Case Report

Case study in drug discovery and development: When it doesn't go to plan Denufosol for the treatment of cystic fibrosis

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ABSTRACT

In current healthcare provision there is an ever growing focus on acquisition cost of pharmacological interventions, and whether they represent value for money. It is often forgotten that for every "breakthrough" drug that makes it onto our formularies, approximately 1000 never get beyond phase 1 clinical trials. Even once this hurdle is passed, there are many more obstacles that stand in the way of a new medication ever being prescribed. The cost of the development of these failed medications is incorporated into the cost of every new product that is brought to market, therefore, every effort is made to ensure, once a product gets to the final stages of development it is used in the most appropriate population using the most appropriate outcomes in order to demonstrate its maximal efficiency.

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CASE STUDY

We present the case of denufosol, a drug that was developed for the treatment of cystic fibrosis. Despite promising results in phase 1 and 2 studies, it failed to achieve clinically meaningful results in final phases of development and thus the potential benefits were never realised. We discuss the potential pitfalls in the trial design and the choice of outcomes used to highlight some considerations for potential investigators, and people considering clinical trial designs

Cystic Fibrosis, also known as mucoviscidosis, is an autosomal recessive disorder caused by an abnormality in both copies of the gene that regulates the cystic fibrosis transmembrane conductance regulator (CFTR) protein. Approximately 1 in 25 adults in the UK carry the faulty gene, however in the heterozygous state there are almost no visible effects. The homozygous recessive state occurs in 1:3,000 live-births in those of Northern European ancestry, although with much less frequency in those of Asian or Black African Origin. The absence of a functioning gene causes a failure of the chloride ion channel in part responsible for creating secretions, such as sweat, digestive fluids and mucus to become too thick. The channel comprises of two domains Trans membrane domains, each containing 6 alpha helices and capable of hydrolyzing (i.e. deriving energy from) ATP. It is regulated by phosphorylation, predominantly by cAMP-dependent protein kinase, and is anchored to the cellular cytoskeleton at the carboxyl terminal.

The channel is primarily responsible for the movement of water and halogens, predominantly chlorine, out of, and in the case of sweat glands into, the cell. In the sweat glands this results in excess amounts of chlorine and thiocyanate (another substrate of the channel) remaining in the gland. The negative charge of the chlorine is neutralized by the cation, sodium, resulting in excessive salt in the sweat, which forms the basis of the Sweat Test for the diagnosis of cystic fibrosis. In other tissues, the principle pathology is caused by a lack of flow of water into the extracellular space. This causes hyperviscosity of the secretions. In the airways this also leads to a deficiency of the Airway Surface Liquid (ASL), in which the cilia operate. In the absence of this ASL, the cilia cannot effectively clear the thick mucus, resulting in blockage of the small airways. Further, the thick nutrient-rich

mucous within the small airways is an ideal medium for bacteria to flourish, resulting in recurrent infections. A similar process occurs in the pancreas resulting in a deficiency of the exocrine components of pancreatic function (the digestive enzymes) and an impairment and eventual failure of the endocrine cells resulting in a disease that has characteristics of both type 1 (particularly in terms of insulin production) and type 2 (in terms of glucagon regulation).

The pathophysiological mechanism that leads to the failure of the channel is a mutation in the CFTR gene at the q31.2 locus of chromosome 7. The most common mutation, accounting for between 66-70% of worldwide cases, higher in those of Caucasian origin, is a deletion of the three-bases that code for phenylalanine in the 508th position of the 1480 amino acids that constitute CFTR. This results in a protein that does not fold properly and is degraded by the cell. There are many other different mutations that result in shortened proteins, proteins with a charged channel that does not allow the anion chlorine to pass through it, proteins that cannot hydrolyze ATP, therefore cannot utilize energy to pump up an osmotic gradient, or proteins that are simply prone to rapid enzymatic cleavage.

The median life expectancy of a person with cystic fibrosis in the UK is currently 41, although it is anticipated that today's children will live substantially longer. The principle cause of death in people with cystic fibrosis relates to the pulmonary complications therefore addressing these has become of paramount importance in the race to beat cystic fibrosis.

Previously therapy for cystic fibrosis was supportive, providing insulin, pancreatic enzymes supplementation, managing chronic infections and considering organ transplantation as required, however with a greater understanding of the pathophysiology of the disease there has been growing interest in targeting the underlying protein deficiencies. There have been some success stories with this, such as Ivacaftor, which acts as the missing potentiator for the ~4% of people with cystic fibrosis who have the mutation that leads to a glycine-aspartic acid switch at position 551 on the amino acid [1]. This oral medication opens the defective channels and can allow a small proportion of patients with cystic fibrosis to live an effectively normal life. This however is only available for one specific genotype of people with cystic fibrosis and can, at best, only treat ~4% of patients [2]. The search for broader management strategies that can be used independent of genotype has been at the forefront of many research team's aims over recent years. Such strategies all share the common aim, namely to restore ciliary clearance of mucus, thereby restoring the passive and innate immunity of the lungs. There are three key mechanisms being explored in order to achieve this: 1) reduce sodium and water resorption from other channels in the lungs, notably the epithelial sodium channel (ENaC); 2) increasing airway surface hydration by applying topical osmotic agents and 3) activating alternative chloride channels thereby supplementing hydration activity by other functioning pathways. One particular receptor that has been of particular interest in achieving the latter mechanism is P2Y (2). This nucleoside receptor is responsible for sodium absorption, chloride conductance and CFTR independent hydration of the airway surface. Non-specific agonists were demonstrated in animal models to enhance ciliary beat frequency and increase mucociliary clearance in animals and subjects with impaired mucociliary clearance. These nucleoside analogues were unstable, and not suitable for pharmacological development [3]. Denufosol was the first stable compound to demonstrate any potential in this area.

Originally investigated as a potential treatment for retinal detachment [4], denufosol is a bi-nucleoside, composed of 2'-desoxycytidine and uridine, linked by 4 units of phosphoric acid. It is a selective agonist of an alternative, non-CFTR regulated chloride-secreting channel and inhibits ENaC, and thus provided an attractive genotype-independent mechanism for improving the ASL. Its nucleoside structure made it only suitable for direct administration and it was prepared as a nebulized inhaled formulation. The preparation required a degree of patient co-ordination and was deemed only suitable for those over the age of 5. Phase 1 studies suggested approximately 18% of the drug would be delivered to the airways when nebulized, substantially higher than other inhaled products, such as beta agonists and inhaled corticosteroids when nebulized [5].

The precedent for using nebulized therapies in cystic fibrosis is well established, indeed inhaled antibiotics such as tobramycin and aztreonam are a normal component of therapies. At such a dose in animal models, denufosol had demonstrated a substantial improvement in ciliary clearance of mucus [6]. Healthy non-smokers underwent dose escalation studies which demonstrated good tolerability, minimal systemic effects, and the only real side effect of note was increased expectoration presumably due to enhanced mucosal hydration and mucociliary clearance [3].

Phase 2 studies explored primarily the safety but also efficacy of administering nebulized denufosol for patients with cystic fibrosis with mild–moderate lung disease [7,8]. In addition to demonstrating excellent safety in a range of degrees of disease, the pooled denufosol study group demonstrated both statistically and clinically significant improvements in FEV_1, FVC, FEF and FEV_1/FVC ratio, all important markers of pulmonary function, compared to placebo at 28 days [7,8]. These trials suggested that the greatest benefit was at higher baseline function, an effect analogous to the legacy effect widely accepted in diabetes, such that those with the least complications have the most to gain out of intensive therapy.

As a result of these successful studies, a phase 3 program was launched exploring the use of nebulized denufosol in normal and mildly impaired lung function; the Transport of Ions to Generate Epithelial Rehydration (TIGER) studies. TIGER-1 assessed the impact of denufosol at 24 weeks on 352 people with cystic fibrosis and only good lung function [9]. There was a statistically significant improvement of 0.045L in FEV_1, although this value would be of dubious clinical relevance. Secondary endpoints, including exacerbation rates and other measures of lung function were not improved, echoing the scepticism regarding the clinical relevance of the benefits demonstrated in FEV_1 [9]. At 48 weeks, however, even the marginal improvement in FEV_1 had been lost, and when TIGER-2 was published exploring the effect in 233 people *vs.* a further 233 placebo controls there was no significant improvement in FEV_1, exacerbation rates, or time to first event [10]. Shortly afterwards, Inspire pharmaceuticals and the Cystic Fibrosis Foundation withdrew the clinical development program.

This has caused many to ask “what went wrong”. For such a promising drug that had demonstrated great success in phase 1 and 2 trials to fail so categorically at phase 3, led to Inspire pharmaceuticals dropping in value by $400 m dollars in one day and being subsequently acquired by Merck. There are several vital stages where things may have been overlooked, mis-interpreted or just ignored that led to this high profile U-turn. The first is the choice of target channel. Originally the P2Y (2) receptor was an attractive target as it modulated a calcium activated chlorine channel that is CFRT independent, and is effectively inert in day-to-day health. It was though that augmentation of this channel could therefore supplant the deficiency. We have subsequently learned, however, that these channels are activated by the shear stress of airway clearance regimes [11]. As a result, these may be a refractory target for patients with cystic fibrosis who frequently undergo chest physiotherapy. It may, of course, have been possible that a higher dose of denufosol would have been effective at in these refractory patients. The dose used of 60mg was chosen due to concerns that higher doses caused excessive coughing and expectoration in Phase 1 studies. These studies, were, however, conducted in healthy individuals. It is entirely plausible that a higher dose may have been required to gain a similar effect in those without functioning CFTR and therefore were exclusively dependent on alternative pathways. Further evidence that a higher dose may have been appropriate is based around the pharmacokinetics and degradation of denufosol.

Denufosol has a half-life of 25 hours ex-vivo, which was reduced to 3.5 hours when ciliated endothelial cells were added to the culture [3,6]. This was heralded as a potential safety benefit, with the reduction in half-life being attributed to endothelial ectonucleotidases, which would limit any systemic exposure from the drug. What was not appreciated until 2 years after the program was publishing its phase 1 data, was an up-regulation of these ectonucleotidases in people with cystic fibrosis causing an ex-vivo further reduction in half-life [12]. It is equally feasible that administration of the

denufosol would have further up-regulated the ectonucleotidases causing a decline in benefit, analogous to the up-regulation of catechol-O-methyl transferase seen in Parkinson's disease patients who are administered Levo-Dopa and experience treatment failure over-time. If this is indeed the case, it opens the potential for future inhaled combination therapies with ectonuceotidase inhibitor and denufosol therapy.

The population identified for the development of the phase 2 and 3 program was those with mild early disease. This was justified on the grounds that these patients would have more to gain and less chance of already having experienced permanent alveolar or bronchiolar damage. The pharmacodynamic measure used to choose this population, however, was "expectorated sputum weight" in the early studies. This is an interesting outcome to choose in any case, as it has very poor reproducibility, and in early cystic fibrosis, where expectoration is not a recognized problem, has limited value. Further, there was no dose response curve to this outcome and the observation that expectorated sputum only increased after the first dose challenges this choice of population.

The lack of dose response follows through to the Phase 2 studies, where all three tested doses of denufosol (20 mg, 40 mg and 60 mg) demonstrated a very similar response, and only when pooled did these show a benefit versus placebo. Two important criticisms remain that the "per protocol" population appeared to benefit less than the "intention to treat", and more importantly, there was no actual improvement in the groups, rather a steep decline in the placebo population [7]. The decline in placebo population was ascribed to regression to the mean, and it was felt that the denufosol was providing protective properties, hence studies were continued. Another phase 2 study in more advanced cystic fibrosis lung disease failed to show any benefit [13], however this was regarded as confirmation of the benefit in early disease alone, rather than questioning the hypothesis that there was a protective effect.

Finally, the clinical research program was discontinued in the basis of a failure to improve FEV_1 in patients with normal or mildly impaired lung function. This begs the question how much improvement was expected in a population that have effectively normal lungs to start with? Additionally in normal lungs is FEV_1 a suitable surrogate to use? To answer the latter question there is an international search ongoing to find a more appropriate surrogate, however at the time TIGER-2 was being conducted FEV_1 was the mandated surrogate outcome that predicts hospitalisation, exacerbations and ultimately death. With regard to the former question, there was a small difference in decline of FEV_1 between those on treatment and placebo, such that FEV_1 declined by 3.04% in the placebo group but only 2.3% in active group. This gives a relative reduction in decline of 24% although with such small absolute effects did not reach statistical significance. There was also a numerical but not significant difference in the exacerbation rate. However, again at such an early stage of the disease, this did not reach statistical significance.

In summary, cystic fibrosis is an autosomal recessive condition characterised by excessively viscous secretions. With the majority of patients dying of pulmonary complications addressing these has become a major research focus. Several mechanisms have been trialled, and indeed a few have made it to market, however to date there is no therapy that has demonstrated benefit in all patients. Denufosol had an attractive mechanism of action stimulating the CFRT-independent Calcium-activated chloride channel via the P2Y (2) receptor. Early studies were promising, however the definitive studies failed to show sufficient benefit to justify continuing the program. This may be a result of underpowered studies in a relatively well population, underestimating the pharmacokinetic effects in the diseased population, or simply that the cystic fibrosis population is refractory modulation of this channel.

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