Identifying the most effective drug combination for the treatment of Cystic Fibrosis

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Abstract:

Cystic fibrosis transmembrane regulator (CFTR) is a cell membrane protein that serves as a chloride ion channel. If defective CFTR protein is synthesized, it interferes with the function of the secreted mucus, changing its consistency. The most common cause of the defective CFTR protein is deletion mutation indicated as F508del. 80% of CF patients carry this mutation and suffer from the symptoms associated with thick mucus. Correctors are artificially designed drugs to minimize the effect of defective CFTR protein. Correctors are commonly used in combination with a potentiator, Ivacaftor. To date, three such corrector-potentiator combinations have been used, namely, LUMA/IVA, TEZA/IVA and ELEXA/TEZA/IVA (Trikafta). More than 85% of CF patients are subjected to this therapy. This paper reviews the effectiveness of these combinations, studying the parameters that indicate lung, liver, and pancreas functions.

Keywords : Cystic Fibrosis (CF), Cystic fibrosis transmembrane regulator (CFTR), Potentiator, Ivacaftor, Correctors

Introduction:

Cystic Fibrosis(CF), is a genetic disease. If both the parents carry a recessive allele for the mutated Cystic fibrosis transmembrane regulator (CFTR) protein, those with homozygous recessive genotype will suffer from CF. The CFTR protein has numerous mutations. The most common mutation is the F508-del. In this, Phenylalanine(amino acid) is deleted at position 508 in CFTR protein. Due to this, many organs such as the lungs, pancreas, sweat glands, liver, intestine, sinuses, and reproductive tract are affected. This mutation causes misfolding of the CFTR protein during its production. In the endoplasmic reticulum, the protein then gets degraded. This transmembrane protein acts as a chloride ion channel, regulating the flow of chloride ions across the membrane. These ions are necessary to maintain the required consistency of the secreted mucus layer. However, due to the mutation, the CFTR protein does not function as a chloride ion channel. Subsequently, a sticky and thick layer of mucus gets formed in various parts of the body. The respiratory tract is the most affected organ, resulting in laboured breathing in most patients.

Correctors are artificially designed chemicals used to correct or minimize the lethal effects of defective CFTR in the body. Corrective action for CF patients is treatment with correctors. There is no cure for CF, but these correctors can help reduce any further complications due to this disease. They do so by stabilizing the CFTR protein and try to prevent further degradation of the protein. They also correct the folding defects of the F508 mutation. They try to help the protein perform its function by improving the transport of the chloride ions. Another chemical referred to as a potentiator is a drug that enhances the function of the defective CFTR protein. They allow the chloride ions to pass through the channel more effectively. A potentiator called Ivacaftor(IVA) augments the channel gating of CFTR on the cell surface (Sagel et al., 2021). It is commonly abbreviated as VX770. Every corrector is combined with the potentiator, Ivacaftor(VX770). The correctors and their functions are tabulated below (Table 1), indicating their probable corrective actions when used for patients. This paper reviews the effectiveness of different combinations when used in patients where all the combinations have employed a common potentiator, Ivacaftor(VX770).

Table 1: CF Correctors and their Functions

No.	**Name of the corrector**	**Abbreviation used**	**Function**	**Reference**
1.	Lumacaftor	VX809, LUMA	Increases trafficking of the F508del CFTR to the cell surface	(Sagel et al., 2021)
2.	Tezacaftor	VX661, TEZA	It binds to the Phe508del CFTR protein, augmenting intracellular processing and trafficking.	(Davies et al., 2018)
3.	Elexacaftor	VX445, ELEXA	It improves the CFTR folding as well as the ion conductance.	(Veit et al., 2021)

What is Cystic Fibrosis (CF)?

Cystic Fibrosis, commonly called CF, is an autosomal recessive disease. Usually, both parents carry recessive alleles for mutated CFTR protein without expressing it phenotypically. If offspring inherit both alleles from their parents, they suffer from CF due to the expression of defective CFTR protein, which affects multiple systems in the body. Thus, this is a genetic disorder expressed as autosomal recessive inheritance. There are various mutations for this disease. The Cystic Fibrosis Transmembrane Regulator (CFTR) protein is a chloride channel that regulates salt and water movement across systems. The most common mutation, present in approximately 80% of CF patients, is the one in which there is a deletion of amino acid Phenylalanine(Phe) at position 508 in the CFTR protein. This Cl^- channel function of the CFTR protein in the epithelial cell apical membrane of the lungs, upper respiratory tract, pancreas, liver, gallbladder, intestines, sweat glands, and the reproductive tract is essential for the osmotic balance of the mucus and its viscosity. Owing to this widespread distribution of the CFTR protein, CF is a multisystem disease.

CFTR protein structure :

Figure 1: Components of CFTR protein: two transmembrane domains (MSD1 and MSD2), two cytosolic nucleotide-binding domains (NBD1 and NBD2), and a single regulatory R-domain

CFTR protein includes two transmembrane domains (MSD1 and MSD2), two cytosolic nucleotide-binding domains (NBD1 and NBD2), and a single regulatory R-domain. MSD1 is linked to NBD1, and MSD2 is linked to NBD2, thereby forming two MSD-NBD complexes united by the R-domain. The MSDs form the channel of the CFTR protein, while the NBDs regulate its opening and closure. As the CFTR channel is an ATP-dependent ion channel, its opening requires R-domain phosphorylation (P) by the protein kinase A (PKA) and ATP binding at the NBDs, leading to their dimerization. This allows the chloride (Cl^-) ions to exit the epithelial cells. Channel closure is triggered by ATP hydrolysis, which results in the separation of the NBD dimer and restoration of the MSD conformation. (Hanssens et al., 2021)

CFTR protein functions:

Cystic Fibrosis is a disease that is caused due to mutations in the CFTR protein. More than 2000 such mutations in the CFTR protein have been identified, which can cause CF. Mentioned below are the significant mutations and their effects that lead to the development of CF.(Lara-Reyna et al., 2020)

1. Class I : These mutations lead to no production of protein.
2. Class II : These mutations are associated with a trafficking block.
3. Class III : These mutations lead to defective channel gating.
4. Class IV : These mutations lead to altered conductance.
5. Class V : These mutations reduce the amount of protein produced.
6. Class VI : These mutations produce a protein which is unstable.
7. Class VII : Due to these mutations, no mRNA is present.

The *ΔF508* mutation (also called *F508del*): The deletion of amino acid Phenylalanine(Phe) at position 508 in the CFTR protein is the most common mutation among CF patients, occurring in approximately 80% of the CF patients. This is a Class II mutation. Due to this mutation, the CFTR protein gets misfolded during its production and it does not reach the cell membrane. It is held in the endoplasmic reticulum and degraded due to its misfolded form. Since it does not reach the cell membrane, it does not function as a chloride ion channel (a channel that regulates the movement of chloride ions in and out of the cell). Because of this, thick, sticky mucus is developed in various parts of the body.

CF correctors have been developed to rectify this mutation to some extent, minimizing the symptoms in CF patients. (Ostedgaard et al., 2011). Table 2 has enlisted the variety of combinations of Correctors and Potentiators used in patients, indicating the desired outcome.

Table 2 : Combination of Correctors and Potentiators used on different systems affected by CF

Affected Organs	Manifestations	Combination of corrector-potentiator	Reference

Reproductive tract	Absence of vas deferens (males), infertility in females	Elexacaftor, Tezacaftor and Ivacaftor	(Putman et al., 2023)
Sweat glands	Elevated sweat chloride	Lumacaftor and Ivacaftor	(Enaud et al., 2023)
Lungs	Airway obstruction, chronic bacterial infection, bronchiectasis, pneumothorax, hemoptysis	Lumacaftor and Ivacaftor	(Enaud et al., 2023)
Sinuses	Sinusitis, polyps	Lumacaftor and Ivacaftor	(Enaud et al., 2023)
Pancreas	Exocrine insufficiency, CF-related diabetes	Elexacaftor, Tezacaftor and Ivacaftor	(Putman et al., 2023)
Liver	Obstructive biliary tract disease	Elexacaftor, Tezacaftor and Ivacaftor	(Duong et al., 2024)
Intestine	Meconium ileus, distal intestinal obstruction syndrome, rectal prolapse	Elexacaftor, Tezacaftor and Ivacaftor	(Putman et al., 2023)

Study of various CF correctors used in combination with Ivacaftor(IVA) in patients:

The effectiveness of the various Corrector-Potentiator combinations is assessed using a sputum sample, which indicates the extent of damage to the respiratory system, and OGTT, which indicates the function of the pancreas and glucose metabolism.

The mucus layer lines the airways in the respiratory system. It plays an important role in maintaining the balance of normal flora in the respiratory tract and traps pathogens. This function of the mucus layer is affected when the consistency of the mucus changes, making it sticky and thick. Airways lined with thick and sticky mucus - commonly seen in CF patients - allow the colonization of fungal and bacterial species that are otherwise kept under control with the help of the ciliary lining of the trachea. *Pseudomonas aeruginosa* is the most common pathogen that colonizes such airways, initiating the host immune response and inflammatory reaction. Therefore, the sputum sample is a very useful indication of the affected respiratory system in CF patients.(Parkins et al., 2018)

Thick and sticky mucus can affect the pancreas leading to CF-related diabetes. The insulin-producing cells in the pancreas get affected due to the mucus build-up in reduced insulin production and impaired glucose metabolism. The pancreas secretes C-peptide in the same quantity as insulin. C-peptide concentrations give an insight into pancreatic function and insulin secretion. Monitoring c-peptide concentrations helps assess the degree of insulin production. An Oral Glucose Tolerance Test(OGTT) is useful for assessing glucose metabolism and insulin secretion. Thus, it helps us to manage blood sugar levels and improve our overall health. (Putman et al., 2023)

1.Lumacaftor :

i. (Colombo et al., 2021)
This is a study to test if the combination of LUMA/IVA has an effect on improving glucose metabolism(pancreas) of a person with CF. In 2021 a study was carried out by C. Colombo et al. The subjects received a modified 3 h oral glucose tolerance test (OGTT), sampling at baseline, and at 30 min intervals for plasma glucose, serum insulin, and c-peptide concentrations to evaluate glucose tolerance. The team found that LUMA/IVA combination had no effect on the glucose levels of a person with CF. Basically, this combination was ineffective in improving the functioning of the pancreas.

ii. (Enaud et al., 2023)

This study was performed to understand the efficiency of the LUMA/IVA combination on treating microbiota-mycobiota and local inflammation(lungs). In 2023 a study was carried out by Raphael Enaud et al. The team collected sputum samples from CF patients aged 12 and older. The team has not been able to understand clearly the effects of the study yet.

iii. (Yaacoby-Bianu et al., 2022)

This study was conducted to understand the impact of LUMA/IVA combination on various organs. The effect of the combination was assessed on pancreatic function, bone metabolism, fertility status and nutritional factors. This study was carried out by Karin Yaacoby-Bianu et al(2022).

To evaluate the functioning of pancreas, an OGTT was performed in the patients without CF related diabetes. To evaluate the effect of combination on bone metabolism, the team kept a track of bone density of the patients. Bone metabolism factors like calcium, phosphorus and vitamin D levels were also evaluated. For understanding effectiveness of combination on nutritional status, body mass index (BMI) and levels of vitamin A, E, and albumin were monitored. The effect of combination on fertility status was understood by assessing reproductive hormones such as LH, FSH, testosterone, and estradiol in all the patients. The team found out that LUMA/IVA therapy had no impact on insulin secretion or glucose tolerance. But, LUMA/IVA therapy did improve the vitamin D absorption in the patients. Since, there was no change in the BMI, the team concluded that this combination had no effect on the nutritional status. There was no change in the sex hormones after treatment with LUMA/IVA therapy in a year, so, it had no impact on the fertility status either.

2. Tezacaftor :

i. (Paterson et al., 2023)

This study was performed to understand the impact of TEZA/IVA use in treatment for adults affected by CF. In 2023 a study was conducted by Iona Paterson et al. The team collected data on lung functioning and body mass index(BMI). There was a significant improvement in BMI. This combination was found to be effective.

3. Triple combination :

i. (Gur et al., 2023)

The objective of this study was to assess the effect of Elexacaftor, Tezacaftor and Ivacaftor(Trikafta) on factors like bone density, body composition and exercise capacity of patients. In 2022 a study was conducted by Michal Gur et al. A sweat test, lung clearance index, spirometry, cardio-pulmonary exercise test, and a six-minute walk test were carried out. The BMI and bone mineral density of patients was also measured. The team found out that there was a surge in BMI and bone mineral density of patients. The team discovered that there was improved respiratory efficacy. The team found a reduction in sweat test results.

ii. (Stastna et al., 2024)

This study was performed to understand the effect of ELEXA-TEZA-IVA(ETI) therapy on the nutritional status and digestive function of patients with CF. In 2024 a study was conducted by Nela Stastna et al. To conduct the study, the body measurement, pancreatic enzymes replacement therapy needs, and gastrointestinal symptoms were evaluated. The team found out that there was an increase in nutritional criterias, reduced requirements of pancreatic substitution in adult CF patients with ETI therapy.

iii. (Loske et al., 2024)

This study aims to find out the effects of ETI therapy on the transcriptome of nasal epithelial and immune cells on children with CF. In 2024 a study was conducted by Jennifer Loske et al. The team cumulated nasal swabs from children with CF. The team found out that this combination improves mucosal immunity and lowers immune cell inflammatory responses in children. The team believes that if the children are given ETI treatment at early stages, it could also restore the epithelial homeostasis.

Discussion :

Trikafta improves lung function by about 14 points whereas LUMA/IVA and TEZA/IVA show about a 4 point and 3-point improvement. LUMA/IVA did not improve the functioning of the pancreas whereas Trikafta was effective in improving pancreatic function. LUMA/IVA combination had no effect on BMI, TEZA/IVA therapy was found to improve BMI but not as much as Trikafta. Trikafta lowered pulmonary exacerbations by a lot. Trikafta improves pancreatic function, BMI and reduces immune cell inflammatory responses. Trikafta is effective

in 90% of CF patients. (https://www.mdpi.com/2075-4426/12/9/1528)(Tice et al., 2021) Trikafta is the only combination developed so far that has helped in overall development of a CF patient. Thus, we can conclude that Trikafta is the most effective and useful therapy discovered till now for CF patients.

Trikafta is the first widely effective treatment for people with F508del mutation. Trikafta has proved to be revolutionary in treatment for CF patients and it has a positive impact on the daily lives of people with CF. Even now, a person with CF used the Trikafta treatment and she reported that it had a positive effect on her body. She said that she was able to speak to people on the phone during walks and did not feel breathlessness(Zaher et al., 2021). Clearly, the Trikafta treatment was extremely successful for her. Using Trikafta is anticipated to bring substantial improvements to the lives of CF patients. This treatment has the potential to prevent the spread of the disease to multiple organs. It might also help small children with CF to reach a typical life expectancy. Trikafta therapy led to fewer visits to the hospitals for CF patients. Trikafta therapy has been proven useful and it has a profound impact on the lives of CF patients.

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