Alternative strategies for F508del-CFTR repair: novel targets for drug discovery approach in Cystic Fibrosis

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Background and Rationale. Cystic Fibrosis (CF) is a life-shortening genetic disorder, caused by mutations of Cystic Fibrosis Transmembrane-conductance Regulator (CFTR) [1]. In particular, the most common F508delCFTR mutant is unable to traffic to and reside at the plasma membrane (PM) [2]. Currently, no highly effective CFTR-repairing therapies are available for F508del-CFTR. For this reason our novel approach aims at targeting the derailed CF intracellular environment, and not directly the mutant CFTR, through a combination of drugs (e.g. cysteamine and epigallocatechin-gallate, EGCG) able to inhibit TG2/PDI activities and specific protein kinases [3].

Hypothesis and Objectives. We aim to 1) Refine new targets as novel therapeutic strategy in CF by exploiting a network of in silico and experimental approaches; 2) Validate the efficacy of novel drug candidates in pre-clinical CF models.

Essential Methods. We used: A) in silico approaches to identify novel chemical entities able to interact with our new protein targets [4]; B) in vitro and in cell methodologies to validate the best candidates from (A); C) In vivo validation into appropriate mouse models.

Results. A) **Refining cysteamine structure:** the chemical optimization of cysteamine led to a novel compound (CT11), active in restoring F508delCFTR function equally to cysteamine in cell models (CFBE41o-), but at a concentration 500 fold lower. These results were confirmed also in ex vivo experiments in nasal cells collected by nasal brushing from CF patients. CT11 was more efficacious than cysteamine also in vivo (F508delCFTR homozygous mice).

B) **New generation of safety tested natural compounds**: our selection of natural compounds led to the discovery of a three natural molecules (AC1, AC2, AC3) able to restore between 50-78% of F508del-CFTR function in cell models (CFBE41o-).

C) **Novel PDI inhibitors active in restoring F508del-CFTR function:** after a preliminary screening campaign we have identified three compounds (SPH1, SPH2 and SEC1) with PDI inhibitory activity, able to restore between 45-60% of F508delCFTR function in cell models. To note that SPH2 is able to increase the level of F508delCFTR band C of almost 90%

Spin-off for research & clinical purposes. The novel compounds identified represent a new frontier for the treatment of Cystic Fibrosis, by a) clarifying the roles of several other protein targets in CF, despite from the F508del-CFTR itself; b) paving the way for novel phase clinical studies with a combination of molecules (or a single drug candidate) able to improve the life of CF patients.

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