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UNIVERSIDADE D
COIMBRA

Carla Alexandra Almeida Fonseca

Relatório de Estágio e Monografia intitulada "Cystic Fibrosis: physiopathology and the latest pharmacological treatments" referentes à Unidade Curricular "Estágio", sob a orientação do Doutor Paulo Jorge da Silva Monteiro e da Professora Doutora Ana Cristina Bairrada Fortuna apresentados à Faculdade de Farmácia da Universidade de Coimbra, para apreciação na prestação de provas públicas de Mestrado Integrado em Ciências Farmacêuticas.

Julho de 2020

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Coimbra, julho de 2020

Eu, Carla Alexandra Almeida Fonseca, estudante do Mestrado Integrado em Ciências Farmacêuticas, com o nº 2015259349, declaro assumir toda a responsabilidade pelo conteúdo do Documento Relatório de Estágio e Monografia intitulada "Cystic Fibrosis: physiopathology and the latest pharmacological treatments" apresentados à Faculdade de Farmácia da Universidade de Coimbra, no âmbito da unidade de Estágio Curricular.

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Coimbra, 15 de julho de 2020.

Carla Alexandra Almeida Fonseca

(Carla Alexandra Almeida Fonseca)

Agradecimentos

No término de mais uma etapa importante da minha vida, não posso deixar de agradecer a todas as pessoas que, direta e indiretamente, me deram força durante todo o meu percurso de MICF.

Com um carinho especial, quero agradecer à minha orientadora Professora Doutora Ana Cristina Bairrada Fortuna por toda a preocupação e disponibilidade nesta fase final. Gratifico também a esperança e incentivo depositados no meu trabalho.

Agradeço a todos os membros da equipa da Farmácia São José pelo excelente acolhimento, preocupação e disponibilidade constante para me ajudar, independentemente das circunstâncias. Em especial, gratifico a confiança e cuidado do Dr. Paulo Jorge da Silva Monteiro e a amizade da Dr.ª Carla Oliveira e do João Miguel Marques. Agradeço ainda aos colegas estagiários pelo ambiente divertido e agradável, realçando a amizade da Carolina Baptista e do Ivo Simões.

Aos meus amigos quero agradecer toda força e coragem, estando sempre presentes quer nos bons quer nos maus momentos.

Agradeço a toda a minha família, particularmente ao meu irmão, pelo incentivo e encorajamento constantes, acreditando sempre no meu empenho e esforço.

A todos, um **enorme obrigada!**

"The future depends on what you do today"

Mahatma Gandhi

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PARTE I

RELATÓRIO DE ESTÁGIO EM FARMÁCIA COMUNITÁRIA

FARMÁCIA SÃO JOSÉ

Lista de Abreviaturas

API – Princípio Ativo do medicamento

COVID-19 – Corona Virus Disease 2019

MICF – Mestrado Integrado em Ciências Farmacêuticas

MNSRM – Medicamento Não Sujeito a Receita Médica

MSRM – Medicamento Sujeito a Receita Médica

SWOT – *Strengths, Weaknesses, Opportunities* e *Threats*

Introdução

No âmbito da unidade curricular "Estágio Curricular", do Mestrado Integrado em Ciências Farmacêuticas (MICF), conforme Diretiva 2013/55/UE, do Parlamento Europeu e do Conselho de 20 de novembro de 2013 (Artº 44º, nº 2)[1], está implícito a realização de um estágio em Farmácia Comunitária. O estágio tem como objetivo a consolidação e aplicação dos conhecimentos adquiridos ao longo do MICF, bem como a aquisição de experiência e prática em contexto real, indispensáveis para a correta futura carreira profissional.

Da realização do estágio em Farmácia Comunitária espera-se o desempenho de funções de aprovisionamento, armazenamento, gestão e indicação farmacêutica de medicamentos e produtos de saúde, a preparação e dispensa de medicamentos, informação e consulta de documentação científica de utilização em Farmácia, interação farmacêutico-utente-medicamento e, quando possível, simultaneamente com o médico ou outros profissionais de saúde. A organização e gestão de Farmácia fazem, de igual modo, parte das funções delineadas para o estágio curricular.

O Estágio em Farmácia Comunitária foi realizado na Farmácia São José, localizada na Avenida Calouste Gulbenkian, lote 5, rés do chão, Santo António dos Olivais, em Coimbra (Figura 1). Foi fundada a 1997 e é dirigida pelo Dr. Paulo Jorge da Silva Monteiro. A Farmácia São José encontra-se autorizada, pela Autoridade Nacional do Medicamento e Produtos de Saúde, I. P (INFARMED, I.P.), a disponibilizar medicamentos via Internet, e apresenta diversos serviços farmacêuticos ao dispor do utente. Destes serviços nomeia-se a administração de injetáveis e vacinas, a monitorização do índice de massa corporal (IMC), colesterol total, triglicerídeos, glicemia, pressão arterial e frequência cardíaca, consultas de nutrição e dietética e, atualmente com menor repercussão, consultas de podologia e serviços de cessação tabágica. Acrescenta-se ainda o facto de ser uma farmácia pertencente ao grupo das Farmácia Portuguesas, o que beneficia utentes aderentes com cartão "Saúda"[3]. O calendário preenchido, com dias promocionais face às diferentes marcas de dermocosmética disponíveis, dias dedicados a grávidas, assim como campanhas de acústica médica, são aspetos característicos da Farmácia São José.

Farmácia São José

Figura 1. Logotipo da Farmácia São José[2].

A Farmácia São José foca-se nas necessidades do utente e da população em geral garantindo o correto uso dos medicamentos. Prioriza o acesso à terapêutica e aos produtos de saúde e, aquando da sua cedência, garante o aconselhamento farmacêutico indispensável para a correta utilização e adesão à terapêutica. Permite ainda uma terapêutica personalizada visto ser uma Farmácia apta à produção de medicamentos manipulados.

A minha integração na equipa profissional da Farmácia São José permitiu-me realizar inúmeras tarefas pelos vários sectores conforme as necessidades. Destaco o atendimento ao público com disponibilização de medicamentos não sujeitos a receita médica (MNSRM) e medicamentos sujeitos a receita médica (MSRM), quer por receita eletrónica, quer por receita manual, assim como em situações de comparticipação especial e situações de protocolos acordados com a Liga Portuguesa Contra o Cancro (LPCC) e Associação de Pais e Amigos de Crianças Com Cancro (ACREDITAR). Realço o foco constante na promoção da saúde publica e bem-estar do utente.

Referente à organização e gestão de Farmácia foram realizados pedidos e receção de encomendas diárias, envio e receção de encomendas diretas acordadas diretamente com os fornecedores, gestão e regularização de devoluções quer com produtos, notas de crédito e até devoluções não aceites, e gestão de *stocks*, nomeadamente no controlo de prazos de validade e na análise das compras e vendas dos produtos de saúde, mesmo tratando-se de produtos esgotados. A organização do receituário, emissão do verbete, resumo de lotes e emissão da fatura para reembolso monetário da comparticipação feita ao utente fez, de igual modo, parte das tarefas efetuadas. No laboratório foram realizadas tarefas de auxílio na produção de medicamentos manipulados, na correta higienização e controlo de qualidade, bem como na gestão dos respetivos *stocks*.

Face à situação excecional de pandemia vivida pela doença provocada pelo novo Coronavírus (COVID-19)[4], durante o período de estágio, experienciei a organização e gestão de Farmácia em clima de stress face à elevada procura de medicamentos e produtos de saúde e, especialmente, de equipamentos de proteção individual (EPI).

Neste documento apresenta-se de seguida uma análise SWOT, (*Strengths, Weaknesses, Opportunities* e *Threats*), que traduz sinteticamente os pontos fortes (*Strengths*), os pontos fracos (*Weaknesses*), as oportunidades (*Opportunities*) e as ameaças (*Threats*) advindas do estágio curricular realizado na Farmácia São José.

Análise SWOT

A análise SWOT permite abordar condicionantes internas, expressas através dos pontos fortes (*Strengths*) e pontos fracos (*Weaknesses*), e condicionantes externas, explícitas nas oportunidades (*Opportunities*) e ameaças (*Threats*). Assim, sinteticamente, apresenta-se de seguida as principais condicionantes do estágio realizado na Farmácia São José (Figura 2).

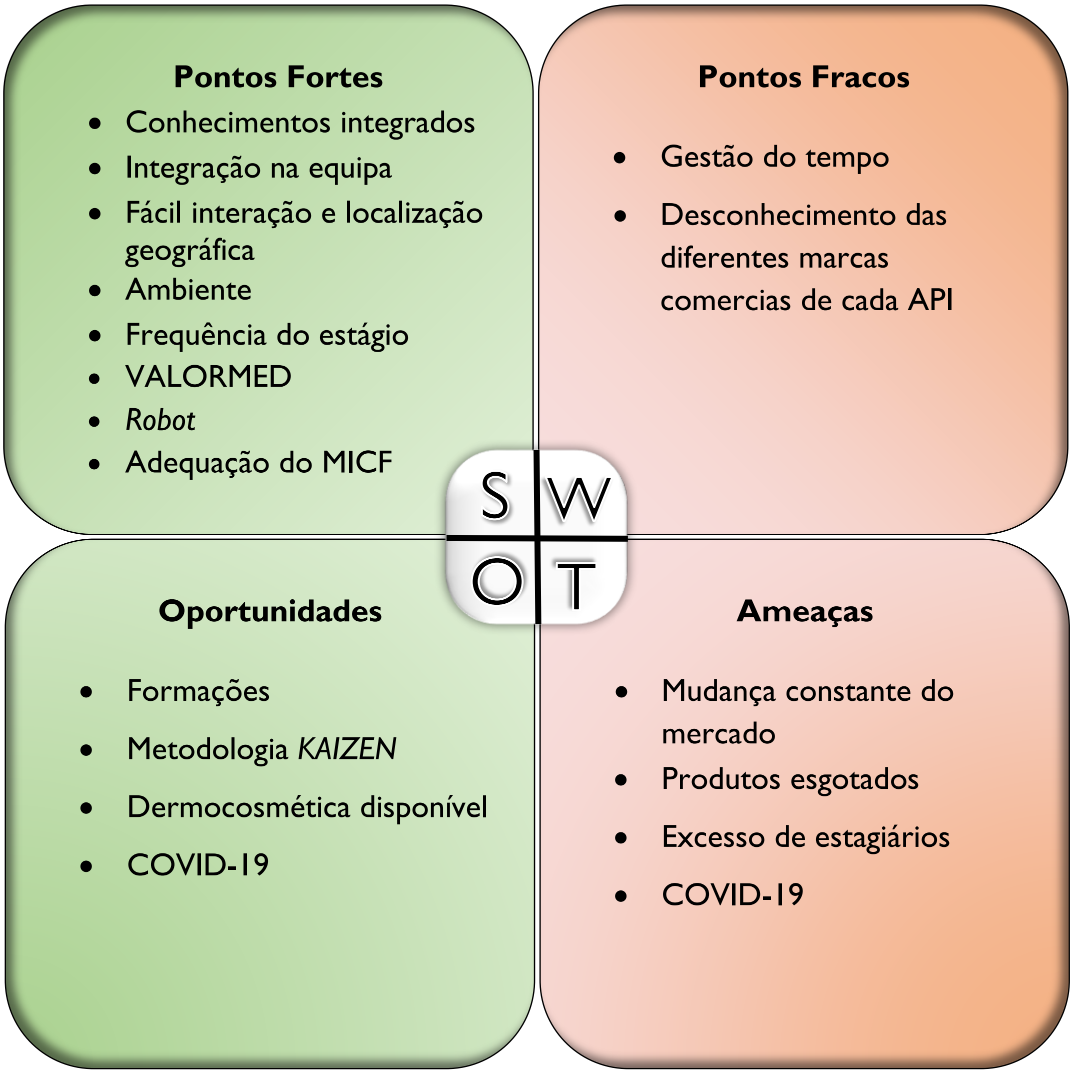

Figura 2. Esquema sintético da análise SWOT ao estágio realizado na Farmácia São José.

1. Pontos Fortes

1.1. Conhecimentos integrados

Os conhecimentos teóricos adquiridos ao longo do curso encontram-se bem integrados com aos conhecimentos necessários para a prática profissional. Foram vários os momentos em que, perante as explicações e aconselhamentos que me eram ensinados pelos membros da equipa de trabalho, me via a recordar e aplicar a teoria ensinada durante o MICF.

Sendo o estágio efetuado em Farmácia Comunitária, foram fundamentais várias unidades curriculares. Enfatizo Indicação Farmacêutica, Farmácia Clínica, Farmacoterapia, Bioquímica Clínica e Farmacologias para a compreensão das terapêuticas, mecanismos de ação, interações farmacocinéticas e farmacodinâmicas, assim como para a compreensão dos efeitos adversos. Todos estes conhecimentos permitiram efetuar um aconselhamento cuidado e personalizado, garantindo o correto uso do medicamento. A unidade curricular de Dermofarmácia e Cosmética foi fulcral, visto que a Farmácia São José apresenta uma forte vertente de dermocosmética ao dispor do utente. Para a realização das atividades no laboratório, no auxílio da preparação de medicamentos manipulados, foram os conhecimentos de Farmácia Galénica, de Gestão e Garantia de Qualidade e das Tecnologias Farmacêuticas que me suportaram para uma correta atividade. Outras unidades curriculares como Farmacognosia, Plantas Medicinais e Fitoterapia foram úteis e aplicáveis no aconselhamento de produtos à base de plantas.

1.2. Integração na equipa

A integração na equipa da Farmácia São José é um ponto forte, visto que apresentou um impacto muito positivo tanto na minha motivação durante a realização do estágio como no meu progresso de aprendizagem e adaptação. A simpatia constante dos membros da equipa, o acolhimento que me fez sentir membro pertencente, a disponibilidade para o esclarecimento de qualquer dúvida que me surgisse qualifica todo o período de estágio. Sobrelevo a confiança depositada no meu trabalho, que me permitiu a aquisição de experiência e capacidade para saber lidar com as eventualidades inerentes. Testemunho a realização de telefonemas para os fornecedores, ou distribuidores para pedidos de encomendas, ou até para reclamações quando os produtos enviados não iam de encontro com a nota de encomenda. Todos estes aspetos contribuíram para uma evolução acima das minhas espectativas e, sobretudo, para a sensação de utilidade e felicidade durante a realização do estágio.

1.3. Fácil interação e localização geográfica

Outro dos pontos fortes do estágio foi a facilidade de interação com o médico prescritor. O facto de a Farmácia São José estar localizada numa zona próxima do Hospital e de clínicas privadas, potencia esta facilidade na comunicação. Em caso de dúvidas de prescrição, estas eram facilmente solucionadas com um telefonema. Inúmeras vezes ocorreu que o utente era o próprio médico prescritor, o que me permitia esclarecer de imediato qualquer dúvida que me surgisse ou até a eventual correção de erros involuntários que pudessem surgir nas receitas manuais.

1.4. Ambiente

A boa interação com os utentes é positivamente influenciada pelo ambiente agradável, traduzindo-se assim em mais um ponto forte do estágio realizado. O facto de se fazer de tudo para que o tempo de espera seja o mínimo possível, assim como o espaço amplo e luminoso são fatores que contribuem favoravelmente.

A Farmácia São José, comumente, é visitada pelos seus utentes fidelizados, o que permite criar empatia com os mesmos. Deste modo, cria-se um ambiente caloroso, de confiança e amizade marcado pela sensação de bem-estar, sendo mesmo um local de desabafo. Várias foram as situações em que os utentes vieram à Farmácia para pedir um conselho, dar um aceno ou desabafar. "Vocês são a minha família" foram palavras que me marcaram, expressas por uma utente emocionada.

1.5. Frequência do estágio

Sendo amplo o horário de funcionamento da Farmácia São José (Tabela 1), tal permitiu criar uma rotina de trabalho, com frequência diária semanal, abrindo a possibilidade de estagiar ao sábado. Antes da pandemia provocada pelo COVID-19, o estágio era realizado por turnos. Durante a manhã realizava atendimento ao publico, sendo as tarefas de organização e gestão de Farmácia realizadas durante a tarde, invertendo a ordem no dia seguinte. Esta organização permitiu-me acompanhar o percurso completo da medicação na Farmácia para o utente. Recordo uma encomenda realizada durante uma manhã de atendimento, com respetiva receção durante a tarde. Neste caso, como o utente veio levantar a sua encomenda na tarde seguinte, tive ainda a oportunidade de lha entregar com o devido aconselhamento necessário.

Tabela 1. Horário de funcionamento da Farmácia São José.[2]

Dia de semana	Horário
Segunda-feira a Sexta-feira	08:30 às 21:00
Sábado	09:00 às 20:00
Domingos e Feriados	Fechada

1.6. VALORMED

A Sociedade Gestora de Resíduos de Embalagens e Medicamentos, Lda., é também um ponto forte, visto que me permitiu consciencializar a população no que concerne ao destino que deve ser dado à medicação e seus resíduos, contribuindo para a educação da saúde pública focada na ecologia ambiental. Ressalvo a boa aderência, existindo utentes que se dirigiam à Farmácia com o principal objetivo de entregar os seus resíduos medicamentosos.

Separadamente, efetuava-se, de igual modo, a recolha de exames complementares de diagnóstico por imagem, como são exemplos os raios-X, a tomografia computadorizada e a ressonância magnética.

1.7. *Robot*

Sendo a receção de encomendas efetuada manualmente, com posterior introdução dos medicamentos no *robot*, tal permitiu a familiarização com as embalagens e, simultaneamente, uma arrumação mais rápida e segura.

A nível do atendimento ao público, o *robot* facilitava a disponibilização dos medicamentos, facultando a medicação sem erros, no que concerne a dosagens e laboratórios de origem, permitindo ainda usufruir do tempo desde o pedido à disponibilização do medicamento pelo *robot* para consultar informações e até possíveis interações medicamentosas. Este fator permitiu assegurar o uso racional da medicação através de um correto aconselhamento.

1.8. Adequação do MICF

O MICF apresenta uma base teórica bem estruturada, ainda que exaustiva face à elevada carga horária.

Sendo os primeiros anos do MICF mais gerais e teóricos, permitem a obtenção de conhecimentos essenciais, independentemente do rumo profissional, para aplicação nas aulas práticas. É através da realização de casos clínicos, atividades laboratoriais, demonstrações de aconselhamentos corretos e modos de agir face às diversas situações e personalidades, que se adquire uma boa formação e preparação para a futura carreira profissional. O constante incentivo ao desenvolvimento de características singulares, *soft skills*, bem como o alerta para a constante evolução do mercado de trabalho contribuem para a melhor capacidade de adaptação e de evolução a nível profissional.

Assim, considero como ponto forte a adequação do MICF às perspetivas futuras para uma carreira profissional visto que me forneceu conhecimentos e estimulou o desenvolvimento de *soft skills*, concedendo-me uma forte capacidade de adaptação e aprendizagem.

Saliento a extrema importância a um fácil acesso à realização de estágios extracurriculares, tanto para a aquisição de experiência, como para o delineamento profissional. Fundamento com a minha experiência face à realização de um estágio de verão em Farmácia Comunitária, que me permitiu contactar com a realidade, facilitando também a consolidação de conhecimentos instruídos posteriormente.

2. Pontos Fracos

2.1. Gestão do tempo

Muito forçado pelos pontos fortes do estágio realizado, um dos maiores pontos fracos consistiu na falta de gestão do tempo. Este aspeto tornou-me incapaz de cumprir horários no sentido em que apresentei dificuldades em deixar uma tarefa incompleta, se existisse a possibilidade de a terminar. Aparentemente seria um ponto forte, mas tal atitude influenciou as pausas para efetuar as refeições e até de fim de dia. Várias foram as alturas em que, no atendimento, mesmo após efetuar o fim de dia no SIFARMA 2000® e respetivo fecho da caixa, no caso de chegarem utentes, continuava a prestar atendimento passando da hora estipulada para o fim de dia, implicando a repetição do processo de fecho. O mesmo se sucedeu com receção de encomendas. Este fator teve repercussões a nível do cansaço sentido, com impacto negativo no desenvolvimento da monografia.

2.2. Desconhecimento das diferentes marcas comerciais de cada API

Condicionado pela falta de experiência e pela constante mudança do mercado farmacêutico, como ponto fraco, destaco o desconhecimento dos nomes de marca existentes para os variados princípios ativos (API) disponíveis no mercado. Este desconhecimento teve repercussão na disponibilização tanto de MNSRM como de MSRM, no entanto, os maiores impasses foram sentidos aquando da abertura da receita eletrónica, em que a medicação está acessível por denominação comum internacional (DCI) e o utente solicitava a medicação através do nome comercial.

3. Oportunidades

3.1. Formações

No decorrer do estágio foram inúmeras as formações disponíveis que contribuíram para um incremento no conhecimento e atualização do mesmo. Desde as formações mais curtas realizadas no espaço da Farmácia por delegados de informação, às formações mais completas. Exemplos são a formação "A PELE, Conceitos de Cosmética e Cosmetologia" a 25 de janeiro de 2020, efetuada por um membro trabalhador da Farmácia São José, Susana de Jesus (Anexo); a formação efetuada pela Arkopharma® e a formação acerca do COVID-19, realizada nas instalações da PLURAL, a distribuidora de produtos farmacêuticos.

Estas formações foram uma oportunidade de aprendizagem e influenciaram positivamente no que respeita ao aconselhamento farmacêutico visto que, ao possuir informação atualizada, estava apta a transmiti-la com maior grau de confiança.

3.2. Metodologia *KAIZEN*

A Farmácia São José concedeu-me a oportunidade de contactar com a metodologia *KAIZEN* e participar de forma ativa em atividades de organização do espaço da Farmácia. Esta metodologia foi introduzida na Farmácia através de uma consultora da *Global Intelligent Technologies, Glintt*. A consultora procedeu a uma análise da eficiência operacional, gestão económico-financeira, gestão dos recursos humanos bem com a gestão comercial e de marketing de modo a garantir a sustentabilidade e um sistema de melhoria contínua. Esta metodologia consciencializou-me para a importância da capacidade de adaptação, espírito critico e espírito de equipa.

3.3. Dermocosmética disponível

Outra das oportunidades que me foram facultadas durante a realização do estágio foi a possibilidade de contactar com inúmeras marcas de dermocosmética, o que promoveu o meu conhecimento na área. Destas marcas destaco Caudalie®, Avène®, Bioderma®, Eucerin®, Filorga®, MatiDerm®, Vichy® e La Roche-Posay®.

Apesar da dificuldade em estar permanentemente atualizada a nível dos produtos disponíveis e respetivas gamas, através da ajuda dos membros da equipa, era dada a possibilidade de escolha ao utente de modo a satisfazer as suas necessidades, sendo assim uma mais valia durante o atendimento.

3.4. COVID-19

Face à situação excecional vivida durante o período de estágio, a pandemia provocada pelo COVID-19 facultou-me algumas oportunidades, apesar das predominantes ameaças que abordarei no tópico seguinte.

Uma das oportunidades consistiu no aprimoramento da capacidade de gestão de reservas de EPI face à situação de procura constante e, simultaneamente, se tratar de produtos rateados, estando disponíveis em quantidades limitadas. Outra das oportunidades foi o desenvolvimento da capacidade de trabalhar sob regras rigorosas face ao plano de contingência Nacional.

4. Ameaças

4.1. Mudança constante do mercado

A constate atualização do mercado consistiu numa ameaça durante a realização do estágio visto que não permitia obter segurança na informação consolidada. As alterações de preços bastante frequentes, alterações no aspeto físico da embalagem dos medicamentos foram aspetos que tiveram impacto, principalmente, durante o atendimento, perante utentes fidelizados. Isto deve-se ao facto destes utentes estarem habituados a determinados preços e aspetos físicos das embalagens e, adicionalmente face à idade avançada de alguns, a mudança tornava-se um entrave.

4.2. Produtos esgotados

A existência de produtos esgotados foi outra das ameaças do estágio. Relato diversas situações em que o utente pretendia um medicamento específico, ou de um laboratório específico, por ser sempre essa a sua preferência, ou face à sensibilidade a excipientes de diferentes laboratórios, e o medicamento pretendido não se encontrava disponível. Este fator desencadeou sensações de insatisfação, tanto para o utente por não encontrar o pretendido, como para o farmacêutico, visto que a satisfação do utente não estava dependente das suas ações.

4.3. Excesso de estagiários

Sendo a equipa da Farmácia São José numerosa, o número de estagiários tornou-se uma ameaça apesar da excelente relação estabelecida. O motivo está no facto de algumas vezes não ser possível efetuar tarefas por não existir computadores livres para a realização das mesmas e na impossibilidade de ter um acompanhamento mais individualizado. Este fator teve maior impacto no período de estágio após o levantamento do estado de emergência face à pandeia vivida pelo COVID-19, o que obrigou a uma gestão dos estagiários por turnos.

4.4. COVID-19

Além das oportunidades mencionadas anteriormente, a situação de pandemia vivida pelo COVID-19 desencadeou uma grande ameaça, a necessidade de interrupção do estágio. Desta forma, todo o processo de aprendizagem foi abalado e mesmo após o regresso, a COVID-19 manteve o impacto negativo, uma vez que a frequência ao estágio teve de ser obrigatoriamente reduzida, principalmente porque a Farmácia apresentava a equipa e horário mais reduzidos. A necessidade de desinfeção total do local de atendimento após cada utente recebido, bem como o número limitado de utentes no interior da Farmácia traduziu-se na redução do número de atendimentos por dia, diminuindo a experiência e probabilidade de lidar com novas situações.

Casos Práticos

O estágio na Farmácia São José permitiu aplicar e testar os conhecimentos adquiridos ao longo destes últimos quatro anos e meio de MICF, bem como adquirir prática em contexto real, da qual advêm os seguintes casos clínicos exemplificativos:

Caso 1

Senhora, de 56 anos, apresenta-se na Farmácia São José queixando-se de "fungo na unha do pé". Menciona que está a chegar o calor e pretende envernizar as unhas para poder usar calçado de verão, no entanto, sente agravamento do estado da unha aquando deste cuidado estético. Solicita algo para o tratamento do fungo que, simultaneamente, lhe permita continuar a envernizar as unhas.

Face à presente situação, apresentei como solução amorolfina a 5% sob a forma farmacêutica de verniz para as unhas. Baseei o meu aconselhamento no facto deste medicamento poder ser de aplicação única semanal. Deste modo expliquei que poderia fazer a aplicação uma vez por semana, salientando a importância de remover o verniz não medicamentoso e limar a unha antes da aplicação do verniz de amorolfina. Aconselhei também a realização do tratamento à noite de modo a que a amorolfina consiga exercer a sua atividade antifúngica durante a noite e, deste modo, na manhã seguinte, poderia realizar o seu cuidado estético novamente. Adicionalmente aconselhei a fazer uma higiene mais pormenorizada do calçado e a evitar ter o pé muito tempo sob um ambiente húmido e pouco arejado para evitar a contaminação das restantes unhas. Alertei ainda para o facto de o tratamento das onicomicoses ser um tratamento longo e demorado até à obtenção de resultados visíveis e que o sucesso do tratamento dependia do não abandono da terapêutica.

Caso 2

Senhora de 42 anos dirige-se à Farmácia com uma receita médica do seu filho de 13 anos mencionando que se tratava de uma prescrição médica para o tratamento da asma do filho. Na receita eletrónica estava prescrito Symbicort Turbohaler®, uma associação de um corticosteroide (80 microgramas de budesonida/inalação) com um broncodilatador agonista β_2 adrenérgico de ação prolongada (4,5 microgramas de fumarato de formoterol/inalação).

Sendo a associação de baixa dosagem e a posologia de uma inalação em caso de necessidade, surge a indicação de que é uma terapêutica para a asma numa fase inicial, pelo que procurei saber junto da senhora se estava a iniciar a terapêutica pela primeira vez. Após confirmação, e uma vez que era a primeira vez que ia utilizar do dispositivo Turbohaler®

recorri de um dispositivo de placebo para demonstrar um correto funcionamento do dispositivo de modo a assegurar a sua correta utilização. Inicie por demonstrar como funcionava o dispositivo (rodar a base até ouvir um *clic*, o indicador de que está pronto a utilizar) e qual o procedimento que o menino deveria seguir para a correta utilização: expiração máxima, colocar a boca no bucal e selar na totalidade com os lábios, efetuar uma inspiração energética e profunda, retirar a boca do bucal, suster a respiração por poucos segundos e expirar lentamente, seguindo-se a correta higienização do dispositivo. Alertei ainda que face à pequena quantidade de fármaco libertado poderá não sentir sabor ou a presença da medicação durante a utilização, não devendo repetir a administração se o procedimento for corretamente efetuado. Adicionalmente informei acerca da necessidade de lavar a boca com água após a inalação a fim de reduzir o risco de candidíase orofaríngea.

Caso 3

Jovem, de 24 anos, queixa-se de uma ferida na planta do pé advinda de uma lesão durante um treino. Solicita algo para alívio da dor e auxílio na cicatrização, indicando que gostaria de continuar com os treinos na atividade desportiva que realiza.

Iniciei a minha intervenção questionando acerca do aspeto físico da ferida uma vez que não me era acessível a sua observação. Sabendo que estava perante uma ulceração numa zona de pressão constante, com alguma vermelhidão, aconselhei Bepanthene Plus®, a associação dexpantenol com cloro-hexidina, de modo a permitir uma rápida cicatrização e a evitar a infeção. Dado que o Bepanthene Plus® apresenta a forma farmacêutica de creme e face à localização da ulceração, tornar-se ia difícil manter o medicamento no local pretendido. Aconselhei, deste modo, a efetuar um curativo aplicando o creme no local e, posteriormente, um penso oclusivo, de modo a manter a forma farmacêutica no local da ulceração, devendo este curativo ser renovado, no máximo de dois em dois dias.

Conclui o atendimento mencionando que, se não observasse melhorias ou se ocorresse inflamação, deveria dirigir-se ao médico.

Considerações Finais

A realização do estágio em Farmácia Comunitária é essencial na formação dos alunos do MICF. Permite aplicar, reforçar e adquirir experiência em contexto real, aspetos estes fundamentais para a futura carreira profissional como Farmacêutico.

O estágio na Farmácia São José superou de forma muito positiva as minhas espectativas. Sobrepõem-se os pontos fortes em relação aos pontos fracos e, apesar de em número as oportunidades estarem em igualdade com as ameaças, dou enfase às oportunidades que me foram facultadas, até porque a maioria das ameaças sentidas não dependeram do local de estágio, mas sim do mercado farmacêutico e da situação excecional vivida pelo COVID-19. Acrescento ainda que o estágio realizado me permitiu desempenhar todas as funções delineadas de aprovisionamento, armazenamento, gestão e indicação farmacêutica de medicamentos e produtos de saúde, preparação e dispensa de medicamentos, informação e consulta de documentação científica de utilização em Farmácia, interação farmacêutico-utente-medicamento e, até, com o médico prescritor e, organização e gestão de Farmácia.

Além da realização das tarefas inerentes, o estágio na Farmácia São José permitiu-me ainda uma das melhores experiências de trabalho focada no utente, tendo por intermediário o medicamento e todo o aconselhamento e informação associada, no intuito de permitir o uso racional da medicação. Aliado a tal, tive a oportunidade de trabalhar com uma equipa incrível, o que tornava o ambiente de trabalho um espaço muito agradável.

Saliento, em jeito de conclusão, a importância da realização do estágio para o enquadramento correto no título de Farmacêutico, um verdadeiro agente de saúde publica que, mesmo em situações graves de pandemias estão na linha da frente, juntamente com outros profissionais de saúde, aptos a ajudar e a promover a saúde publica.

O Farmacêutico é o especialista do medicamento, mas a sua correta atividade profissional é muito mais abrangente. Ser Farmacêutico é amizade, é confidencialidade, é pensar no outro, é ser família.

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Anexo

Certificado de Formação

Certifica-se que:

_____Carla Alexandra Almeida Fonseca____,

participou na acção de formação subordinada ao tema "A PELE, Conceitos de Cosmética e Cosmetologia", realizado no dia 25/ 01/2020 , com a duração de 5 horas.

Susana de Jesus
(Formadora)

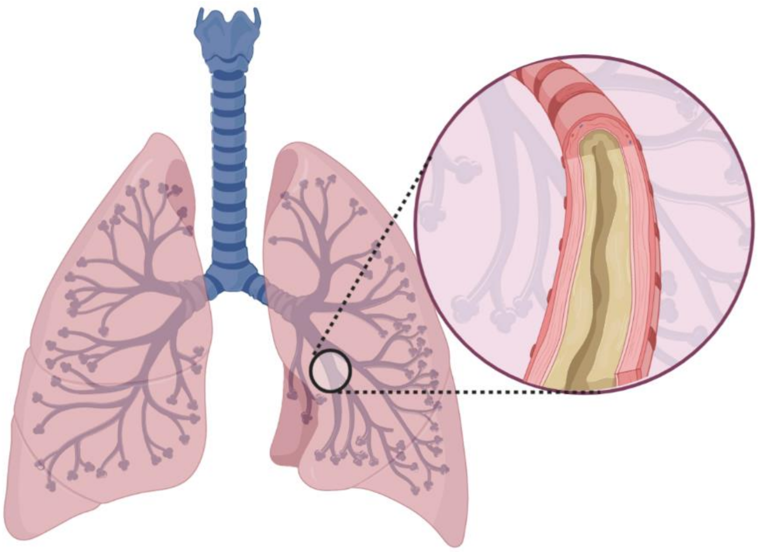
Programa de Formação

- A Pele, estrutura e funções
- Princípios de Cosmétologia
- Elementos de Cosmétologia
- Ingredientes Activos
- Tipos de Pele
- Estados de Pele
- Envelhecimento
- Zona Contorno de Olhos
- Patologias
- Intolerâncias Solares
- Proteção Solar

PARTE II

MONOGRAFIA

"CYSTIC FIBROSIS: PHYSIOPATHOLOGY AND THE LATEST PHARMACOLOGICAL TREATMENTS"



List of Abbreviatures

AA – Arachidonic Acid

AAV2 – Adeno-associated virus serotype 2

ABC – ATP-Binding Cassette

AMPK – AMP-activated Protein Kinase

ANO1 – Anoctamin-1

ASO – Antisense Oligonucleotide

ATP – Adenosine Triphosphate

cAMP – cyclic Adenosine Monophosphate

CF – Cystic Fibrosis

CFTR – Cystic Fibrosis Transmembrane conductance Regulator

Cl- – Chloride anion

DIOS – Distal Intestinal Obstruction Syndrome

DNA – Deoxyribonucleic Acid

ENaC – Epithelial sodium Channel

FEV1 – Forced Expiratory Volume in 1 second

IKBKB – Inhibitor of IKKβ

IKKβ – IkB kinase

IL-8 – Interleukin 8

K+ – Potassium cation

LA – Linoleic Acid

miRNA – microRNA

mRNA – messenger RNA

Na+ – Sodium cation

NBD – Nucleotide-Binding Domain

NF-kB – Nuclear Factor kappa B

PKA – Protein Kinase A

RNA – Ribonucleic Acid

TM – Transmembrane

TMD – Transmembrane Domain

TOM1 – Target Of Myb1

TSB – Target Site Blocker

UTR – Untranslated Regions

Abstract

Cystic Fibrosis (CF) is the lethal autosomal recessive genetic disease, caused by a mutation in the cystic fibrosis transmembrane conductance regulator gene (*CFTR)*. This gene encodes the CFTR protein, a distinctive membrane transport of the ATP-binding cassette (ABC) superfamily of proteins that functions as a chloride channel, allowing the balance and transport of chloride through the apical membrane of epithelial cells. Due to its ubiquitous location, mutations in the *CFTR* gene trigger multiple changes both in ion transport and in metabolic pathways, affecting various organs as it will be herein exploited. However, pulmonary impairment is the most characteristic comorbidity of CF, and the respiratory failure is the main cause of death.

In the presence of a positive diagnosis of CF, a primary therapy is instituted for symptomatic prevention and relief. Associated with this therapy, CFTR modulators can be used, the new therapeutic approaches that allow the correction or potentiation of the deficit CFTR channel. In an attempt to overcome the disadvantages of CFTR modulators, studies are being developed based on the application of biotechnology techniques including gene therapy, gene editing, RNA therapy and therapeutic microRNAs, as well as on the investigation of the potential of the intranasal administration route.

Keywords: Cystic Fibrosis, CFTR, CFTR modulators, biotechnology, inhalation.

Resumo

A Fibrose Quística (CF) é uma doença genética autossómica recessiva letal, provocada por uma mutação no gene regulador da condutância transmembranar da Fibrose Quística (*CFTR*). Este gene codifica a proteína CFTR, um transportador membranar pertencente à superfamília de proteínas transportadoras dependentes de ATP (ABC) e que funciona como um canal de cloro, permitindo o equilíbrio e transporte deste ião através da membrana apical de células epiteliais. Face à ubíqua localização deste transportador, mutações no gene *CFTR* desencadeiam múltiplas alterações quer no transporte dos iões quer em várias vias metabólicas, afetando vários órgãos como será aqui explorado. No entanto, é o comprometimento da função pulmonar a comorbilidade mais característica da CF, sendo a falha respiratória a principal causa de morte.

Na presença de um diagnóstico positivo de CF, é instituída uma terapêutica primária para prevenção e alívio sintomático. Associada a esta terapêutica pode recorrer-se aos moduladores do CFTR, novas abordagens terapêuticas que permitem corrigir ou potenciar o canal CFTR deficitário. Na tentativa de colmatar as desvantagens dos moduladores do CFTR estão a ser desenvolvidos estudos baseados na aplicação de técnicas de biotecnologia, como a terapia génica, edição génica, terapia de RNA e microRNAs terapêuticos, bem como na investigação do potencial da via intranasal.

Palavras-chave: Fibrose Quística, CFTR, moduladores do CFTR, biotecnologia, inalatória.

Introduction

Cystic Fibrosis (CF) is a lethal autosomal recessive genetic disease for which there is no cure (Figure 1). It is more prevalent in Caucasians, but it also affects other races and ethnicities[1,2]. In Europe, its incidence is around 1 in every 2000-3000 births[3,4] with an average prevalence of approximately 32000 patients[5,6]. Worldwide, the prevalence is close to 85000 patients[5,6].

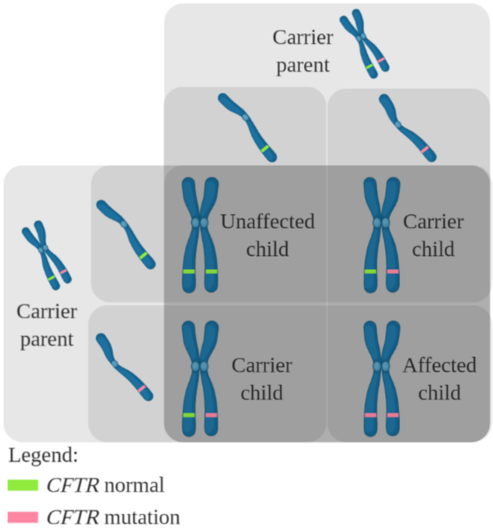


Figure 1. Representation of the genetic transmission of CF, an autosomal recessive disease. As each descendant inherits one gene from each parent, if both carry the mutated gene, the probability of having CF is 1 in 4 offspring[2].

The disease originates from a mutation in the cystic fibrosis transmembrane conductance regulator gene, the *CFTR* gene, which encodes a chloride channel structuring protein. This is essential for the osmotic balance and maintenance of a normal transport of electrolytes in the apical surface of the epithelial cells from lungs, intestines, pancreas and other exocrine glands, such as the sweat glands and epididymis in the male reproductive system. The Congenital bilateral absence of the vas deferens can occur, expressing 98% of cases of CF male infertility[1,7,8,9]. Mutations on this ion channel gene will compromise CFTR function, causing numerous pathophysiological changes, which characterize the CF. Among them, the ionic imbalance and the hyperviscosity of the respiratory mucus stain out, decreasing the mucociliary clearance, promoting bacterial colonizations and establishing cycles of infection and/or inflammation that may culminate in the degradation of the pulmonary epithelium, originating the pulmonary manifestations[7]. In view of the vast location of the CFTR channel, CF is a multisystem disease since several other exocrine organs are often affected[1,10].

This disease has a high impact on patients' quality of life, not only because of its wide range of complications, but mainly because it is a lethal disease, observing a decreasing of the average life expectancy[1]. Considering the patients who do not receive a lung transplant, respiratory failure secondary to progressive lung disease is the main cause of death[11].

CF is a progressive disease and therefore it requires specific care and treatments to improve quality of life, encompassing the increment of airway mucus clearance, the control of infections and correction of nutritional deficits, to allow the increase of the average life expectancy of patients. This factor, more visible in countries with well-founded health and well-being systems, made possible an increase of cases in adult people, no longer being a predominantly infant disease[11]. Currently, in developed countries, the number of adults with CF tends to increase 75% by 2025[9,12], and the average life expectancy of these patients is expected to exceed the 40 years of age[11,13]. In countries with lower resources and access to healthcare, average life expectancy is only around the second decade of life[6,11].

The positive development in recent years regarding the quality and average life expectancy of CF patients is based on the development and evolution of technologies. Associated with a primary therapy that allows the prevention and relief of CF symptoms, new molecules are emerging to modulate *CFTR* mutations. However, the several obstacles raised from this strategy still promoting the development and application of biotechnology, in order to find a definitive and safe solution to cure CF[2,4].

CFTR expression

CF is caused by a mutation in the *CFTR* gene, with approximately 2000 identified mutations that trigger the disease[1,8]. Phenylalanine deletion at position 508, Phe508del, is the most common mutation, being present at least in one allele in 70% of the patients with CF[1,14,15].

The *CFTR* gene is expressed on chromosome 7, in the long arm (q), of region 3, band 1, in sub-band 2 (7q31.2). From the transcription of this gene comes the CFTR mRNA, which is translated into the CFTR protein with 1480 amino acids[1,4]. The translation of this protein takes place inside the endoplasmic reticulum, where it undergoes glycosylation and complex winding processes for structuring the functional CFTR protein[16]. This is then transported to the apical membrane of epithelial cells through vesicles from the Golgi Complex (Figure 2, from 1 to 3)[16]. The CFTR protein is, hence, expressed in epithelial cells of numerous organs, as lungs, upper respiratory tract, pancreas, liver and gallbladder, intestines, sweat ducts and other exocrine ducts like vas deferent in the male reproductive system, affecting fertility[9].

CFTR protein belongs to the adenosine triphosphate (ATP)-binding cassette (ABC) transporters family encompassing a chloride channel that is responsible for the transport of chloride in the apical cell membrane of epithelial cells, consuming energy from ATP. Depending of the activity of the basolateral membrane of the cell involved, CFTR channel also transports bicarbonate ions, but with a lower electrochemical gradient than for chloride ions[1,5,17,18]. These ions, mainly the bicarbonate ion, play an important role in the acid/basic balance maintaining the physiological pH on the surface of the epithelial cells where CFTR is expressed. In the lungs and upper respiratory airways, the bicarbonate ion is essential to sustain the physiological pH of liquids on the surface of the airways, to promote a competent innate immunity system, and to influence mucus viscosity, since the complete mucin expansion, a mucus structuring protein, require bicarbonate ions. In pancreas, bicarbonate secretion is required for a correct nutritional absorption, granting a normal intestinal function. In sweat glands, CFTR is expressed in both apical and basolateral membranes of the duct for normal chloride secretion and absorption, preserving a normal hydroelectrolytic balance and normal body temperature in association with other channels, for sodium and water secretion and absorption[18].

CFTR mutations disturb chloride and bicarbonate levels, decreasing the pH of surface liquids of the compromised organ and, consequently, affecting the innate immunity that increases the infections frequency. Mucus viscosity is also altered, and the reason is the deficit of bicarbonate ions that makes the mucin more compact, resulting in a thicker and more viscous mucus. Chloride reabsorption in the sweat glands can also be affected, ensuing in excessive chloride secretion with hydric imbalance because chloride secretion remains normal in response to cholinergic stimulation. Thus, different CF comorbidities appear according to the affected organ (Figure 3)[1,5,17].

The CFTR channel, in addition to its activity as an ionic channel, is also associated with other signaling pathways and is exposed to secondary messengers that activate CFTR coupled heterotrimeric proteins, such as AMP-activated protein kinase (AMPK), triggering phosphorylation cascades. Therefore, all cellular metabolism can be compromised: from the balance between autophagy, proteostasis and apoptosis to the metabolism of fatty acids and cholesterol[19]. Noteworthy is also the effect of the CFTR on the regulation of epithelial sodium channel (ENaC), because the inactivity of the CFTR channel causes a hyperactivity of the ENaC, resulting in an excess of sodium resorption. Thus, there is a high concentration of sodium in the intracellular medium that affects the hydroelectrolytic balance, increasing the movement of water molecules to the medium intracellular and, therefore, dehydration of the epithelial surface occurs[5,16].

CFTR protein

Like most ABC transporters, the CFTR consists of two transmembrane domains (TMD), the TMD1 that is formed by 6 transmembrane (TM) segments 1-6, and the TMD2, composed of the TMs 7-12 segments. The TMD1 is linking to a cytosolic nucleotide-binding domain (NBD), the NBD1 and, the TMD2 is linking to a NBD2, forming two complexes, TMD-NBD, which are united by a single regulatory domain. Regulatory domain consists of serine/threonine residues that allow the activation of the protein kinase A (PKA) dependent phosphorylation pathway, essential for CFTR to function as an ATP-dependent ionic channel. Thus, the TMD forms the porous channel, the NBD controls the closure/opening of the channel through the ATP connection, and the regulatory domain is responsible for its activation via PKA-dependent phosphorylation and for interaction with the other domains[5,10,16].

During CFTR normal function, channel opening requires external stimulation mediated by the increase of cyclic adenosine monophosphate (cAMP) that will activate the PKA. In turn, the PKA will trigger to the regulatory domain phosphorylation, while the presence of MgATP leads to the NBD dimerization, maintaining the robust activity of the CFTR[5,19]. In opposition, the hydrolysis of ATP translates into the separation of NBD, decreasing chloride channel opening frequency[10]. When ATP binding occurs, with consequent dimerization of the NBD, there are a *flip-flop* movement in the NBD-TMD interface. As a result, the substrate binding site is exposed, alternately to the intracellular and extracellular medium, due to the conformational alteration of the TMD that occurs preferentially in the TM4-6 subunits, allowing the complete cycle of ionic transport (Figure 2, from A to C)[5]. The exposure of the chloride binding site and the positive charge inside the pore, provided by the amino acids that form the channel, attract the cytoplasm ions and its enter into the pore, resulting in the movement of ions by passive diffusion. It is also known that the pore has the capacity to accommodate approximately 180 water molecules but, in the chloride transport, the ions needs to be dehydrated to pass through the narrowest region of the channel, with posterior rehydration occurring at the outlet of the channel[5,10,19]. The closure of the channel is then triggered by ATP hydrolysis, which results in the separation of the NBD dimer, reestablishing the conformation of the TMD that culminates in the closing of the pore[5].

CFTR mutations

Mutations that may occur in CF do not all cause defects in the CFTR protein with disease development[10]. However, others mutations will alter the conformation, mode of interaction or stability of one or several domains of the CFTR protein, and will compromise the normal mechanism of ion and water transport from the intracellular to the extracellular medium, as well as signaling pathways in which the CFTR protein is involved[5,19]. In this way, the distinct *CFTR* mutations were classified in six classes according to the severity with which they trigger the disease and the mechanism that is changed (Table 1 and Figure 2, from I to VI). Class I to III mutations have the greatest impact[4], and the most common mutation, Phe508del, belong predominantly to class II[1,4,11].

Table 1. Synthetic representation of the processes and mechanisms involved in the different mutation classes as well as the most prevalent mutation[1,4].

Mutation class	Target mechanistic process	Consequence	Cause	Most common mutation[10]
I	Protein synthesis	Complete absence of CFTR protein	*Nonsense* or *frameshift* mutation culminating in the early termination of mRNA due to premature termination codon insertion[15]	Gly542X (10% of CF patients)
II	Protein maturation	Absence of migration of normal CFTR protein to apical membrane[6]	Deficient posttranslational modifications in the endoplasmic reticulum, originating an incorrect folding of the NBD1. The aberrant structure formed is marked for degradation in the ubiquitin-proteasome system by the physiological mechanisms of the cells[11]	Phe508del (70% of CF patients)
III	Channel regulation	Decreased protein activity	Deficient ATP binding to NBD that reduce the dimerization capacity of NBD and, consequently, the conformational change of TMD. As a result, the frequency of channel opening decreases, despite its expression and location are normal[5,15]	Gly551Asp (4% of CF patients)
IV	Channel conductivity	Decreased ion flow and channel opening time	Mutation between TMD1 and TMD2 that affects the arginine charge, compromising the attractiveness of negatively charged chloride ions[5]	Arg117His (3% of CF patients)
V	Protein synthesis and functioning	Decreased functioning of CFTR	Instability of mRNA and/or immature protein by *splicing* errors, which results in a marked decrease in normal CFTR protein in the apical membrane[11,15]	Ala455Glu (3% of CF patients)
VI	CFTR stability in membrane	Decreased CFTR stability	Abnormal C-terminus of the CFTR protein that increases CFTR *turnover,* changing its stability in the apical membrane[6,20]	Gln1412X

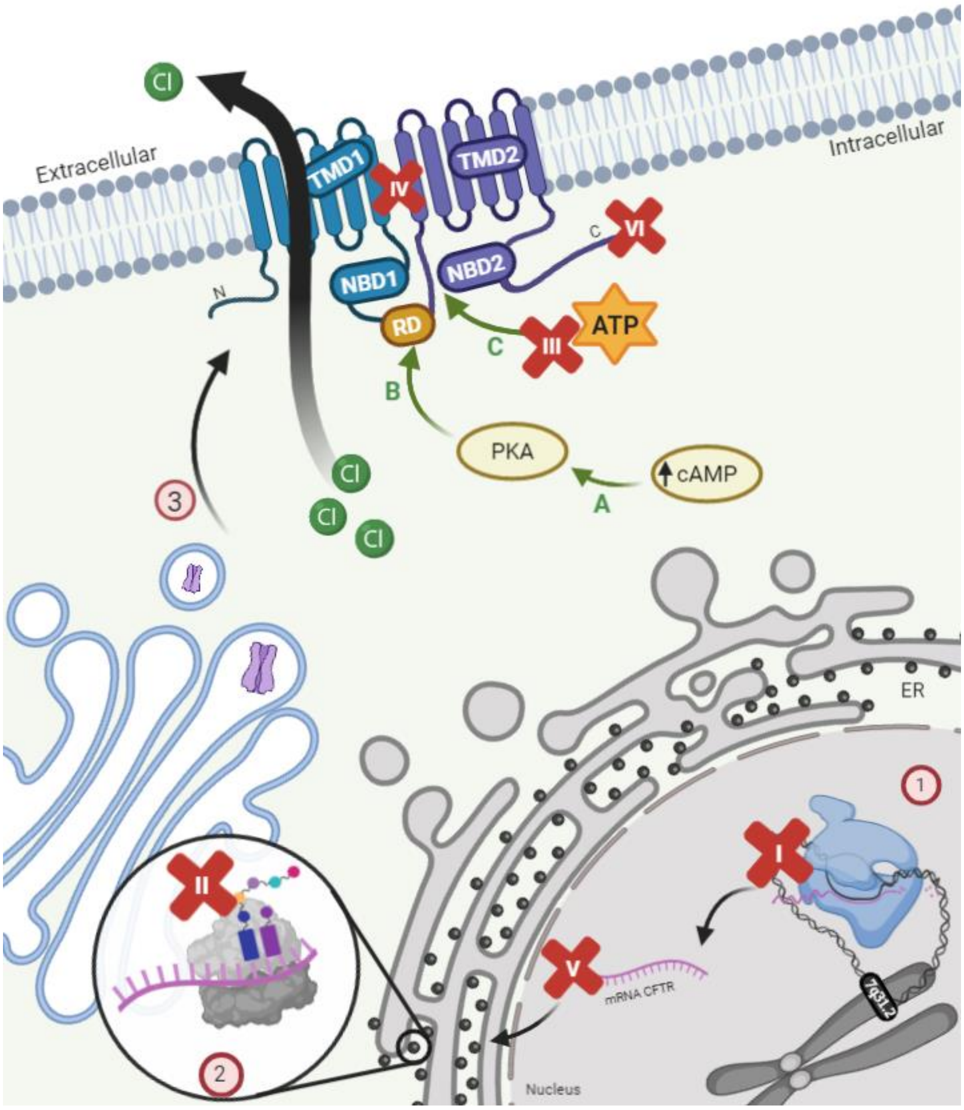


Figure 2. Representation, from 1 to 3, of the CFTR protein mechanism of expression, its activation, of the letter A to C, as well as the mutation classes from I to VI. **1-** Transcription of the *CFTR* gene, located at 7p31.2, in mRNA CFTR. **2-** Translation of mRNA into endoplasmic reticulum, where it undergoes glycosylation and specific windings for structuring the functional CFTR protein. **3-** Transport of the CFTR protein to the apical epithelial membrane, through the Golgi complex, functioning as a chloride channel. **A-** Increase of cAMP with respective PKA activation. **B-** Activated PKA will phosphorylate the regulatory domain. **C-** From regulatory domain phosphorylation, in the presence of ATP, there is the dimerization of the NBD leading to a *flip-flop* movement on the NBD-TMD interface, which results in the conformational alteration of the TMD, and consequent opening of the channel and chloride transport occurs. **I-** Alteration in protein synthesis resulting in the absence of CFTR channel in apical membrane. **II-** Alteration in protein maturation, resulting in its degradation, and therefore the migration of normal CFTR to the apical membrane is not observed. **III-** ATP link change decreasing CFTR activity. **IV-** Mutation between TMD1 and TMD2 with decreased channel conductivity. **V-** Instability of mRNA and/or immature protein leading to decreased CFTR in the apical membrane. **VI-** CFTR with abnormal C-terminal, which leads to increased *turnover,* thus increasing protein instability. RD-Regulatory Domain. ER-Endoplasmic Reticulum[1,5,11,16].

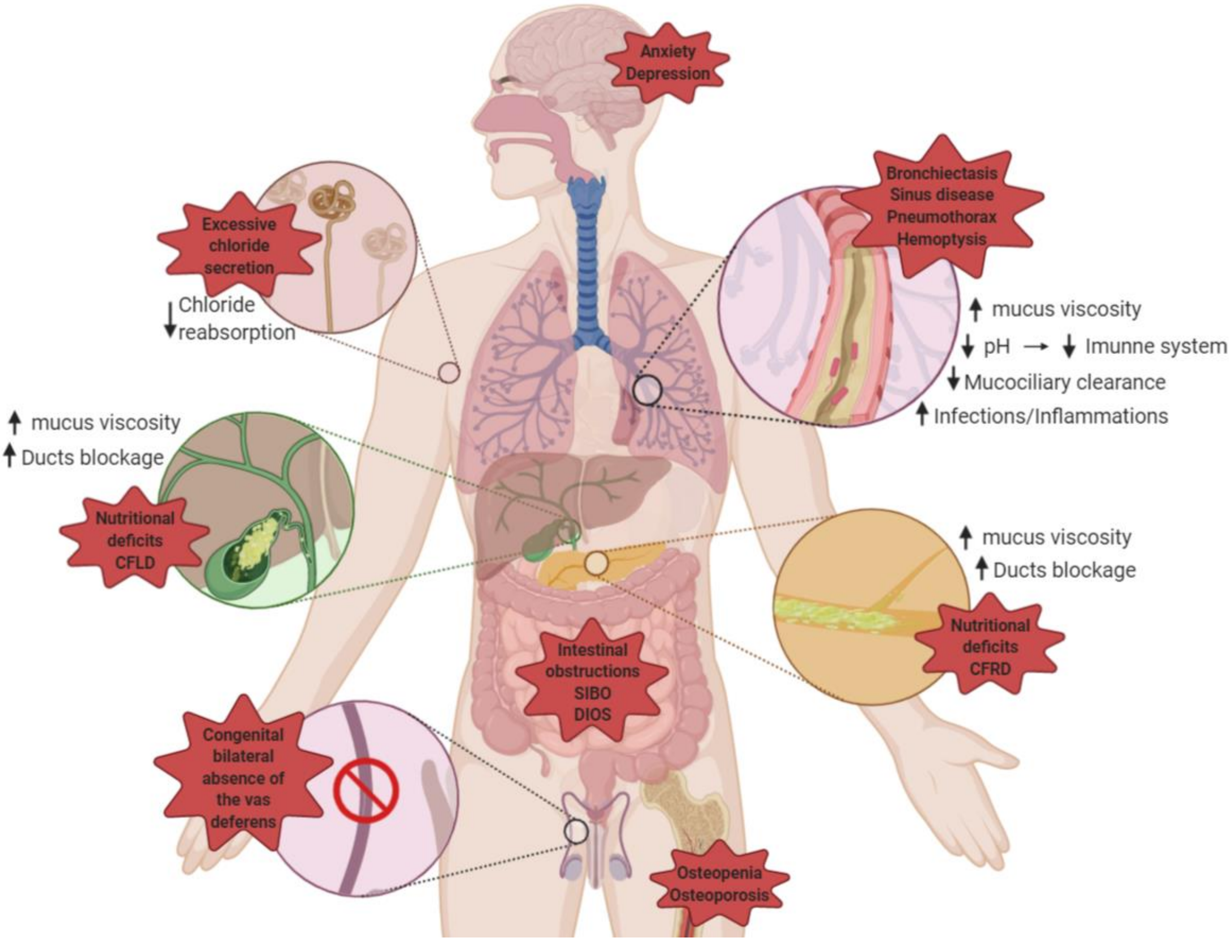


Figure 3. Representation of the different CF comorbidities according to the organ compromised by the *CFTR* mutation and its implicit mechanisms. As CFTR is expressed in epithelial cells of the lungs, upper respiratory tract, sweat glands, pancreas, liver and gallbladder, intestines and in different organs of the reproductive system, as an example the vas deferens, it is a multisystemic disease. Osteopenia, osteoporosis, anxiety and depression are a consequence of other comorbidities, of applied therapeutics and limited lifestyle. SIBO-Small Intestinal Bacterial Overgrowth [9,11,18,21].

CFTR dysfunction and multisystem CF disease

CF is caused by mutations in the *CFTR* gene, which results in a decrease in the function of the CFTR chloride channel. This channel has the ability to interact with other ionic channels and other cellular pathways and is present in the apical membrane of epithelial cells, located in numerous tissues and exocrine organs, resulting in numerous complications that characterize this disease (Figure 3)[1,3,11].

Amongst the CF complications, the ones that cause the most morbidity and mortality are those that occur at the respiratory system[1,3]. The implicit mechanism is based on CFTR channel dysfunction, leading to ion imbalance with consequent pH and mucus viscosity dysregulation, compromising innate immunity and normal mucociliary clearance. As such, there are accumulations of mucus and chronic infections and/or inflammation that, if recurrent and constant, terminate in the destruction of the pulmonary epithelium. The reason for this epithelial destruction is the fibrous development of subepithelial tissues, impairment the respiratory function, despite the activation of physiological repair and remodeling mechanisms[1]. Examples are bronchiectasis, chronic infections that can cause pneumonia, sinus disease, hemoptysis and pneumothorax, all of which contribute to respiratory failure[4,11].

Bronchiectasis is also caused by extensive infiltration of neutrophils that release elastase, responsible for deregulating innate immunity, increasing mucus production and digesting peptides and pulmonary proteins, culminating in tissue destruction. Also occurs the infiltration of degranulating neutrophils, that is nucleic acid liberators and cytoplasmic matrix proteins, responsible for increasing mucus viscosity (Figure 4)[4,11]. These phenomena, if recurrent, will also increase the susceptibility to contract bacterial infections. The most common is caused by *Pseudomonas aeruginosa, Stenotrophomonas maltophilia* and *Achromobacter* ssp., but the most lethal is caused by *Burkholderia cenocepacia*[11,22]. Sinus disease, rhinosinusitis and nasal polyps can serve as a reservoir for pathogens that, as soon as they have the necessary conditions, infect the lungs. Hemoptysis is more recurrent in older patients and may be triggered by previous pneumothorax, and the occurrence of these increases the likelihood of recurrence[9].

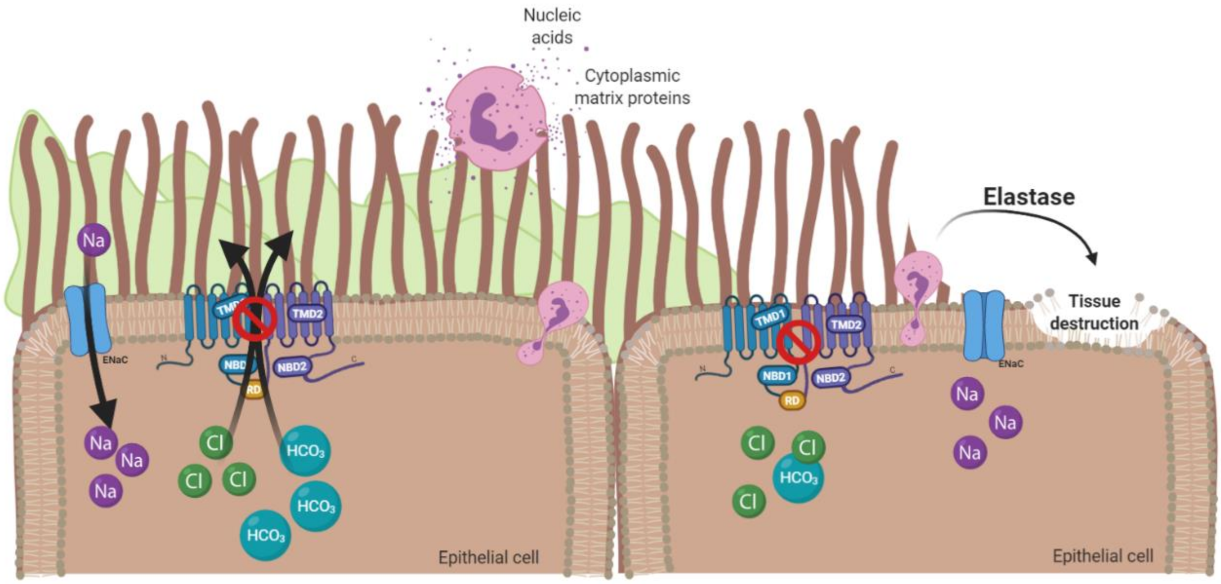


Figure 4. Representation of the imbalance that occurs in pulmonary epithelium level due to mutations in the CFTR channel. In the presence of a mutation that compromises the flow of Cl^- and HCO_3^- ions by epithelial cells to the extracellular space, a pH change occurs on the epithelial pulmonary surface, reducing the action of innate immunity. The absence of HCO_3^- also causes imbalances in the function and viscosity of mucus, so that it becomes thick and little fluid compromising the mucociliary clearance. The hyperabsorption of Na^+ via epithelial sodium channel (ENaC) will lead to insufficient water secretion to the pulmonary epithelial surface, also contributing to mucus hyperviscosity and dehydratation. There is also massive infiltration of neutrophils that release elastase, which results in tissue destruction, as well as the infiltration of granular neutrophils that release nucleic acids and cytoplasmic matrix proteins contributing to the mucus hyperviscosity. This neutrophil infiltration characterizes bronchiectasis[4,6,11].

At the digestive system level, several complications occur due to the blockage of the ducts of the adjacent exocrine organs, namely pancreas and gallbladder, by the thick mucus. The obstruction of pancreatic and biliary canaliculi results in nutritional deficits, mainly in lipids and fat-soluble vitamins (vitamins A, D, E and K). Lipid intolerance and weight loss also occur, since bile salts and pancreatic enzymes not released in CF are essential for the correct absorption of lipids in the duodenum[4,9]. These obstructions may also cause, according to the blocked duct, liver and biliary diseases, diabetes and pancreatic insufficiency[2,4]. CF related liver disease (CFLD) is the most lethal non-respiratory complication of CF, since it can cause stagnation of biliary secretion, with duct obstruction and consequent hepatic cirrhosis[9]. CF related diabetes (CFRD) is distinct from type 1 and 2 diabetes for the reason that, as type 1 diabetics, presents pancreatic β cells reduction and consequent decrease in insulin production, but without the characteristic ketoacidosis, because patients are able to produce enough endogenous insulin to avoid this situation. CFRD is also characterized by high glucose levels on airway surface fluids, providing a favorable environment for bacterial growth and proliferation, increasing the pulmonary exacerbations[9].

These comorbidities can trigger further complications, such as intestinal obstructions, small intestinal bacterial overgrowth and distal intestinal obstruction syndrome (DIOS)[4]. DIOS is characterized by an ileo-cecal obstruction and an imbalance of minerals in the bloodstream in the face of excessive loss of salts by sweat, thus being a complication in more advanced states of the disease. This hydroelectrolyte imbalance, in turn, can manifest itself in dehydration, arrhythmias, fatigue, weakness, insolation and, more rarely, can lead to death[4].

Blocking the pancreatic and biliary ducts also change the metabolism of unsaturated fatty acids, caused by increased expression and activity of desaturases of fatty acids. In the most advanced disease states, especially when there is pancreatic insufficiency, metabolic changes are more significant. These changes occur fundamentally in cells with compromised CFTR function, since this channel is simultaneously associated with kinases and phosphatases that activate other signaling pathways. In this case, activation of AMPK, a heterotrimeric protein composed of an α-catalytic subunit and two regulatory β and γ subunits, may occur, either by increasing calcium that activates calcium/calmodulin-dependent protein kinase kinase β (CaMkkβ), or by intracellular AMP that actives the liver kinase β1(LKB1). This results in a phosphorylation cascade that activates the PPAR-α, through its co-activator 1γ (PGC-1γ), culminating in increased expression and activity of the Δ6-desaturase and Δ5-desaturase enzymes (Figure 5)[3,23]. There is, then, a decrease of linoleic acid (LA; 18:2n-6) and docosahexaenoic acid (DHA; 16:1n-7) and an increase of LA metabolite, arachidonic acid (AA; 20:4n-6), of the palmitoleic acid (POA; 16:1n-7) and acid mead (MA; 20:3n-9). Such changes may result in abnormalities in pulmonary immunity and/or inflammations, because the eicosanoid metabolites of AA [prostaglandins (PGs), leukotrienes (LTs) and lipoxins (LXs)] are consequently altered, modifying the biophysical properties of epithelial membranes, making the functionality of membrane proteins questionable[3,23].

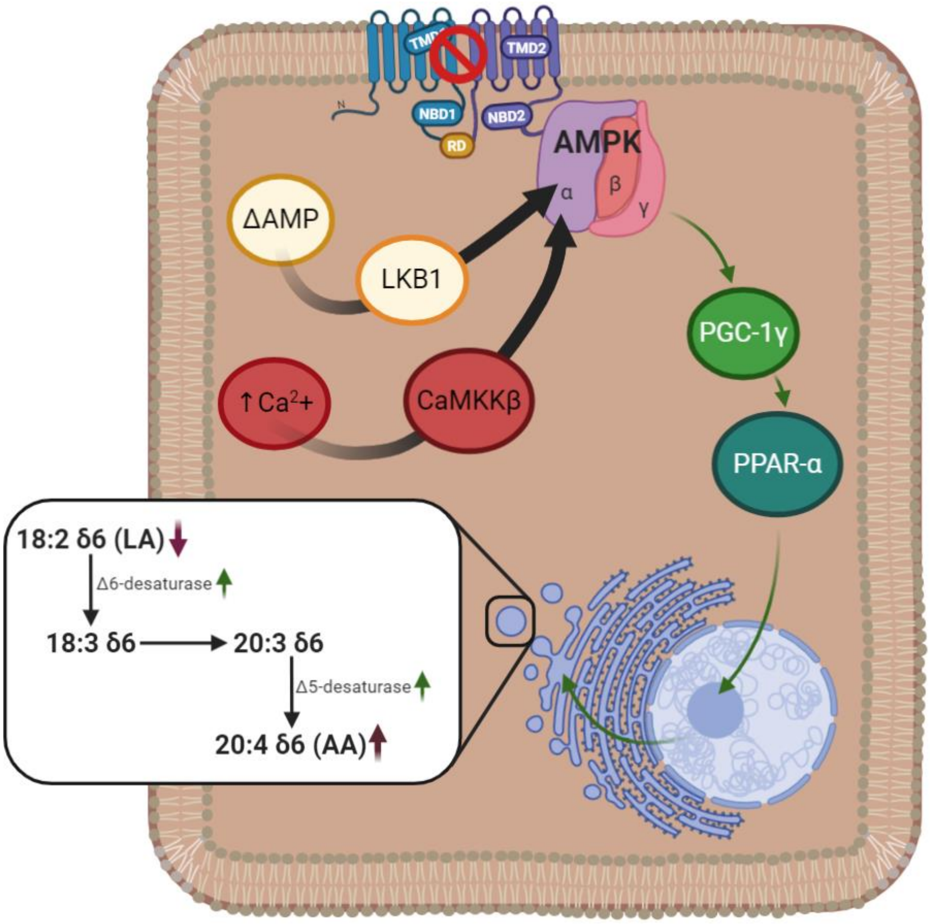


Figure 5. Representation of the CFTR channel dependent desaturases synthesis mechanism in hepatic epithelial cell. This channel is associated with AMPK, a heterotrimeric protein composed of an α-catalytic subunit and two regulatory β and γ subunits, which interacts and regulates CFTR activity. AMPK is activated either by increasing calcium that activates calcium/calmodulin-dependent protein kinase kinase β (CaMkkβ), or by intracellular AMP by activation of liver kinase β1(LKB1). This increased AMPK activity triggers, via phosphorylation, CFTR regulation mechanisms and activates PPAR-α, through its co-activator 1γ (PGC-1γ) which culminates in the increased activity of the Δ6-desaturase and Δ5-desaturase that will convert LA into AA, decreasing LA and increasing AA[3,23].

During the fetal development of CF patients, the reproductive system can suffer malformations, since CFTR is expressed both in the male reproductive system, in organs such as epididymis, seminal vesicles and vas deferens, and in the female reproductive system, with presence in the uterus and vagina. As a result, the congenital bilateral absence of the vas deferens may occur in men, decreasing ejaculate volume, and of the seminal vesicles, which are responsible for the acid pH of the lower ejaculate volume. In female, congenital absence of the uterus and vagina may occurs. Bicarbonate ion pay an important role in the reproductive system, as it is involved in motility and capacitation of sperm to fertilize the egg, being present in the ejaculate and, in high concentrations, in the uterus. In this way, *CFTR* mutations affect fertilization and, due to changes in mucus viscosity and mucous membranes hydration, prevent normal sperm penetration, and the reason is the absence of less viscous mucus during ovulation to allow sperm penetration through cervical mucus[9,18,21].

There may also be impairment of the sweat glands, due to excessive chloride secretion in sweat[13], as well as osteopenia and osteoporosis[2,11]. Osteopenia is associated with delayed puberty, chronic inflammation, inactivity, as well as other comorbidities which do not allow the proper reabsorption of calcium and vitamins D and K, for example[8,9,11]. Associating the fact that they have to avoid sun exposure in view of the photosensitivity of antibiotics that they use recurrently as a preventive therapy and for states of inflammation, there is the worsening osteopenia, with possible progression to osteoporosis[8].

In addition to all these comorbidities, states of depression and anxiety may arise in view of the high impact that CF has on patient's lives, either by limited lifestyle or by symptoms and adverse effects of medication, as for all the time that is necessary to dispense for complete treatment[11]. The prevalence of depression in children and adolescents, analyzed in 2018, is around 73.2%, increasing in adults (up to 80.1%). Anxiety has an identical prevalence, 72.5% in children and adolescents and 79.5% in adults[24]. This adds the need for a diagnosis as early as possible to be able to institute therapeutics and prevent the progression of the disease, since the comorbidities tend to worse with advancing age[9].

Diagnosis

The best way to prevent disease progression and consequent complications is through early diagnosis of CF[11,25,26].

Knowing that CF is marked by changes in the unsaturated fatty acids values, which, together with their metabolites, correlate with the severity of the disease, these endogenous molecules have been considered as biomarkers and present a high potential for the diagnosis and monitoring of the CF[3]. However, although there are many advances, with the possibility of good biomarkers, diagnostic techniques are very variable, depending on the conditions and access to healthcare available to patients[11,27].

In most health programs, a newborn screening is performed, based on an immunoreactive trypsinogen (IRT) blood test, as trypsinogen may be increased in the presence of the pancreatic obstruction. Sweat test and genetic tests for mutations research in the *CFTR* gene can also be performed. Thus, in the presence of a positive IRT, the sweat test follows, and genetic testing may be necessary for confirmation, in as much as false positives occur[3]. However, given the lack of consensus regarding diagnostic guidelines, a guideline based on genetic testing and sweat test was established, in 2015, defining an initial research into the most frequent *CFTR* mutations associated with sweat test, with further confirmation, if necessary, through research into rare *CFTR* mutations[27].

In 2017, a consensual guideline defined the sweat test as primary diagnosis, after a positive newborn screening, signs and/or symptoms characteristic of CF or family history. If sweat test is greater than or equal to 60mmol/L, it is a diagnosis of CF. If sweat test is less than 30 mmol/L, it is an unlikely CF. In case of sweat test with values between 30 and 59 mmol/L, genetic analysis is performed, in which the presence of two *CFTR* mutations indicates diagnosis of CF, absence of *CFTR* mutations indicates unlikely CF, and the presence of one *CFTR* mutation indicates that physiological testing of *CFTR* is required, such as Intestinal Current Measurement and Nasal Potential Difference, indicating diagnosis or non-compliance, respectively, with dysfunction or preservation of CFTR function[28].

Treatment

Although currently there is no cure for CF, a primary therapeutic scheme, encompassing pharmacological and non-pharmacological therapies, was developed for the relief of symptoms and complications caused by the disease in early-diagnosed patients. The primary therapy will then allow preventing and alleviating the symptoms caused by the disease (Table 2) [2,4].

In addition to therapy, it is essential a good pharmacotherapeutic follow-up to try to maintain the healthy state of CF patients, so doctors, nurses, pharmacists and other health professionals assume an important role in the lives of CF patients[2,4]. Thus, disease progression is decreased and, in presence of respiratory failure, the most serious complication, the need for lung transplantation, or even another organ, can be avoided[26].

Table 2. Representation of primary therapy in CF patients and respective therapeutic indication, dosage, administration route, adverse effects and important precautions for the correct use of the drugs[4,6,11,24,32,36]

Drug	Dosage; Administration route	Mechanism of action	Adverse effects	Additional information
		Respiratory system		
		To prevent and control lung infections: Antibiotics		
Aztreonam	75 mg, 3 i.d., in 28-day cycles; Inhaled*	Bactericidal activity by inhibiting bacterial cell wall synthesis, with action on *Pseudomonas aeruginosa*[29]	Cough; Nasal congestion; Pharyngolaryngeal pain; Chest discomfort; Bronchospasm.	>6 years old; Follow the order: bronchodilator, mucolytic and aztreonam in case of simultaneous use of the mentioned drugs.
Tobramycin	300 mg, 2 i.d., in 28-day cycles; Inhaled*	Bactericidal activity by disruption of protein synthesis with consequent alteration of cell membrane permeability[30]	Cough; Bronchospasm; Nephrotoxicity; Ototoxicity; Muscle changes.	≥6 years old; To moderate or severe lung disease; *Local release and less risk of systemic adverse effects.
Azithromycin	250 mg, 3 times a week; Oral or intravenous	Inhibition of bacterial protein synthesis: binding to the 50S subunit of the ribosome without peptide translocation[31]	Diarrhea; Nausea; Vomiting; Nephrotoxicity; Ototoxicity.	>6 years old; Do not use in patients with active non-tuberculous mycobacteria.
		To control of the airway's inflammation: Nonsteroidal anti-inflammatory drugs (NSAIDs)		
Ibuprofen	20-30 mg/Kg, 2 i.d; Oral	Prevents neutrophil aggregation and migration[32]	Abdominal pain; Constipation; Edema; Neutropenia; Prolonged bleeding time.	6-17 years old; FEV_1 ≥60%; $Dose_{max.}$=3200 mg/day; Prevents loss of lung function.

Adjuvants in the respiratory process				
6% hypertonic saline	4 ml/dose, 2 i.d.; Nebulization	Restore airway hydration and increase the mucociliary clearance	Cough; Bronchospasm; Chest tightness; Pharyngitis; Hemoptysis; Sinusitis.	>6 years old; Only for chronic use.
Dornase alfa (DNase recombinant human)	2.5 mg, 1 or 2 i.d.; 24 weeks; Nebulization	DNase cleaves the extracellular DNA present in excess in the pulmonary mucus, decreasing their viscosity and elasticity[4,6]	Chest pain; Conjunctivitis; Pharyngitis; Hoarseness; Voice alterations[33]	>6 years old; Increased cough reflex in the face of decreased mucus viscosity; To moderate or severe lung disease.
Exercise and physiotherapy		Positive expiratory pressure devices		Aid in respiratory cycles and mucociliary clearance[4,34]
Percussion vest		High frequency chest wall oscillation devices		
Digestive system				
Adjuvants for intestinal obstructions				
Rehydration and osmotic laxatives	Oral	Rehydration and increased fecal volume that stimulate bowel movement		Prevent or treat partial obstructions[4]
Enemas	Rectal	Hyperosmolar contrast Enemas		Total obstructions recurrent in DIOS[4]
Balanced electrolyte intestinal lavage solution enema	Rectal	Intestinal lavage with balanced electrolyte solution containing diatrizoate meglumine and diatrizoate sodium		Total obstructions with vomiting; Polyethylene glycol 3350 for 6 to 12 months, if vomiting is recurrent[4]
Adjuvants in the insufficiency pancreatic				
Pancreatic enzyme replacement therapy (PERT)	500-2500 lipase units per body kilogram per meal; Oral	Proteases, lipases and amylases, that in alkaline duodenal medium increase lipid and nutrients absorption with increase the mass body.	Ulcerations: Fibrosing colonopathy (if excessive doses will be administered)	Before meals; Do not chew/crush the capsules; If swallowing difficulties: open and apply the contents of the capsule to soft and non-alkaline foods[4,35]
To establish the normal nutritional and hydroelectrolytic status: Supplementation				
Nutritional	All main meals; Oral	Highly caloric and protein and highly rich in fats (supplementation rich in DHA and low in omega 3 and 6 fatty acids has greater benefits)[3]		Essential for bone mineralization, correction of nutritional deficits and maintenance of body mass[2,3,4];

Vitaminic	Oral	Fat-soluble vitamins, such as vitamins A, D, E and K		Careful choice of supplementation fatty acids; Vitamin D supplementation adds positive effects on lung function, inflammation, intestinal microbiome and the immune system[8]
Mineral	Oral	Mineral salts, namely fluoride and zinc		

More recently, new therapeutic approaches are emerging: correctors of the mutated CFTR, aiming at the structuring of the normal CFTR protein, and the potentiators, which intend to improve the activity of the mutated CFTR protein[2,4].

The biggest disadvantages of these new therapeutic approaches regard their therapeutic effects that are dependent on the mutation type of each patient, and the fact that currently there is no single successful therapy for all classes[1,4,13]. Furthermore, there are limitations on the need of simultaneously performing symptomatic therapy, high potential to develop drug-drug interactions at Cytochrome P450 3A4 (CYP3A4) level and adverse effects, namely the elevation of transaminases, cataract, oropharyngeal pain and upper respiratory tract infection[4].

Kalydeco® (Ivacaftor), a CFTR potentiator, was the first new therapeutic approach to be developed. It has a great ability to improve the pulmonary function as observed by the forced expiratory volume in one second (FEV_1) which improved by approximately 10%[6,37]. Ivacaftor also reduces sweat chloride concentration and the frequency of pulmonary exacerbations, and increase body mass index and patient's quality of life. This drug is particularly efficient in class III mutations, increasing the activity of the CFTR channel by maintaining the channel opened for a longer period of time[4,5]. Importantly, a multicenter, randomized, double-blind, placebo-controlled study of Ivacaftor, carried out in CF homozygous for the Phe508del mutation aged 12 years or older, presents a non-significant response in heterozygous to the most common Phe508del mutation, acting more effectively under the Gly551Asp mutation[38].

In order to cover a greater number of CF patients, the CFTR corrector Lumacaftor was developed, demonstrating biological activity on the Phe508del mutation, the most common class II mutation, in a randomized, double-blind, placebo-controlled study with adults CF patient who were homozygous for the Phe508del mutation[39]. Lumacaftor directly binds to the CFTR protein, modulating its TMD1conformation and avoiding the folding defect caused by the mutation in the NBD of the CFTR protein, which is, hence, transported to the apical surface[40]. However, despite its greater scope, a double-blind, placebo-controlled based on three successive cohort studies with CF patients demonstrated that the improvement results were not significant if the Lumacaftor was administered alone, which requires high doses, further increasing the risk of chest tightness or dyspnea[41].

Therefore, the combination of Lumacaftor and Ivacaftor (Orkambi®) was tested since the former would correct the structure and transport of CFTR protein to the apical membrane while Ivacaftor would potentiate the normal activity of the CFTR channel[4,5,22]. This drug combination revealed to be effective in patients with the Phe508del mutation, according with a double-blind, placebo-controlled based on three successive cohort studies with CF patients[41], allowing the reduction of exacerbations, decreased number of hospitalizations, increasing body weight and improving the pulmonary function (as given by FEV1)[4]. However, the improving effects were lower than that observed with Ivacaftor alone[4,37]. Furthermore, the metabolism of Ivacaftor is increased when administered with Lumacaftor, requiring higher doses[40].

Later, Tezacaftor, a CFTR corrector belonging to the second-generation CFTR modulators, was developed revealing to successfully correct the folding and transport of the CFTR protein to the apical membrane, increasing chloride transport to the extracellular medium of the epithelial cells where CFTR is expressed. Similarly to Orkambi®, Symdeko® emerged as the association of Ivacaftor and Tezacaftor. Although both combinations have similar effects on improving complications, Ivacaftor/Tezacaftor shows lower recurrence of severe adverse effects and pharmacological interactions, demonstrating greater tolerability and safety in homozygous patients for the Phe508del mutation[37,40]. Nevertheless, and in spite of the lower risk of developing drug-drug interactions, Tezacaftor is also a substrate of CYP3A4[13,40].

More recently, the triple therapy combining two CFTR correctors and one CFTR potentiator revealed to be more effective than duple associations in homozygous patients with the Phe508del mutation. Moreover, triple therapy also has benefits for patients with a single Phe508del mutation, covering 90% of CF patients[37,40]. Examples are the combinations VX-659-Tezacaftor-Ivacaftor and VX-445 (Elexacaftor)-Tezacaftor-Ivacaftor[14,40].

A new class of modulators, CFTR amplifiers, also emerges as a recent therapeutic option, still under study, in the face of its amplifying action in the transference of mRNA, and there are combinations with this new class under development (Figure 6)[13,40].

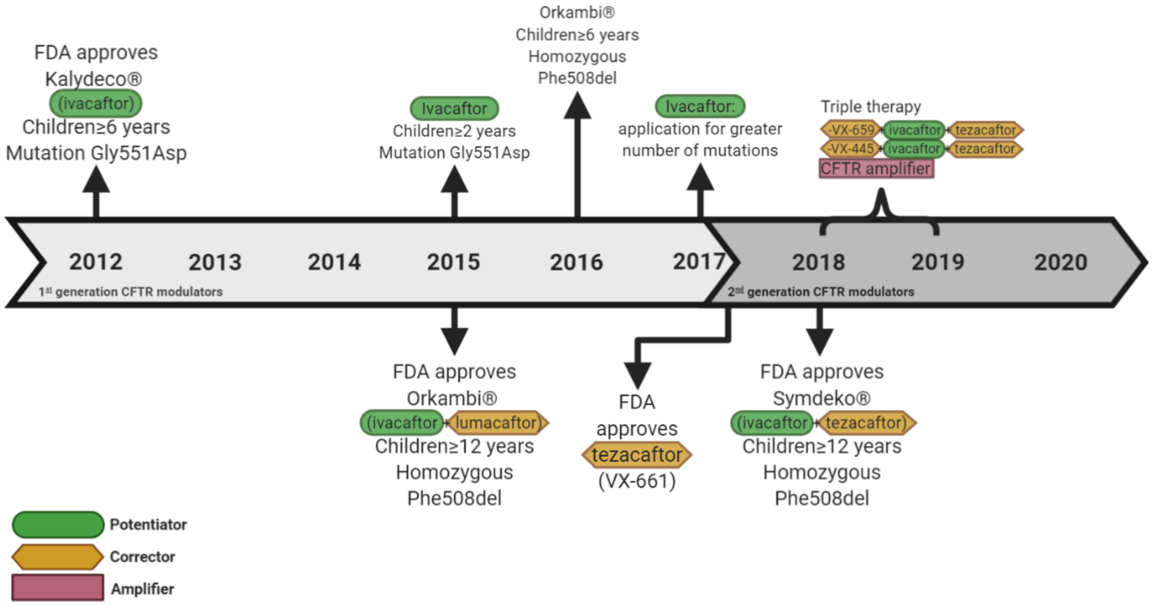


Figure 6. Temporal representation of the development of new therapeutic approaches (CFTR modulators)[4,10,13,22,37].

The stimulation of proteins and alternative channels to the CFTR pathway for the transport of ions and fluids is another mechanism currently under investigation, since it allows correcting the pH imbalance that occurs under the total absence of the CFTR channel. The Anoctamins channels, Anoctamin-1 (ANO1), also called transmembrane protein 16A (TMEM16A), and Anoctamin-6 (ANO6), or transmembrane protein 16F (TMEM16F), allow the movement of chloride ions, and can be activated by outward rectifying chloride channels (ORCC) and calcium-activated chloride channels (CaCC). In view of sodium hyperabsorption via ENaC experienced by the absence of the CFTR channel, inhibitors of ENaC channels can also be used to regulate ENaC activity, such as Amiloride, Benzamil and Phenamil. In the presence of residual activity of the CFTR channel, stimulation of this channel using alternative channels is also an option (Figure 7)[26].

Another alternative example is the read-through agents, that present application for stop codons in *nonsense* mutations, i.e., in class I mutations. The inhalation administration of read-through agents was tested and allowed some correction of defects in the chloride transport decreasing the risk of ototoxicity and renal toxicity due to prolonged use[10]. These agents will interact with the ribosome and, in the mutated site, will add amino acids that not correspond to the premature mutation codon, so that the polypeptide chain is transcribed in its entirety, resulting in full-length functional protein[6,10,26]. Aminoglycoside antibiotics, namely tobramycin, demonstrate ability to perform the mentioned mechanism in studies using yeast *S. cerevisiae* strains by interaction with the ribosome, reducing the control of translation, allowing the incorporation of amino acids with less affinity, but this mechanism is dependent on the premature termination codon present[10,16,26,42].

Application of biotechnology

The classic therapy, despite the very promising advances, still exhibiting many limitations. Of these, the need to know the mutated *CFTR* gene and the heterogeneity of patients is highlighted[1], resulting in *CFTR* mutation classes not covered by classical therapy[43]. The application of biotechnology appears in an auspicious approach, as it allows the restoration of the functions of the CFTR channel regardless of the type of mutation present. Examples are the gene therapy, gene-editing in which CRISPR/Cas9 technology is an example, RNA therapy and therapeutic microRNAs, in which antisense oligonucleotides are inserted (Figure 7)[6,34].

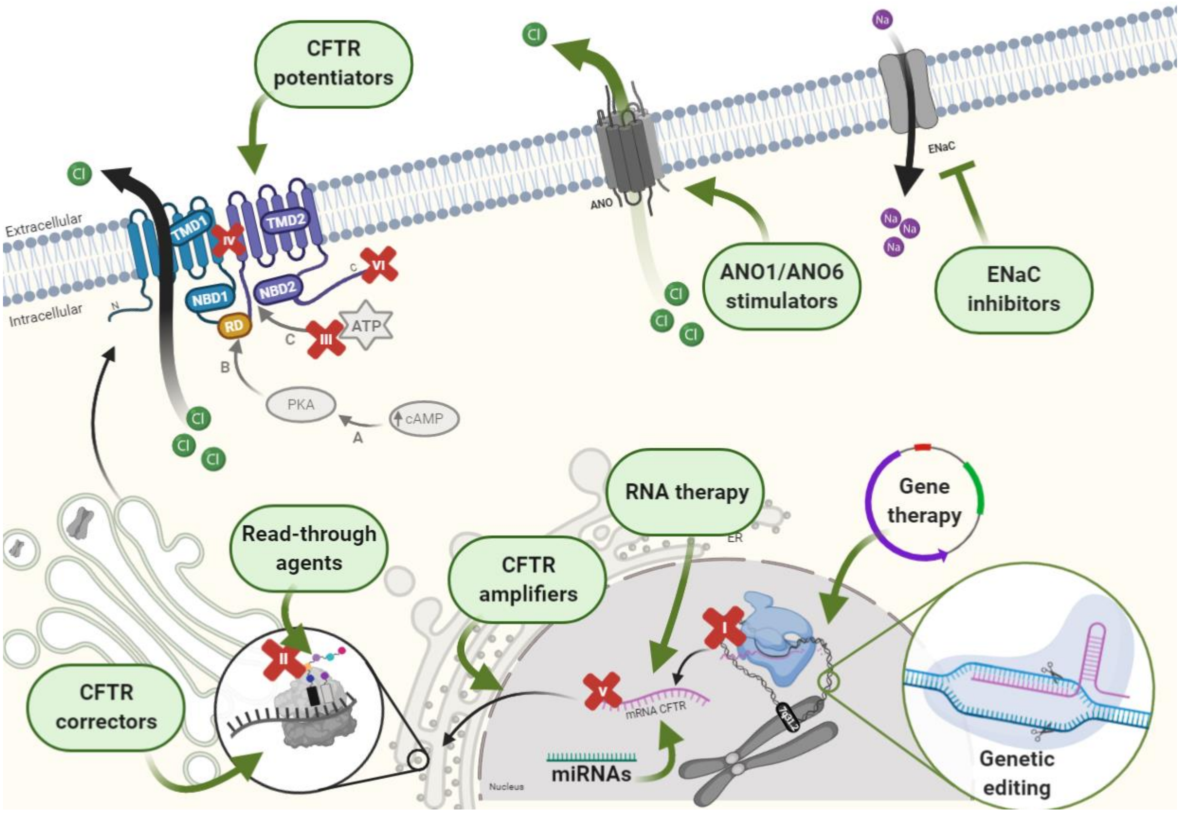


Figure 7. General representation of the place action of the main therapeutics for CF.

The main administration routes of the afore mentioned therapies are the oral and parenteral route, where the intravenous (IV) and subcutaneous (SC) are included. However, the one that appears to be more promising, but further studies still needed is the inhaled route. Indeed, because the therapeutic target is the lungs, the approach of targeting the biophase is expected to decrease systemic exposure, reducing the risk of adverse effects. The disadvantage to the inhalation route is related to the fact that this route can be obstructed, hindering its administration, and there is a need to use transporters to reach the desired target[1].

Gene therapy

Gene therapy consists in restoring the synthesis of normal CFTR in lung cells[10]. It is based on the insertion of normal *CFTR* complementary DNA (cDNA) into a plasmid DNA, with subsequent transfection in lung cells using a viral or non-viral vector[6,16,26]. These viral vectors have shown several obstacles, namely their fast mucociliary clearance and their difficulty to attain pulmonary cells because of the thick mucus layer. Consequently, vector tropism decreases and the effect is short-term, requiring repeated administrations. Multiple administrations lead to the undesirable development of neutralizing antibodies[6,10,16,26]. For instance the Adeno-associated virus serotype 2 (AAV2) has shown low levels in the expression of the *CFTR* transgene. New approaches have been developed to improve AAV2 action, explicitly the use of Human Bocavirus-Type-1 (HBoV1) capsid to involve AAV2; the lentivirus, a viral vector with a great capacity for integration into the genome, revealed potential to increase transepithelial expression of the long-lasting CFTR channel, which decreases the probability of immunogenicity[43].

Regarding non-viral vectors, liposomes have demonstrated safety, efficacy and ability to insert large DNA molecules[43]. An example is the nebulization administered liposome gene complex, which has moderately positive effects, according to the study reported by ALTON *et al.* 2015[44].

The formulation of plasmid DNA nanoparticles with biodegradable polymers (β-amino esters) is other technic to increase the efficiency of transfection performed by non-viral vectors. The advantage is the polyethylene glycol (PEG) surface of the nanoparticle that allows greater penetration into the airway mucus. The substitution of plasmid DNA by mini DNA circles, formed only by the transgene and regulatory elements, has been studied to increase the nanoparticle's ability to release the genetic material, but there is still no certainty about its advantage. Exosomes, derived from the natural formation of extracellular vesicles from donor cells, are another of the developing techniques that have demonstrated the ability to transfer both mRNA CFTR from exosomal cytoplasm and from glycosylated exosomal membrane bound *CFTR*[43].

Gene editing

Genetic editing is based on technologies that will accurately correct the specific *CFTR* gene mutations, or/and inactivate genes that overexpress ENaC channels. Genetic editing uses nucleases, which resort guide RNAs to break the double DNA chain at the target site of the mutation. Endogenous DNA repair mechanisms are activated, and the double DNA chain is repaired by homologous recombination in presence of the donor DNA or by non-homologous recombination. Among the nucleases that can be used, it is the clustered regularly interspaced short palindromic repeats associated with Cas9 nuclease (CRISPR/Cas9) which presents greater simplicity, chromosomal specificity through guide RNAs and low cost. Zinc Finger nucleases have been tested in the ability to insert the CFTR cDNA fragments, covering exons 11-27, into exon 11, in order to stimulate a functional correction in the ion transport capacity of the cells, but more safety studies have to be carried out in view of the possibility of changing the expression of the *CFTR* gene[43]. Given the risk of nuclease action in non-target sites, which may result in other dangerous mutations, nuclease-free approaches are studied, such as the use of Peptide Nucleic Acids to repair the Phe508del mutation[13,43].

RNA therapy

RNA therapy is another technique that has been developed and consists of chemical modification of mRNA to restore CFTR protein levels. mRNAs are chemically modified *in vitro* by incorporating modified nucleosides. They have lower immunoinflammatory potential, greater stability and greater expression capacity, which allows greater safety when compared to modified DNA genes, since they also do not present the risk of integration into the chromosome. For its delivery in epithelial cells, liposome or polymeric non-viral vectors are used through different techniques from those used in DNA therapy, because the mRNA has a very short half-life time and its expression is transient, implying repeated administrations to maintain the expression of CFTR and, therefore, techniques that do not develop immunogenicity[43]. Globally, the liposomal systems are favorable, and it can be used for the administration of the Cas9 nuclease, making the transient expression a safety advantage[43].

MicroRNAs therapy

MicroRNAs therapy (miRNAs) is based on the study of miRNAs, which are small chains of 20 to 25 non-coding nucleotides able to suppress or induce the expression of genes according to their location and place of action, to directly and indirectly modulating the transcription, translation and maturation processes of proteins. Thus, studies have been developed in order to discover potential miRNA that affect the expression of *CFTR* in CF[1,7].

MicroRNAs in *CFTR* expression

Several miRNAs revealed to be involved in the expression of *CFTR* in the CF, however, its regulatory action varies according to the tissue specificity and patient's phenotype. They repress the translation and/or induce the degradation through the binding to the 3'-untranslated regions (3'-UTRs) of the terminal part of the RNA that are associated to several polymorphisms, which may compromise the efficacy of miRNAs based therapeutics[1,7,34]. In addition, how it was observed that the regulation of the CFTR channel at the pulmonary level is widely expressed before birth, decreasing abruptly after birth, the microRNAs therapy efficacy may be variable according of the patient age. In fact, the negative regulation of *CFTR* expression in adult lung cell lines by miR-101 occurs, having no effect on fetal bronchial epithelial[1,45].

Up to day, it has been demonstrated that the expression of the *CFTR* is directly regulated by miR-145, miR-223 and miR-494, while the miR-509-3p can directly repress it and the miR-138 indirectly stimulated it through SIN3 transcriptor regulator family member A (SIN3A). The overexpression of miR-138 increases the expression and activity of CFTR channels on the surface of epithelial cells from CF patients with the Phe508del mutation (Table 3)[1,34].

MicroRNAs in inflammatory process

It has also been demonstrated that miRNAs expression may be involved in inflammatory processes, airway obstructions and pulmonary level infections in CF[1]. An analysis of RNA sequencing showed that 50% of unregulated mRNA are directly or indirectly related to the NF-kB pathway, with miR-199a-3p being a downregulated miRNA in human primary air-liquid interface cultures and in bronchial explants of CF patients[7].

MiR-199a-3p has a negative regulation mechanism. Briefly, miR-199a-3p will bind to the 3'-URT regions of the mRNA that codifies the IKKβ inhibitor (IKBKB), an NF-kB inhibitor. In this way, miR-199a-3p overexpression increases the IKBKB expression that results in the inhibition of the NF-kB pathway. On the other hand, miR-199a-3p downregulation hyperactivates the NF-kB pathway because the NF-kB inhibitor is underexpressed. How the activation of the NF-kB pathway culminates in the production of interleukin 8 (IL-8) and other pro-inflammatory mediators that contribute to the state of chronic pulmonary inflammation, it is possible to control the inflammatory process by controlling the miR-199a-3p expression (Figure 8)[7].

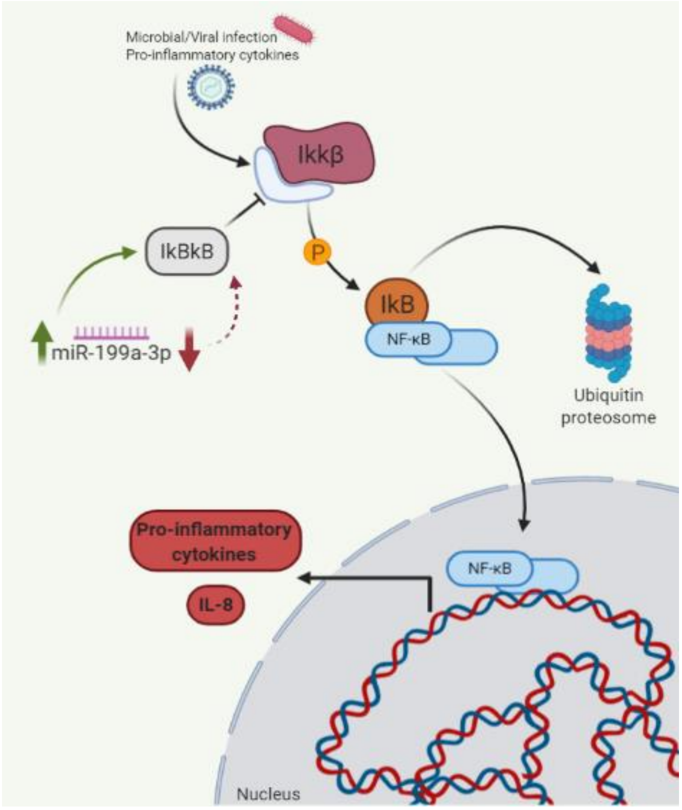


Figure 8. Representation of the NF-kB pathway that occurs in the airways in patients with CF and miR-199a-3p action in its regulation. NF-kB pathway can be activated by microbial and/or viral infections, as well as by pro-inflammatory cytokines, which will activate the β subunit of IKB kinase (Ikkβ). Ikkβ will phosphorylate the kB inhibitor (IkB) bound to the NF-kB, resulting in degradation via ubiquitin-proteasome of IkB and consequent NF-kB translocation to the nucleus. NF-kB, a dimeric transcription factor that is activated by binding the kB subunit to DNA, leads to the production of pro-inflammatory cytokines, IL-8 and activation of polynuclear neutrophils by regulation of the transcription of DNA genes in the nucleus of cells. NF-kB pathway can be negatively regulated by miR-199a-3p, since miR-199a-3p overexpression increases the activity of the Ikkβ inhibitor (IkBkB) that inhibits the NF-kB pathway by interaction with the β subunit of Ikkβ. The miR-199a-3p downregulation decreases IkBkB activity, overactivating the NF-kB pathway, due to inhibition deficit. The interaction of miR-199a-3p occurs in the 3'-URT region of the mRNA that encodes the IkBkB[7].

Other miRNAs demonstrate involvement in maintaining the state of inflammation (Table 3):

- downregulation of miR-93 and miR-17, through binding to the 3'-URT region of mRNA that encoding for IL-8[1,34];
- downregulation of miR-509-5p and miR-494, which are associated with hyperactivation of the NF-kB pathway[1];
- miR-31 downregulation, that stimulates cathepsin 5 activity (CPSS), resulting in pro-inflammation and tissues deterioration by prostheases[1,34];
- miR-199a-5p modulates the expression of caveolin protein 1 (CAV1), responsible for the resolution of the state of inflammation[1,34];
- miR-155 directly alters the expression of Src Homology-2 domain containing inositol 5-polyphosphatase 1 (SHIP1) and indirectly altering the expression of IL-8, contributing to the inflammatory state and tissue fibrosis[1,34];

- miR-221 modulates inflammation caused by stress in endoplasmic reticulum, through activation transcription factor 6 (ATF6)[1].

MicroRNAs in the airways

As for the mucociliary clearance, it may be compromised by miR-449 overexpression because miR-449 is expressed in multiciliary cells of the pulmonary airway[1,46]. Human studies have shown that miR-449 overexpression may interact with several targets, namely the centriolar protein Cp110, the Notch pathway and modulate the expression of small GTPases[46]. For normal expression in the pulmonary airways of multiciliary cells, repression of Cp110 is necessary for basal cells maturation. The active Notch pathway allows the centriole amplification and multiple motile cilia formation, as well as the formation of the actin web reorganization. GTPases also play an important role in the formation of multiciliary cells by controlling the cytoskeletal dynamics of actin. This way, mucociliary clearance is affected because miR-449 overexpression represses the cell cycle and the Notch pathway, and for the others mentioned targets, altering the normal formation of multiciliary cells in the pulmonary airways[1,46].

Airway obstruction may result from overexpressed miR-101, which leads to low expression of CFTR protein with consequent ion and pH imbalance that result in obstruction by the thick mucus layer. The miR-146a seems to be also responsible for the thick mucus layer, as it modulates the *MUC5AC* gene that encodes the MUC5AC, the main mucins produced by the goblet cells in the tracheobronchial surface epithelium[1].

Dysregulation in immunity can be felt in the face of downregulation of miR-126, which leads to the hyperactivity of the target of Myb1 (TOM1) that deregulates the expression of receptors on the epithelial surface because TOM1 transports the ubiquitin labelled proteins to proteasome, where they are degraded. MiR-181b can also affect immunity, changing the phagocytic ability of macrophages, acting on the lipoxin A_4 receptor/formyl-peptide receptor type 2 (ALX/FPR2)[34,47].

Therapeutic strategies

Once a miRNA demonstrates to be closely linked with a pathology, they can be considered a new pharmacological target. Hence, controlling the expression of the *CFTR* gene and other intermediate elements of involved in CF are mainstream therapeutic strategies currently under development. The miRNAs inhibitors and the antisense oligonucleotides (ASOs), which include miR sponges and target site blockers (TSB), decrease the expression of overexpressed miRNA and function gains in CF. In addition, miRNAs can be used to mimic and restructure the function loss, however their formulation is currently hampered[1].

Thus, ASOs are simple chains consisting of oligonucleotides with 2'O-methyl modified ribose and a phosphorothioate backbone. ASOs present an oral and systemic route that is not suitable, making the main inhalation route, with aerosol administration[10]. They act by binding to the miRNA target or pre-mRNA and, complementarity, preventing gene transcription. Although they can be used to correct *splicing* defects, which are characteristic in class V, ASOs can regulate the expression of various genes, developing side effects[1,6].

The TSBs, through their specific binding in the 3'-UTR region of the CFTR mRNA, will prevent the connection of miRNA, which in the specific case of miR-101 and miRNA-145 culminates in an increase of expression and activity of the CFTR[1]. Other example is the intranasal administration in animals of a specific TSB that prevents miR-9 binding in the 3'-UTR region of mRNA that encodes the ANO1, an alternative chloride channel. Thus, increase expression and activity of chloride ion, cell migration and mucociliary clearance regardless of calcium concentration[1]. It is noteworthy the relevance of the specific binding of miRNAs inhibitors, since non-specificity can lead to numerous adverse effects in relation to non-target gene modification[1].

Hence, in view of the growing knowledge about miRNAs and in order to optimize safety of inhibitions, new methods and chemical modifications appears in the existing therapeutics strategies. The use of locked nucleic acids (LNA) has greater thermodynamic stability and resistance to detection by nucleases thanks to a methylene bridge inserted into the ribose ring, causing greater. The insertion of phosphorothioate groups in the ASO allows to increase the resistance to hydrolysis by the 3'exonucleases and to improve the binding affinity. MiRNAs sponges are small nucleotidic chains with specific sequences complementary to many miRNAs. When the target miRNA finds its complementary sequence, it binds to the miRNA sponge and, permanently, prevents the miRNA from acting on its target[1]. The use of nanoparticles, on the other hand, improve the release affinity of miRNA[1].

The stimulation of proteins and alternative channels to the CFTR pathway, for the transport of ions and fluids in order to correct the pH imbalance, can also be modulated through the expression of miRNAs. Na^+-K^+-$2Cl^-$-cotransport protein is an example and can be modulated by miR-183[1]. The chloride channel ANO1 is another example, negatively modulated by the expression of miR-9[1,19,34]. For the sodium channel ENaC, as it is in hyperactivity in CF in the face of the non-functioning of the CFTR channel, arises the need of its repression, which can be done by modulating miR-183 and small interfering RNA (siRNA) therapies (Table 3)[1,43].

Table 3. Synthetic representation of miRNAs with altered expression in CF, which will exert action, directly or indirectly, on the respective targets[1,34].

Involved process	microRNA	Target	Outcome
***CFTR* expression**	↑ miR-101	*CFTR*	Decreased expression and activity of CFTR channels by negative regulation
	↑ miR-145 ↑ miR-223 ↑ miR-494	*CFTR*	Direct regulation of CFTR channels expression and activity
	↑ miR-509-3p	*CFTR*	Direct repression of CFTR channels expression and activity
	↑ miR-138	SIN3A	Indirect regulation of CFTR channels expression and activity
Inflammatory process	miR-199-3p	IKBKB	Negative indirect regulation of the NF-kB pathway
	↓ miR-93 ↓ miR17	IL-8	Maintenance of the inflammatory state
	↓ miR-509-5p ↓ miR-494	NF-kB	Hyperactivity of the NF-kB pathway
	↓ miR-31	CPSS	Stimulation of the CPSS that culminates in pro-inflammation and tissues deterioration by proteases
	↑ miR-199a-5p	CAV1	Decreased the ability to resolve inflammatory state
	↑ miR-155	SHIP1	Direct alteration of SHIP1 expression and indirect alteration of IL-8 expression, resulting in maintenance of the inflammatory state and tissue fibrosis
	↑ miR-221	ATF6	Inflammation caused by stress in the endoplasmic reticulum
Mucociliary clearance	↑ miR-449	Via Notch and small GTPases	Alteration in the production of normal multiciliary cells
Airway obstruction	miR-146a	*MUC5AC*	Alteration of the MUC5AC production contributing to the formation of a thick mucus layer
Immunity	↓ miR-126	TOM1	Dysregulation of receptors on the cells surface due to TOM1 hyperactivity, which is involved in the endosomal trafficking of ubiquitinated proteins
	↑ miR-181b	ALX/FRR2	Decreased phagocytic ability
Chloride channels	miR-9	ANO1	Decreased *ANO1* expression and regulation of chloride activity
	miR-183	ENaC and other chloride cotransporters	Alteration in the expression of the chloride channel involved

Conclusions and future prospective

Currently with no cure, CF is an autosomal recessive disease triggered by a mutation in the *CFTR* gene. Several mutations occur in this gene, compromising the transcription of the structuring CFTR protein, which physiological mechanism becomes limited in a wide variety of cells. Consequently, CF is characterized by pulmonary impairment and several comorbidities that affect various organs.

Knowledge of the mechanism underlying the expression of the *CFTR* gene and the subsequently metabolic pathways involved is of great importance, not only to allow the development of curative therapies, but also to control metabolic alterations, avoiding the progression and intensification of comorbidities. Early diagnosis also contributes to preventing disease progression.

The great technological development has improved the quality and average life expectancy of CF patients, but it is still not enough. It is necessary to ensure resources and healthcare for all patients and to continue to develop therapies with greater efficacy and safety, and could be administered to all CF patients independently of the mutation types.

With the development of CFTR modulators, it was possible to improve pulmonary function and decrease the risk of exacerbations and hospitalizations. Nevertheless, this therapy depends on *CFTR* mutation type of the patient. Moreover, the high risk of developing pharmacokinetic and pharmacodynamic interactions, the occurrence of possible adverse effects and the high cost hinder the therapy access for all patients. The application of biotechnology is an alternative, through gene therapy, gene editing, RNA therapy and application of therapeutic microRNAs. These techniques allow possible solutions to mutations classes that are not covered by CFTR modulators, opening the possibility of a permanent restructuring of the normal cell function. However, further studies are still needed to allow better drug delivery to the therapeutic targets, greater safety during their administration and accessibility to all patients.

Optimization of the administration route is also expected to increase therapy efficacy and decrease side effects, namely when thinking about the inhalation route to attain the lungs. Indeed, the inhaled route is showing promising advantages in term of proximity to the therapeutic target, lower risk of adverse effects, but more studies are needed to address the access limitations caused by mucus hyperviscosity and mucociliary clearance.

In addition, there is the need for a personalized therapy, associated with the optimization of existing therapies, so that CF is no longer the most lethal autosomal recessive disease in Caucasian individuals, allowing them to increase their quality of life and have a normal average life expectancy when compared to that of the healthy population.

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