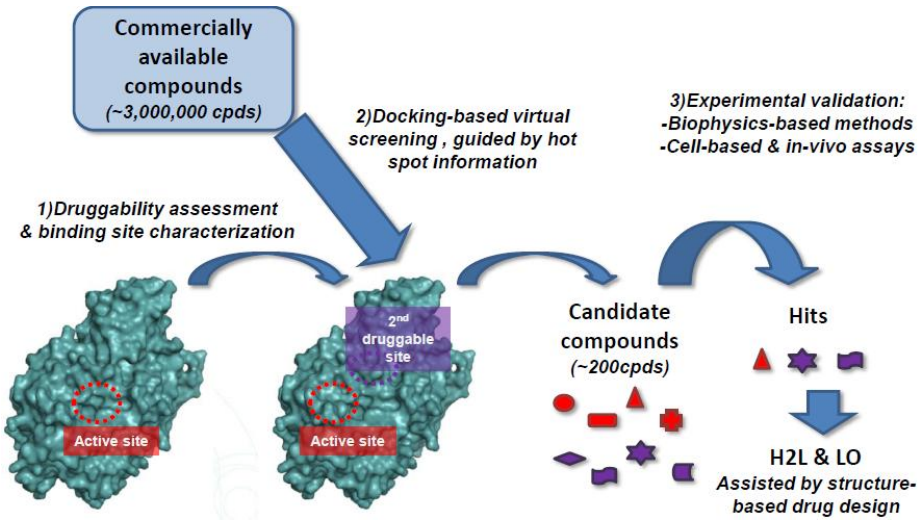

Fig. 1. Self-explanatory graphic of the methodology used to discover non-competitive pharmacological chaperones.

3 Beta-galactosidase deficit disorders (GM1 – gangliosidosis and Morquio B).

Two lysosomal storage disorders, GM1 and Morquio B, are produced by the deficiency of the enzyme beta-galactosidase due to mutations in the GLB1 gene. This enzyme cleaves beta-galactoses from different substrates which are then accumulated in the body. GM1 Gangliosidosis is a neurodegenerative disease that affects severely the CNS and other organs. The disease is classified into three clinical types according to the age of onset and the symptom severity: type I (infantile form), type II (late infantile/juvenile form) and type III (adult form) [8]. Morquio B disease is characterized by typical massive skeletal changes, cornea clouding and deficient cardiac function.

Several compounds have been found as pharmacological chaperones for GM1 being the most advanced NOEV [9] and 6S-NBI-DGJ [10]. Interestingly, although both compounds bind to the active site, they show different responsive profile when tested on a panel of GLB1 mutations [11].

3.1 Screening

We applied our methodology to identify druggable binding sites different from the active-site. We use the published 3D structure obtained by X-ray crystallography and refined to 1.8 Å resolution [12]. A druggable cavity was identified initially with an empirical model implemented in fpocket [4,5]. The druggability of the cavity was then confirmed with a physics-based method consisting in molecular dynamics simulations of the protein in organic-aqueous solvent mixtures [6,7]. The same method was used to identify key interaction sites (binding hot spots), which were used as

pharmacophoric restraints to guide docking. A virtual collection of several million commercially-available compounds were evaluated with the docking program rDock using the standard scoring function, pharmacophoric restraints and a high-throughput protocol[13]. The best scoring compounds were visually inspected and a subset of them (virtual hits) was selected based on the plausibility of the binding mode and chemical diversity considerations. Virtual hits where identified and tested on the DSF assay (Differential scanning fluorimetry). The DSF technique was also used as primary screening to optimize the hits. The hits identified were small molecules with excellent ligand efficiency having potency in the μM range (Fig.2). Those molecules were non-sugar like molecules with a SAR (structure–activity relationship) consistent with the predicted binding mode on the new binding site.

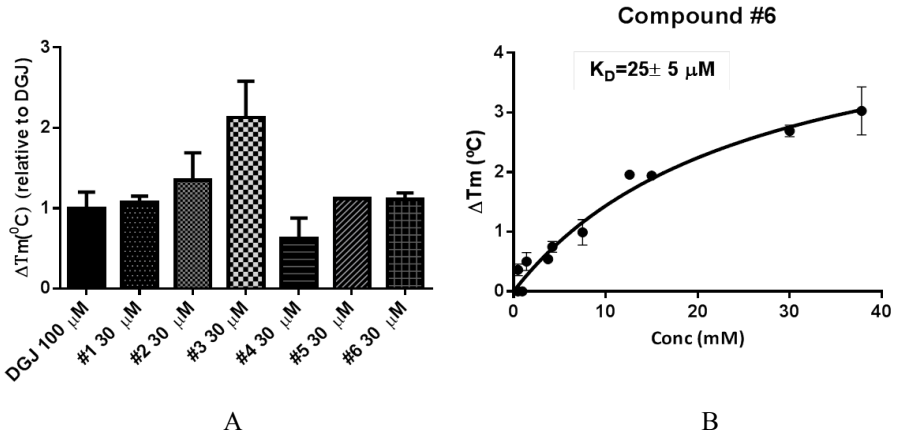

Fig. 2. A) Compounds tested on the DSF assay. ΔTm is expressed as relative to reference compound DGJ. B) Kd for compound #6.

Several chemical series has been identified and round 80 compounds have been synthesized until now on the optimization process.

3.2 Enzyme inhibition.

Contrary to substrate-competitive PC's, our compounds did not present inhibitory activity on the enzyme. To test the enzyme inhibition we compared our compounds with reference compounds such as DGJ and NN-DGJ on an inhibition experiment, both with purified hGLB1 and lysates from human fibroblasts (Fig.3). Similar results were obtained.

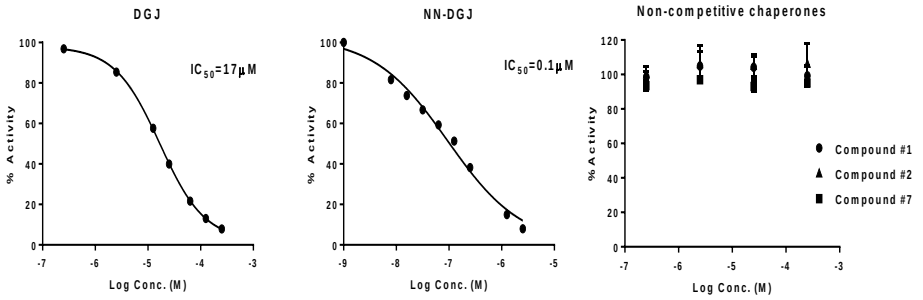

Fig. 3. Inhibitory activity on the enzyme with lysates from normal human fibroblast. A) Reference compound DGJ. B) Reference compound NN-DGJ. C) Non-competitive pharmacological chaperones #1, #2, #7.

3.3 Enzyme enhancement in cells.

We tested enzyme enhancement activity of our compounds on a panel of COS cells transfected with hGLB1, either wild type (WT) or pathologic mutants.

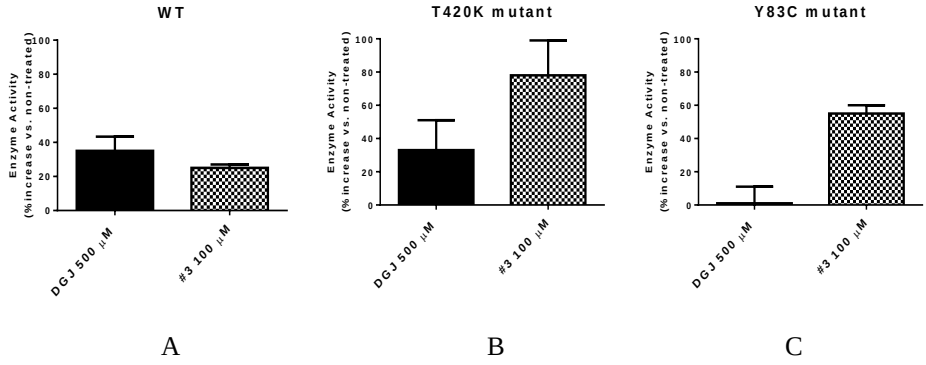

Fig. 4. Enzyme enhancement activity of compound #3 on COS cells transfected on : A) WT protein. B) T420K mutated protein. C) Y83C mutated protein (Morquio B).

Results showed consistent enzyme enhancement activity in cell-based assays either with WT or with selected mutants at 100 µM. The enzyme enhancement activities are on the same range as with DGJ but at 5X lower concentration (Fig.4). Enzyme enhancement activity by using GM1 patients and in mutant mouse fibroblasts is ongoing.

Apparently, responsiveness to mutations is also related to the binding site and the degree of unstabilizing effect of the specific mutation. The profile of responsive mutations and the magnitude of enzyme enhancement may be rather different among non-

competitive and substrate competitive compounds. Combination of pharmacological chaperones targeting different binding sites may offer an avenue for broadening their therapeutic window and increasing the number of responsive mutations.

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