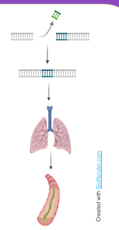
Improving Cystic Fibrosis Drug, Trikafta, to Improve Patient Outcome

Taylor Joron, Jan Mak, Abby Snape and Alex Schneider

Abstract

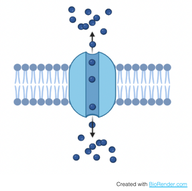
- Cystic fibrosis (CF) is a genetic condition
- characterized by a buildup of mucus resulting in lung disease, shortened life span and diminished quality of life
- 4300 people affected in Canada, 90% have the common F508del mutation

- Trikafta is a combination of 3 drugs (Ivacaftor, Tezacaftor, Elexacaftor) currently being used in the treatment of patients with F508del
- We propose multiple modifications to Ivacaftor and Tezacaftor to improve binding to the CFTR protein to increase their effectiveness



Introduction

- Cystic fibrosis is caused by failure of the cystic fibrosis transmembrane conductance regulator (CFTR), a protein channel that regulates the balance of fluids in the lung (1,3)
- Failure results in mucus building up and causing lung disease and other serious effects such as pancreatic insufficiency, frequent respiratory infection and malnutrition (1,3)
- Current therapies extend life expectancy from early childhood to 48 years and beyond (2)
- Most common mutation is deletion of phenylalanine at position 508 (F508del) (7)
- F508 deletion destabilizes the protein and makes it difficult for the protein to assemble into its functional conformation (7)



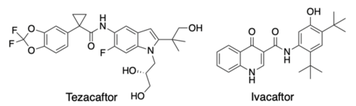


Figure 1: The chemical structures of Trikafta's three components.
Shown are the original structures before modifications

- Stabilization of other areas on the protein can rescue the mutant protein (6)
- Trikafta is a triple combination therapy consisting of Ivacaftor, Tezacaftor, Elexacaftor (7)
- We predict that modifications to Ivacaftor and Tezacaftor will improve their binding to the CFTR protein, potentially improving patient outcomes.

Ivacaftor Modifications

What does it do?

- Ivacaftor is a CFTR **Potentiator** which holds the CFTR channel open longer
- When the CFTR is open, it can regulate fluid balance in the lungs
- Thus, Ivacaftor allows CFTR to continue regulating fluid balance
- Ivacaftor interacts with several loops of the CFTR and the cell membrane to stabilize the protein
- Sustains open conformation

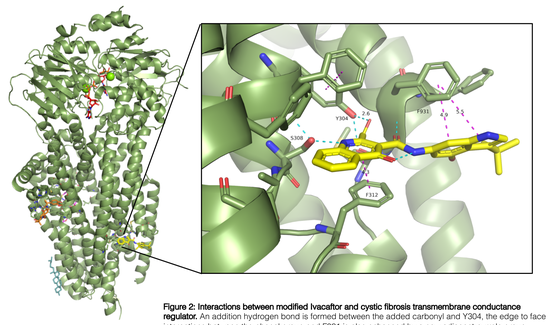
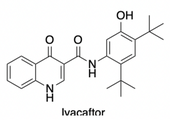


Figure 2: Interactions between modified Ivacaftor and cystic fibrosis transmembrane conductance regulator. An addition hydrogen bond is formed between the added carbonyl and Y304, the edge to face interactions between the phenol group and F931 is also enhanced by a new adjacent pyrrole group. Modified Ivacaftor is shown in yellow, π-interactions are shown in pink and hydrogen bonds are shown in cyan. Only key interactions between Ivacaftor and residues S308, F312, Y304 and F931 are shown above.

Modification #1

- Introduction of carbonyl group
- This addition will allow for the formation of a hydrogen bond between the modified Ivacaftor and CFTR
- This bond is a hydrogen bond with the amino acid, Tyrosine at position 304 of CFTR
- This forms a shorter bonding distance of 2.6 Å between hydrogen and Y304 oxygen
- The shorter bond distance may form a stronger hydrogen bond



Modification #2

- Addition of pyrrole group
  - Better sustainability of open conformation
  - Increased stabilizing effect by π-interactions

Figure 3: Structure of Ivacaftor and modifications proposed. A carbonyl is added to the oxyquinoline moiety, and a pyrrole group is added adjacent to the phenol ring.

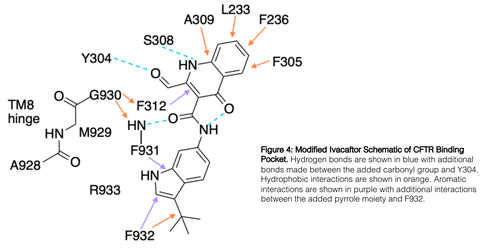


Figure 4: Modified Ivacaftor Schematic of CFTR Binding Pocket. Hydrogen bonds are shown in blue with additional bonds made between the added carbonyl group and Y304. Hydrophobic interactions are shown in orange. Aromatic interactions are shown in purple with additional interactions between the added pyrrole moiety and F932.

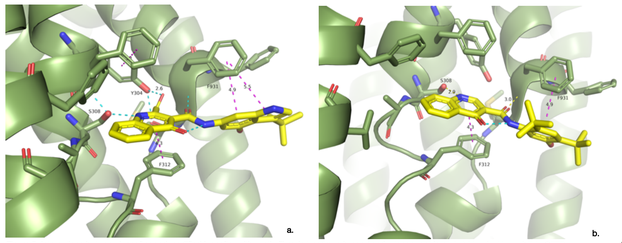


Figure 5: Interactions between Ivacaftor and modified Ivacaftor with cystic fibrosis transmembrane conductance regulator. (a.) Modified Ivacaftor in CFTR pocket. An additional hydrogen bond is formed between the added carbonyl and Y304, the edge to face interactions between the phenol group and F931 are also enhanced by a new adjacent pyrrole group. π-interactions are shown in pink and hydrogen bonds are shown in cyan. (b.) Ivacaftor binding in the CFTR pocket. Only key interactions between residues S308, F312, Y304 and F931 are shown above.

Tezacaftor Modifications

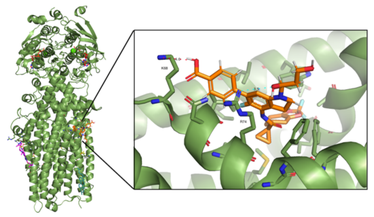


Figure 6: Modified Tezacaftor binding in cystic fibrosis transmembrane conductance regulator. Modified Tezacaftor binding in CFTR pocket. Hydrogen bond between the drug's terminal hydroxyl group and R74 is shown in cyan with a distance of 3.5 Å. Salt bridge formation between the additional carboxylic acid and K68 is shown in red with a distance of 4 Å.

What does it do?

- Tezacaftor is a **Type I Corrector** which binds CFTR directly to ensure folding into proper functional shape
- CFTR with F508del folds into non-functional forms that typically get degraded
- Tezacaftor prevents mutated (F508del) CFTR from being targeted by the cell's quality control mechanisms and stops them from being degraded
- Increases amount of properly folded CFTR levels in the cell membrane

Modification #1

- Addition of N-3-hydroxyl propanol
- This may form a hydrogen bond with amino acid Arginine at position 74 (R74) on CFTR
- Oxygen on Tezacaftor and nitrogen on Arginine form the hydrogen bond which preserves main bond in Tezacaftor interaction
- Bond distance of 3.5 Å
- Nearby oxygen and nitrogen may strengthen hydrogen bond
- Increases stabilization and affinity for CFTR protein

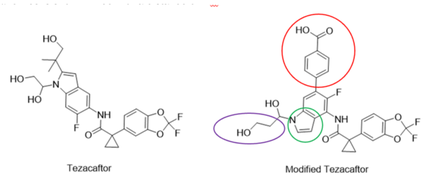


Figure 7: Tezacaftor and Modified Tezacaftor. Modifications in the orientation of the indole ring, addition of N-3-hydroxyl propanol, and benzoic acid branch.

Modification #2

- Reorientation of indole ring
  - Allows for addition of new bonding interaction between drug and CFTR, benzoic acid branch
  - This allows for salt bridge formation with amino acid Lysine at position 68 (K68) on CFTR
  - This will increase binding affinity

Modification #3

- Addition of benzoic acid branch
  - Allows for salt bridge formation with K68
  - This bonding distance is 4 Å
- Found in Lumacaftor, an older CFTR corrector
  - This addition was shown to have higher binding strength and affinity to CFTR
- Increases rigidity of structure, which increases the likelihood of binding

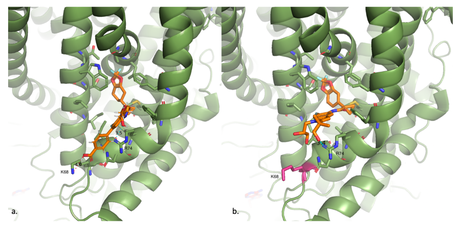


Figure 9: Tezacaftor and Modified Tezacaftor's Interactions with Cystic Fibrosis Transmembrane Conductance Regulator. (a.) Modified Tezacaftor binding in CFTR pocket. Hydrogen bond between the drug's terminal hydroxyl group and R74 is shown in cyan with a distance of 3.5 Å. Salt Bridge formation between the additional carboxylic acid and K68 is shown in red with a distance of 4 Å. (b.) Tezacaftor binding in the CFTR pocket, with hydrogen bonding shown in cyan with a distance of 3.4 Å. K68 is highlighted in pink.

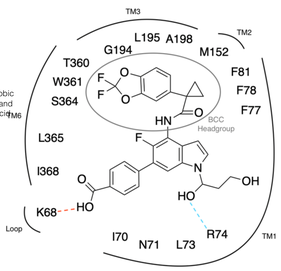


Figure 8: Modified Tezacaftor Schematic of CFTR Binding Pocket.
Conserved BCC Headgroup is circled in grey, surrounded by hydrophobic residues. Hydrogen bond between the drug's terminal hydroxyl group and R74 is shown in cyan and salt bridge formation between the benzoic acid and K68 is shown in red.

References

(1) De Boeck, K. (2020) Cystic fibrosis in the year 2020: A disease with a new face. *Acta Paediatr* **109**, 893-899
(2) McBennett, K.A., Davis, P.B., and Konstan, M.W. (2022) Increasing life expectancy in cystic fibrosis: Advances and challenges. *Pediatr Pulmonol* **57 Suppl 1**, S5-s12
(3) Rosales-Reyes, R., Vargas-Roldán, S.Y., Lezana-Fernández, J.L., and Santos-Preciado, J.I. (2021) Pseudomonas Aeruginosa: Genetic Adaptation, A Strategy for Its Persistence in Cystic Fibrosis. *Arch Med Res* **52**, 357-361
(4) Liu, F., Zhang, Z., Csanády, L., Gadsby, D.C., and Chen, J. (2017) Molecular Structure of the Human CFTR Ion Channel. *Cell* **169**, 85-95.e88
(5) Kleizen, B., van Vlijmen, T., de Jonge, H.R., and Braakman, I. (2005) Folding of CFTR is predominantly cotranslational. *Mol Cell* **20**, 277-287
(6) Fiedorczuk, K., and Chen, J. (2022) Mechanism of CFTR correction by type I folding correctors. *Cell* **185**, 158-168.e111
(7) Fiedorczuk, K., and Chen, J. (2022) Molecular structures reveal synergistic rescue of Δ508 CFTR by Trikafta modulators. *Science* **378**, 284-290
(8) McPhail, G.L., and Clancy, J.P. (2013) Ivacaftor: the first therapy acting on the primary cause of cystic fibrosis. *Drugs Today* **49**, 253-260
(9) Yu, H., Burton, B., Huang, C.J., Worley, J., Cao, D., Johnson, J.P., Jr. et al. (2012) Ivacaftor potentiation of multiple CFTR channels with gating mutations. *J Cyst Fibros* **11**, 237-245
(10) Deeks, E.D. (2016) Lumacaftor/Ivacaftor: A Review in Cystic Fibrosis. *Drugs* **76**, 1191-1201
(11) Davies, J.C., Moskowitz, S.M., Brown, C., Horsley, A., Mall, M.A., McKone, E.F. et al. (2018) VX-659–tezacaftor–ivacaftor in patients with cystic fibrosis and one or two Phe508del alleles. *New England Journal of Medicine* **379**, 1599-1611
(12) Liu, F., Zhang, Z., Levit, A., Levring, J., Touhara, K.K., Shoichet, B.K. et al. (2019) Structural identification of a hotspot on CFTR for potentiation. *Science* **364**, 1184-1188
(13) Van der Plas, S.E., Kelgtermans, H., De Munck, T., Martina, S.L., Dropsit, S., Quinton, E. et al. (2018) Discovery of N-(3-carbamoyl-5, 5, 7, 7-tetramethyl-5, 7-dihydro-4 H-thieno [2, 3-c] pyran-2-yl)-l H-pyrazole-5-carboxamide (GLPG1837), a novel potentiator which can open class III mutant cystic fibrosis transmembrane conductance regulator (CFTR) channels to a high extent. *J. Med. Chem.* **61**, 1425-1435
(14) Hadida, S., Van Goor, F., Zhou, J., Arumugam, V., McCartney, J., Hazlewood, A. et al. (2014) Discovery of N-(2, 4-di-tert-butyl-5-hydroxyphenyl)-4-oxo-1, 4-dihydroquinoline-3-carboxamide (VX-770, ivacaftor), a potent and orally bioavailable CFTR potentiator. *Journal of medicinal chemistry* **57**, 9776-9795
(15) Negoda, A., Hogan, M.S., Cowley, E.A., and Linsdell, P. (2019) Contribution of the eighth transmembrane segment to the function of the CFTR chloride channel pore. *Cellular and Molecular Life Sciences* **76**, 2411-2423
(16) Donald, J.E., Kulp, D.W., and DeGrado, W.F. (2011) Salt bridges: geometrically specific, designable interactions. *Proteins: Structure, Function, and Bioinformatics* **79**, 898-915
(17) The PyMOL Molecular Graphics System, Version 2.5.4, Schrödinger, LLC.
(18) PDB-8EIQ. Molecular structures reveal synergistic rescue of del508 CFTR by Trikafta modulators. Fiedorczuk, K., Chen, J. (2022) *Science* **378**: 284-290 doi: 10.2210/pdb8EIQ/pdb
(19) BioRender.com (2022). Retrieved from https://app.biorender.com/biorender-templates