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REVIEW ARTICLE

Ataluren: A Novel Targeted Therapeutic Approach for the Treatment of Cystic Fibrosis

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ABSTRACT

A lethal autosomal recessive genetic disease, cystic fibrosis (CF) is most common in Caucasians and the prevalence varies with the ethnic origin of the population. Patients with CF lack adequate levels of the CF transmembrane conductance receptor (CFTR) protein, a chloride channel necessary for normal function of the lung, pancreas, liver and other organs. Nonsense (premature stop codon) mutations in mRNA for the CFTR cause CF in approximately 10% of patients. Ataluren, a protein restoration therapy, is designed to promote the translational read-through of premature stop codons that helps to overcome the nonsense mutation and enable the production of a full-length, functional CFTR protein. Insertion of a near cognate amino acid at a premature stop codon allows the protein translation to continue until one of the several stop codons normally present at the ends of mRNA transcript is reached and properly utilized. It is a "corrector" drug specific for type I mutation defects. Ataluren is a novel, orally bioavailable drug that addresses the molecular basis of the disease. Phase III Clinical trials have shown it to be efficacious and well tolerated. It represents a breakthrough in the pharmacological advances for a rare genetic disease.

KEYWORDS: ataluren, non sense mutation, read-through

INTRODUCTION:

Once thought to be an extremely rare disease in the Indian subcontinent, availability of newer improved genetic and biochemical testing point towards a probable increased incidence of cystic fibrosis (CF). Owing to diverse ethnic origin, Indian patients show evidence of extensive allelic heterogeneity as compared to Mediterranean and European population. [1, 2, 3]

Clinically, classical CF is characterized by faulty chloride transport leading to accumulation of dehydrated and hyper viscous mucus that compromises mucociliary clearance and makes airways more vulnerable to infection (particularly with *Pseudomonas aeruginosa*) and inflammation, ultimately leading to airway destruction, respiratory failure and death. There are protracted periods of clinical stability erupted by pulmonary exacerbations, often triggered by a viral infection and defined by increased cough, weight loss, low grade fever, increased sputum volume, and decrements in pulmonary function. [4, 5] CF transmembrane conductance regulator (CFTR) dysfunction leads to multiorgan manifestations including pancreatic insufficiency, hepatic dysfunction, intestinal malabsorption and reproductive abnormalities. Sweat with elevated chloride (>60mmol/L) is nearly pathognomonic. Atypical CF and CF related disorders include congenital absence of vas deferens, isolated idiopathic pancreatitis, chronic rhinosinusitis, nasal polyposis and idiopathic bronchiectasis.

CF is caused by genetic mutations in the CFTR gene, located on the long arm of chromosome 7 (7q31.2) that undergoes transcription and is translated into CFTR protein that moves to the cell membrane, where it mainly functions as the predominant chloride channel. [4] CFTR provides a pathway for chloride, gluconate and bicarbonate transport with a permeability selectivity of $Br^- > Cl^- > I^- > F^-$.

The CFTR protein is a single polypeptide chain, containing 1480 amino acids. Over 1900 mutations have been described in the CFTR gene, which are broadly categorized into six classes involving truncation, processing, activation, channel conductance, splice mutations and protein instability. [6, 7] .While class IV and V are milder forms, severe phenotypes are seen in classes I, II, III and VI.

Class I mutations (~ 10%) such as G542X result from premature termination codons (PTC) that cause premature truncation of normal protein translation resulting in an inability of the channel to reach the cell membrane.

Class II mutations result from trafficking defects that cause misfolding of CFTR protein so that it is not transported to cell surface; it remains in endoplasmic reticulum and is degraded by proteasome. However, little residual CFTR is maintained as seen in ΔF508, the most common mutation in humans (66-70%) worldwide, where a deletion (Δ) of three nucleotides results in a loss of the

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amino acid phenylalanine (F) at the 508th position on the protein leading to a partially functional anion channel.

Class III mutations (~2-3%) result from gating mutations that are characterized by full length CFTR reaching the cell surface but exhibiting reduced ion transport activity owing to abnormal channel gating as seen in G551D-CFTR, where glycine is substituted with aspartic acid at amino acid 551. The channel does not open properly leading to impaired chloride transport.

In class IV (<2%) mutations such as R117H, CFTR protein reaches cell membrane and some of the protein is functional. However, channel narrowing hampers chloride transport.

Class V (splicing) mutations are least common and result in improper processing of mRNA leading to reduced number of CFTR proteins that reach the surface, but they are able to transport chloride effectively.

Class VI mutations result in impaired conductance of ions other than chloride. Defect in protein stability lead to reduced membrane residence time due to reduced half-life of complex-glycosylated truncated CFTR.

The life expectancy of CF patients is reduced (median survival is 37.4 years), and the treatment burden to maintain health is high, indicating a need for better therapies. Current therapeutic options treat downstream disease process as a result of defective CFTR without addressing the underlying genetic defect. Inhaled tobramycin, recombinant human deoxyribose nuclease (dornase alfa), azithromycin and hypertonic saline are used to improve lung function and reduce pulmonary exacerbations. [7]Therapeutic strategies aimed at rescuing the abnormal protein that is either synthesized in reduced amounts or has poor anion conductance has lead to development of novel drugs. CFTR modulators are pharmacological agents intended to repair the CFTR protein. A compound that enhances CFTR trafficking to the membrane is termed a “corrector” (for mutation classes I and II) while an agent that increases the flow of ions through activated CFTR channels is named as a “potentiator” (for mutation classes III-VI).

There is now a promising pipeline of disease modifying agents currently under evaluation. Ataluren allows read through of premature stop codons and is in Phase III clinical trials for treatment of truncation mutations (class I mutation defect). VX 809 (lumacaftor) is a corrector that modulates CFTR folding and trafficking so that the channel can reach the cell surface (class II mutation defect). Ivacaftor (VX 770) is a first- in-class drug for treatment of CF in patients aged 6 years or older who have a G551D mutation in the CFTR gene (class III mutation defect). [8]

The life expectancy of these patients remains well below normal, and the treatment burden to maintain health is high, indicating a need for better therapies. Current treatment options treat downstream disease process as a result of defective CFTR without addressing the underlying cause of disease. Inhaled tobramycin, recombinant human deoxyribose nuclease (dornase alfa), azithromycin and hypertonic saline are used to improve lung function and reduce exacerbations. [7]Therapeutic strategies aimed at rescuing the abnormal protein that is either synthesized in reduced amounts or has poor anion conductance has lead to development of novel drug candidate molecules.

Non-sense mutations are single-nucleotide alterations in DNA that directly change sense to nonsense codons, causing a premature stop of the mRNA translation process. Stop codons may also be generated by mutations that alter the reading frame of mRNA, either as a result of insertion and/or deletion of a number of nucleotides not evenly divisible by three or of an abnormally spliced mRNA. PTC lead to the formation of truncated proteins that do not function properly. Approximately 1800 inherited human diseases are caused by nonsense mutations. The frequency of PTCs is rather variable, ranging from 5% to 70% of reported mutations for different diseases. [9, 10]

Mutation targeted therapies is a new paradigm for treatment of human genetic diseases by correcting the effect of mutations at the DNA or RNA level rather than at the protein level. This approach is not specific to CF but includes other diseases caused by PTC, such as Duchenne’s muscular dystrophy, hurler’s syndrome, ceroid lipofuscinosis, nephropathy cystinosis and expression of mutated p53. [6]

Nonsense mutations activate a RNA surveillance system, known as nonsense mediated decay (NMD), to destroy PTC-containing transcripts in order to prevent the synthesis of truncated portions that might produce dominant negative effects and waste cellular resources. NMD is a major mechanism of nonsense transcript elimination and governs the patients’ response to read-through compounds. [10] Developing a method to specially disrupt NMD of a disease causing mRNA without influencing normally degraded mRNA might greatly improve the read-through efficiency of targeted nonsense mutation by read–through compounds.

Ataluren (PTC 124) {3-[5-(2- fluorophenyl) – [1,2,4] oxodiazol -3-yl]-benzoic acid)} [Figure 1] is a 284-Da orally delivered compound designed to selectively promote ribosome read through of premature stop

codons, but not normal termination codons. It elicits little off-target activity.

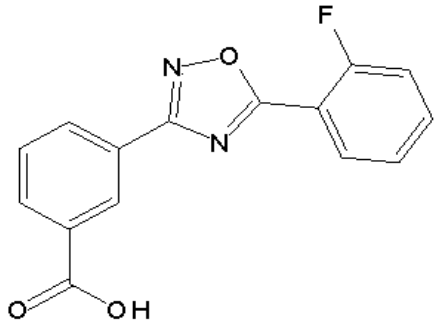

Figure 1: Molecular structure of Ataluren

MECHANISM OF ACTION:

Nonsense mutations create premature stop codons (e.g. in a C>T mutation: CAG, which codes for a glutamine, becomes TAG or UAG, which stops translation at that point, resulting in a truncated unstable protein). [10]Ataluren enables stop codon read-through by introducing an amino acid at the premature stop codon to help continue the mRNA translation. This phenomenon is called "stop codon read-through". Ataluren binds to the 60S ribosomal subunit and promotes suppression of UGA stop codons as well as read-through of UAG and UAA stop codons, though less efficiently. Aminoglycosides also cause read-through but unlike ataluren, they can interfere with NMD. In the presence of ataluren, PTCs are ignored, real stop signals are read and the amount of full-length protein increases in patients carrying class I (stop) mutations. [9]

Absorption of ataluren is slowed with high-fat meals, and there has been observed a diurnal variation resulting in greater exposure after evening doses. [11]

Preclinical data has confirmed enhanced stability of CFTR mRNA. In a mouse model for CF, Du *et al* showed that subcutaneous injection or oral administration of Ataluren to CFTR-1- mice expressing a human CFTR-542X transgene suppressed the G -542 X nonsense mutation and restored a significant amount of human CFTR protein and function. [12]

CLINICAL STUDIES DONE WITH ATALUREN:

In a study by Sermet-Gaudelus *et al*, 30 children were administered suspension of vanilla flavored granules of ataluren. The study included two 28- day cycles, each of 14 days treatment followed by 14 days without treatment. In one cycle, patients received lower dosing regimen [4, 4, 8mg/kg three times daily(TDS)] ; in the other cycle, patients received higher dose (10,10,20 mg /kg TDS). Ataluren induced nasal chloride transport response (at least a- 5mV improvement) or hyperpolarization (value more electrically negative than – 5mV) in 50 % and 47% patients respectively. Additionally, ataluren significantly increased the proportion of nasal epithelial cells expressing apical full-length CFTR protein. [13]

In another evaluation, both low and high doses given for 12 weeks improved total chloride transport with a combined mean change of -5.4mV ($p<0.001$). On-treatment responses and hyperpolarisations were induced in 61% ($p < 0.001$) and 56% ($p = 0.002$) of patients. [14]

In a Phase II study in 19 patients in Israel, patients received ataluren at 16 mg /kg/day in 3 doses everyday for 14 days followed by 14 days without treatment. In the second cycle, patients received 40 mg/ kg ataluren in 3 doses everyday for 14 days followed by 14 days without treatment. Ataluren treatment was associated with increase in intrinsic, stimulated and total chloride transport in nasal potential difference (NPD), slight increase in weight and no change in sweat chloride values. Adverse effects included constipation and dysuria; however, liver enzymes were stable. [15]

A similar study in US did not demonstrate improvement in CFTR function. Reasons for failure include challenge of NPD studies in multicenter trials (addressed by improvement in testing method), relative susceptibility of the W1282 X mutation found in Israel and genetic founder effects, including the degree of CFTR mRNA expression at baseline. [6]

A Phase III, 48-week, double-blind, placebo-controlled trial conducted across 11 countries enrolled 238 patients randomly assigned to either ataluren (10 mg/kg morning, 10 mg/kg midday, 20 mg/kg evening) or placebo (morning, midday, evening). The primary endpoint, the relative change from baseline in %-predicted FEV1 (forced expiratory volume in one second) at 48 weeks, showed a positive trend favoring ataluren versus placebo. An analysis of the relative change from baseline in %-predicted FEV1 demonstrated an average difference between ataluren and placebo of 2.5% (-1.8% vs. -4.3%; p= 0.0478).A substantial treatment effect was seen in the patients not receiving chronic inhaled antibiotics at baseline; the Week 48 difference between the ataluren and placebo arms in FEV1 was 6.7% (-0.2% change on ataluren vs. -6.9% change on placebo).

The secondary endpoint, the rate of pulmonary exacerbations (ie, the number of pulmonary exacerbations in 48 weeks) also showed a positive trend in favor of ataluren, with the rate in the ataluren group being 23% lower than the placebo group (p=.0992). In the patients not receiving chronic inhaled antibiotics, the pulmonary exacerbation rate in the ataluren group was 43% lower

than the rate in the placebo group. These results show a consistent treatment effect of ataluren on both pulmonary function and exacerbation rates. However, the tertiary endpoints of sweat test and NPD did not show an effect between ataluren and placebo. [16]

Ataluren has also been evaluated in Duchenne's Muscular dystrophy (DMD), which is a X-linked pathology due to absence of dystrophin in muscle fibers due to > 4700 DMD mutations that almost always result in a premature stop codon due to frameshift or nonsense mutations . [17]

ADVERSE EFFECTS:

Ataluren has shown remarkable tolerability profile in the trials so far. The most common adverse events were typical for CF and included pulmonary exacerbation, cough, and upper respiratory tract infection, which occurred at similar frequencies in the ataluren and placebo arms. [16] High doses cause headache, dizziness, and gastrointestinal adverse effects. Repeated doses of 50 mg/kg/dose twice daily showed reversible liver enzyme elevations and a few patients had increases in serum creatine kinase.

CURRENT PERSPECTIVES:

Available treatments in CF are helpful but cannot halt inevitable end organ damage. The time needed to administer multiple medications by nebulization over protracted periods each day adds substantial burden to the lives of both children and adults. Early introduction of a new oral therapy designed to address the underlying cause may offer opportunity to address disease manifestations and reduce treatment burden at a point when pathology is still tractable to intervention. However, adherence may be lower since it requires thrice daily administration. Genotyping followed by individualized ataluren administration to patients with class I defect represents the dawn of the era of personalized medicine.

Translation read through is a promising approach for the treatment of hereditary diseases that may complement the use of chaperones for missense mutations. The food and drug administration (FDA) and the European Commission have granted ataluren Orphan Drug status for the treatment of nonsense mutation CF and nonsense mutation Duchenne and Becker muscular dystrophy. The FDA has also granted ataluren Subpart E designation for expedited development, evaluation, and marketing for CF and dystrophinopathy and Fast Track designation for the development of treatment for nonsense mutation dystrophinopathy.

There is theoretical possibility that read- through strategies awake retrotransposons and endogenous retroviruses disabled by PTCs. Potential modulation of human polymorphisms or acquired changes resulting from PTC in the human population maybe expected. Patients on long-term ataluren therapy should be monitored for development of malignancies and other genetic diseases.

CONCLUSION:

CF is an inherited disorder caused by a defective gene. Mutation-class specific pharmacological approaches target at rescuing the poorly biosynthesized or dysfunctional CFTR protein. Ataluren is a first-in-class oral drug that permits ribosomes to read through premature stop codons in mRNA to produce functional CFTR protein in class I mutation. It has been granted Orphan Drug status by FDA. It represents a paradigm shift in the management of CF-from agents used to treat symptoms to a drug that targets the basic underlying defect.

REFERENCES:

1. Kabra SK, Kabra M, Lodha R, Shastri S. Cystic fibrosis in India. Pediatr Pulmonol 2007; 42: 1087-94.
2. Prasad R, Sharma H, Kaur G. Molecular basis of cystic fibrosis disease: an Indian perspective. Indian J Clin Biochem. 2010; 25: 335-41.
3. Ashavaid TF, Raghavan R, Dhairyawan P, Bhawalkar S. Cystic Fibrosis in India: A systematic review. JAPI. 2012; 60: 39-41.
4. Lubamba B, Dhooghe B, Noel S, Leal T. Cystic fibrosis: Insight into CFTR pathophysiology and pharmacotherapy. Clin Biochem. In press 2012.
5. Boucher RC. Cystic fibrosis. In: Longo DL, Kasper DL Jameson JL, Fauci AS, Hauser SL, Loscalzo J, editors. Harrison's principles of internal medicine. 18th ed. Volume 2. New York: McGraw-Hill; 2012. p. 2147-51.
6. Sloane PA, Rowe SM. Cystic fibrosis transmembrane conductance regulator protein repair as a therapeutic strategy in cystic fibrosis. Curr Opin Pulm Med. 2010; 16: 591-7.
7. Pettit RS. Cystic fibrosis transmembrane conductance regulator- modifying medications: the future of cystic fibrosis treatment. Ann Pharmacother. 2012; 46: 1065-75.
8. Aditya S. Ivacaftor in cystic fibrosis: the first disease modifying agent.IJBCP 2012; 1:225-9.
9. Torra R, Oliveira JP, Ortiz A. UGA hopping : a sport for nephrologists too ? Nephrol Dial Transplant. 2010 ; 25 : 2391-5.
10. Hu H, Gatti RA. New approaches to treatment of primary immunodeficiencies: fixing mutations with

chemicals. Curr Opin Allergy Clin Immunol. 2008; 8: 540-6.

11. Hirawat S, Welch EM, Elfring GL, et al. Safety, tolerability, and pharmacokinetics of PTC124, a nonaminoglycoside nonsense mutation suppressor, following single- and multiple-dose administration to healthy male and female adult volunteers. J Clin Pharmacol 2007;47:430–44.

12. Du M, Liu X, Welch EM, Hirawat S, Peltz SW, Bedwell DM. PTC 124 is an orally bioavailable compound that promotes suppression of the human CFTR - 542X nonsense allele in a CF mouse model . Proc Natl Acad Sci USA. 2008; 105: 2064-9.

13. Sermet-Gaudelus I, Boeck KD , Casimir GJ, Vermeulen F, Leal T, Mogenet A, *et al*. Ataluren (PTC124) induces cystic fibrosis transmembrane conductance regulator protein expression and activity in children with nonsense mutation cystic fibrosis. Am J Respir Crit Care Med. 2012; 182:1262-72.

14. Wilschanski M, Miller LL, Shoseyov D, Blau H, Rivlin J, Aviram M, *et al* . Chronic ataluren (PTC 124) treatment of nonsense mutations cystic fibrosis. Eur Respir J. 2011; 38: 59-69.

15. Kerem E, Hirawat S, Armoni S, Yaakov Y, Shoseyov D, Cohen M, *et al.* Effectiveness of PTC 124 treatment of cystic fibrosis caused by nonsense mutations : a prospective phase II trial. Lancet. 2008; 372: 719-27.

16. Top-line Data From Phase 3 Trial of Ataluren in Patients with Nonsense Mutation Cystic Fibrosis Show Promising Results Available from www.prnewswire .com / ... /top-line-data-from-phase-3-trial-of-ataluren. [Last cited on 2013,Mar 13]

17. Pichavant C, Aartsma-Rus A, Clemens PR, Davies KE, Dickson G, Takeda S, *et al* . Current status of pharmaceutical and genetic therapeutic approaches to treat DMD. Mol Ther. 2011; 19: 830-40