DISCOVERY AND DEVELOPMENT OF A NOVEL NANOMOLAR F508-del CFTR CORRECTOR FOR THE TREATMENT OF CYSTIC FIBROSIS

Bertozzi, F.;[a] Rodríguez-Gimeno, A.;[a] Berti, F.;[a] Giraudo, A.;[a] Bertozzi, S.M.;[b] Ottonello, G.;[b] Armirotti, A.;[b] Caci, E.;[c] Ferrera, L.;[c] Tomati, V.;[c] Sondo, E.;[c] Pesce, E.;[c] Pedemonte, N.;[c] Galietta, L.J.V.;[d] Bandiera, T.[a]

[a]D3-PharmaChemistry, Istituto Italiano di Tecnologia (IIT), Via Morego 30, 16163, Genova, Italy; [b]Analytical Chemistry Facility, Istituto Italiano di Tecnologia (IIT), Via Morego 30, 16163, Genova, Italy; [c]U.O.C. Genetica Medica, Istituto Giannina Gaslini (IGG), Via G. Gaslini 5, 16147, Genova, Italy; [d]Telethon Institute of Genetics and Medicine (TIGEM), Via Campi Flegrei 34, 80078, Pozzuoli (Napoli), Italy.

fabio.bertozzi@iit.it

Cystic Fibrosis (CF) is a lethal genetic disease caused by mutations in the CF Transmembrane conductance Regulator (CFTR) chloride channel, resulting in reduced anion conductance on epithelial cells of multiple organs. Nearly 2000 mutations of the CFTR gene have been identified;[1] the most frequent is the deletion of phenylalanine at position 508 (F508-del). This mutation causes a severe defect in protein folding and stability, and affects the gating behavior. An effective treatment for F508-del CF patients requires at least a *corrector*, to increase CFTR levels at the cell surface, and a *potentiator,* to increase the opening frequency of the mutant CFTR channel.[2] At the moment, only two correctors for the treatment of CF patients bearing the F508del-CFTR mutation have been approved, i.e. *lumacaftor* (*VX-809*) and *tezacaftor* (VX-661), in combination with a potentiator, *ivacaftor* (*VX-770*). However, these drugs are unable to effectively rescue the folding defects of F508-del CFTR and, thus to substantially ameliorate CF clinical phenotype. Therefore, there is a clear need to continue developing new CF therapies.[3]

Using a high-throughput functional phenotypic assay, based on the Halide-Sensitive Yellow Fluorescent Protein (HS-YFP),[4] a collection of about 15,000 maximally diverse commercial small-molecules was screened in two different cell types (FRT and CFBE41o-) stably expressing F508del-CFTR. This activity led to the identification of some primary hits, belonging to different chemical classes. One of these chemo-types was investigated extensively. Rounds of chemical modifications of the hit and functional evaluation in different secondary assays provided the information to build the Structure-Activity Relationships (SARs) within this novel chemical class. Hit-to-Lead and Lead-Optimization campaigns led to compounds with high potency and efficacy in rescuing the activity of F508-del CFTR in bronchial epithelial cells from CF patients homozygous for the F508del mutation, as measured by electrophysiological assays. The best correctors displayed a very good efficacy and potency in the low nanomolar range. Among them, few analogs showed drug-like properties suitable for further development upon evaluation in *in vitro* DMPK assays. This work allowed the discovery of a novel, potent CFTR corrector[5] that is currently under preclinical development investigation.

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