

A Paradigm-Shift in Molecular Therapy of Cystic Fibrosis? Gene Therapy versus Pharmacological Correction of Protein Function

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Editorial

Since the early 1990ies Cystic Fibrosis (CF), an autosomal-recessive disease due to mutations in the Cystic Fibrosis Transmembrane Conductance Regulator (CFTR) gene and its protein product, is seen as one of the main disease-targets for gene therapy [1]. About 2000 different CF-causing gene-mutations have been identified [2]. Some mutations are particularly common in certain populations, most notably a trinucleotide deletion called DeltaF508, which is observed in 70-80% of the CF-alleles in Caucasians [3]. The presently most pursued gene therapy strategy for autosomal recessive diseases such as CF aims at introducing a functional copy of the mutated gene into the affected cells of the patient. This gene sequence should then provide sufficient non-mutated protein as required for normal cell function. This strategy has the great advantage that it should work for any kind of CFTR-mutation, irrespective of its exact sequence.

Although this gene addition approach has made great progress over the last 15 years, in particular as *ex vivo* gene therapy for hereditary immune deficiencies [4], it has so far not lead to convincing clinical success in CF [1]. The main stumbling block in gene therapy is delivery. In particular our still limited success in the development of vectors that ensure safe, efficient and sustained gene transfer to the specific target cells, and in overcoming the immunological defences of the patient against the vector or the therapeutic protein.

However, the detailed molecular analysis of the CFTR gene that was inspired by the idea of molecular diagnostics and gene therapy has led to a profound insight into the functions of the CFTR protein and to the way in which different mutations disrupt its normal function. Based on this knowledge CF-mutations have been grouped into 6 different classes according to the molecular mechanisms by which the mutations interfere with the structure and function of the CFTR-protein from its synthesis, posttranslational folding and modification, transport and cell-membrane insertion to its function as a cAMP regulated chloride channel [5]:

Class I mutations lead to absence or instability and degradation of CFTR mRNA or the CFTR protein. Class II mutations cause an erroneous CFTR-processing and subsequent faulty intracellular protein transport to the cell membrane. This class includes the DeltaF508 mutation. Class III "gaiting" mutations do not interfere with the synthesis or intracellular localization of the protein, but disrupt the normal opening and closing function of the CFTR channel in the cell membrane. Thus, the normal epithelial chloride transport is hindered. Class IV CFTR mutations lead, without disturbing the protein localization or opening and closing of the channel, to changes in the flow rates of the chloride ions. Class V mutations reduce the synthesis of CFTR protein and class VI mutations cause an accelerated

degradation of the otherwise correctly localized and functioning protein.

This knowledge led to the alternative therapeutic strategy of trying to reverse the functional faults of the mutated proteins by use of small molecular molecules, able to modify and improve the different impaired CFTR-functions. These small compounds are able to reach the tissues and enter the affected cells much easier than the larger gene therapy constructs.

First attempts in this direction were aimed at overcoming the effect of nonsense mutations (Class I mutations), which act as non-physiological premature stop codons causing incomplete polypeptide synthesis. In 2003 the aminoglycoside-antibiotic Gentamicin was shown to restore some electrophysiological functions of CFTR in the nasal mucosa cells of patients with CF caused by CFTR nonsense mutations [6]. The antibiotic acts by interfering with the correction function of the ribosome during protein synthesis by overriding the mutation stop signal, allowing incorporation of an incorrect amino acid and thereby the synthesis of the full CFTR polypeptide chain [7]. Clinical studies with the more effective and less toxic "over-riding" drug Ataluren [8], that can be applied orally, have shown hopeful therapeutic results for the treatment of stop codon mutations causing CF http://www.cff.org/pipeline_mobile/details.cfm?id=47.

Because of the prevalence of the DeltaF508 mutation in our population, Class II mutations, which disturb the posttranslational folding and modification of CFTR and consequently its membrane insertion, are an obvious pharmacological target. An *in vitro* high-throughput screening of 164000 chemical compounds and subsequent chemical modification of active substances led to a substance called VX809 [9]. This compound can correct the impaired folding and post-translational glycosylation of the DeltaF508-CFTR and thereby achieve transport, membrane integration and the performance of physiological CFTR functions to about 14% of the value in normal cells. In patients this would be enough to transform severe CF disease into a less severe form. However, in a clinical trial so far only a reduction of the chloride secretion of sweat cells of patients could be observed, but no correction of the pathological electrophysiological changes in the nasal epithelium and no improvement of the patients pulmonary function tests or their clinical symptoms was found [10]. A recent study on the molecular mechanism of correction of the Delta F508 folding defect by VX809 indicates that the correction is only partial, and that the combination with other low molecular weight folding correctors that act on other domains of CFTR achieve a better folding. It is therefore conceivable that a combination with other Class II mutation-specific correctors could be applied [11].

So far all of this sound very much like the familiar reports from the various CF-gene therapy trials: some more or less distinctive step

forward, but still not good enough to progress to a reliable clinical therapy.

However, it appears now that for the correction of channel opening defects (Class III mutations) a real breakthrough has been achieved, which is so far the most dramatic clinical success in the development of small molecules for pharmacological correction of CFTR mutations. A low-molecular compound called Ivacaftor (VX770), which potentiates the ion channel function of CFTR and keeps it open for a longer time can correct the effect of class III mutations (gating-mutations) such as G551D. Ivacaftor enhances the cAMP-dependent chloride ion flow through the CFTR chloride-channel. In cell culture experiments Ivacaftor caused a >10-fold increase in chloride transport from an initial value of about 10% in CF-cells without CFTR-mutation. Interestingly, Ivacaftor has a corrective effect on all known "gating" mutations [12]. Patients with at least one G551D allele mutation and a clinically unequivocal diagnosis of CF had no adverse effects to Ivacaftor in a Phase II clinical trial conducted on the basis of these results. In these patients the sweat chloride concentration normalized and they showed significant improvements in lung function tests in contrast to patients who received placebo [13]. In subsequent phase III clinical trials G551D patients were divided into age groups under [14] and over 12 years [15] and treated for 48 weeks with orally administered Ivacaftor. In both patient groups a considerable and long-lasting improvement in lung function tests and the sweat chloride concentration, a decreased incidence of acute clinical deteriorations as well as a substantial weight gain was observed. Ivacaftor (Kalydeco) is already approved in the US and EU as a medicine for the treatment of CF patients with the G551D mutation from 6 years onwards and, after testing in several Phase III clinical studies, it was recently also approved by the FDA for children aged 2-5 years <http://www.drugs.com/history/kalydeco.html>. It has also been applied with clinical benefit to DF508 homozygous CF patients as combination therapy with Lumacaftor, a folding corrector [16].

Ivacaftor (Kalydeco) is the first drug approved for the treatment of cystic fibrosis, which acts on the causal, i.e. disease-causing protein alteration of the patient. However, only the future will show if it is capable of improving existing disease symptoms long-term and, above all, if it can prevent the clinical manifestation and/or progression in young children. Despite these limitations, there is now real hope that new drugs will expand the range of functional mutation correction and spread to other monogenic diseases.

Does this mean a paradigm-shift from gene therapy to low molecular mutations-specific function-corrector molecules for CF? In the short term this may well be the case. However, in the longer run the potential of gene therapy to treat any case of CF irrespective of the mutation by addition of the same normal gene sequence to the affected cells remains a worthwhile goal that stimulates further research on safe and effective gene delivery. In addition new gene therapy strategies that aim at mutation specific gene-correction are also driving the need for effective gene delivery. This approach is particularly important for the treatment of dominant genetic diseases in which the mutated gene product often has a toxic effect, but it may also be attractive for the prevalent Delta F508-CF mutation. All these strategies and their

combination with cell therapy approaches, which aim to use ex vivo corrected stem cells to repopulate affected tissue with healthy cells, may contribute to finding curative therapies for Cystic Fibrosis. Therefore, although all eyes are now on low molecular function-correctors, don't lose sight of gene and cell therapy approaches.

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