Investigating the Cause and Focusing on Treating Heart Failure-Related Target Organ Damage

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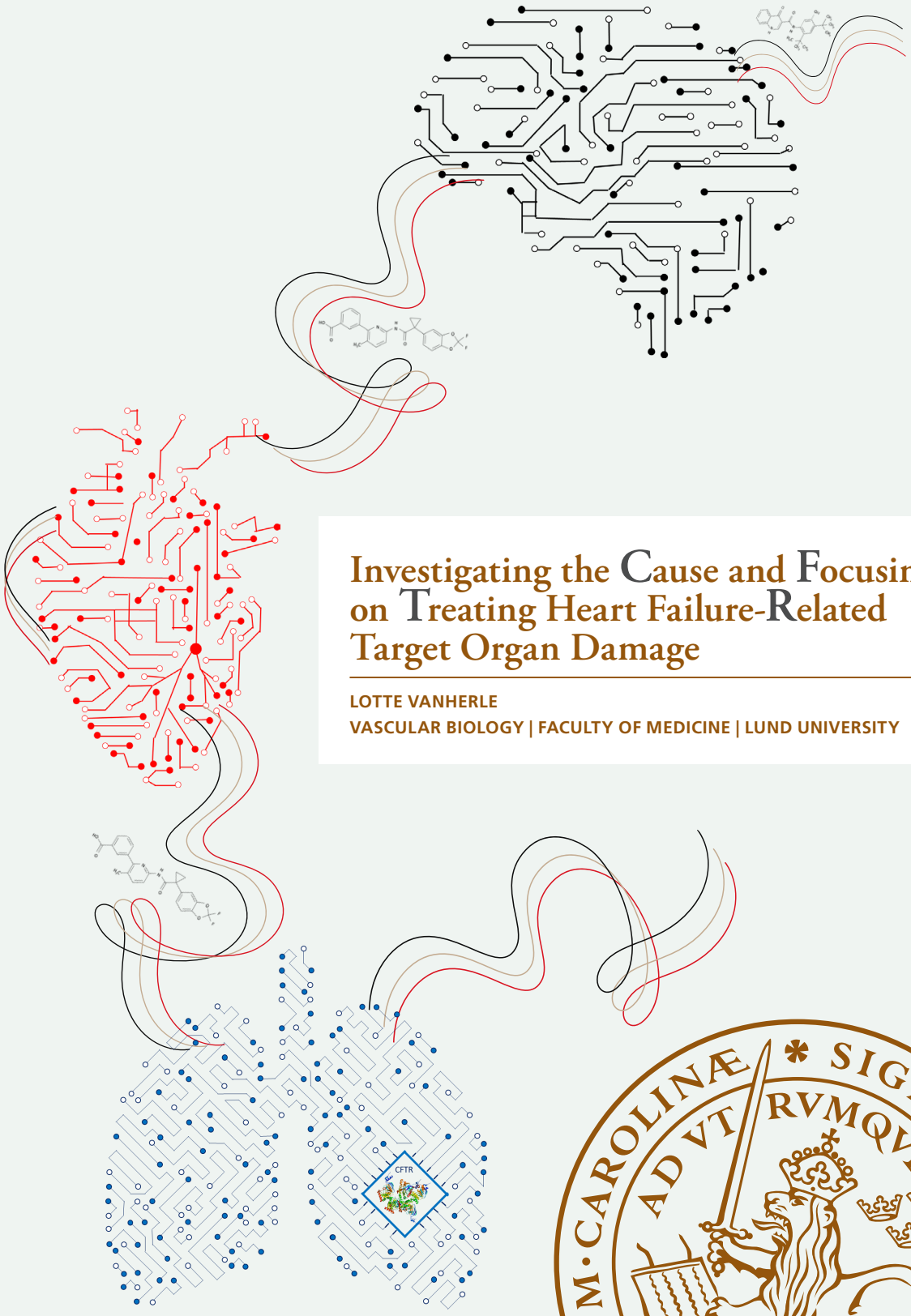

Investigating the Cause and Focusing on Treating Heart Failure-Related Target Organ Damage

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VASCULAR BIOLOGY | FACULTY OF MEDICINE | LUND UNIVERSITY

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LOTTE VANHERLE studied Biomedical Sciences at the University of Hasselt (Belgium) and received a Master´s degree in Clinical and Molecular Sciences. She started her PhD at Lund University within the Vascular Biology group in 2019. Her work focused on elucidating the molecular mechanisms behind cognitive impairment and pulmonary inflammation during heart failure.

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*Investigating the **C**ause and **F**ocusing on **T**reating Heart Failure-**R**elated Target Organ Damage*

Investigating the Cause and Focusing on Treating Heart Failure-Related Target Organ Damage

Lotte Vanherle

DOCTORAL DISSERTATION

Doctoral dissertation for the degree of Doctor of Philosophy (PhD) at the Faculty of Medicine at Lund University to be publicly defended on September 25th, 2023, at 09.00 in Belfragesalen, Department of Experimental Medical Science, Lund, Sweden.

Faculty opponent

Prof. Dr. Christopher Sobey
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Investigating the Cause and Focusing on Treating Heart Failure-Related Target Organ Damage

Abstract

Over the last decades, survival rates after myocardial infarction (MI) have improved significantly, resulting in increasing numbers of patients that develop chronic heart failure (HF). Due to increased longevity, HF patients often develop complications in vital organs aside from the heart, including the brain and the lung. As molecular mechanisms that link the failing heart with such complications are not well understood, effective and safe treatment options are missing.

In a mouse model of chronic HF, a downregulation of the cystic fibrosis transmembrane regulator (CFTR) in heart, lung, and brain tissue has previously been reported. Work presented as part of this thesis extends this concept and describes cell- and tissue-specific CFTR alterations during HF. CFTR expression seems to be an important modulator of HF-associated target organ damage, as (1) proper CFTR expression in the cerebral vasculature is critical for cerebral vascular tone regulation and hence, adequate cerebral blood flow, (2) microglial activation links to neuron-specific downregulation of CFTR with implications for neuronal structure and memory function, and (3) reduced CFTR surface expression on non-alveolar macrophages associates with a pro-inflammatory phenotype in the lung during HF. The importance of CFTR is supported by observed beneficial therapeutic effects of different CFTR correctors. Remarkably, the choice of CFTR modulator (corrector vs. potentiator) critically determined therapeutic outcome. While increasing CFTR cell surface expression using CFTR correctors attenuated vascular, neuronal, and pulmonary alterations associated with HF, potentiating CFTR channel open times without increasing expression yielded opposing results dependent on cell type and target organ.

In conclusion, the herein presented data suggests that restoring CFTR expression could be a promising therapeutic approach to treat damage that occurs in target organs during HF. Moreover, several CFTR therapeutics are readily available for clinical application, which may facilitate a swift repurposing for target organ damage treatment during HF.

Key words
Heart failure, cystic fibrosis transmembrane regulator, target organ damage, brain, lung

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To my family

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Abstract

Over the last decades, survival rates after myocardial infarction (MI) have improved significantly, resulting in increasing numbers of patients that develop chronic heart failure (HF). Due to increased longevity, HF patients often develop complications in vital organs aside from the heart, including the brain and the lung. As molecular mechanisms that link the failing heart with such complications are not well understood, effective and safe treatment options are missing.

In a mouse model of chronic HF, a downregulation of the cystic fibrosis transmembrane regulator (CFTR) in heart, lung, and brain tissue has previously been reported. Work presented as part of this thesis extends this concept and describes cell- and tissue-specific CFTR alterations during HF. CFTR expression seems to be an important modulator of HF-associated target organ damage, as (1) proper CFTR expression in the cerebral vasculature is critical for cerebral vascular tone regulation and hence, adequate cerebral blood flow, (2) microglial activation links to neuron-specific downregulation of CFTR with implications for neuronal structure and memory function, and (3) reduced CFTR surface expression on non-alveolar macrophages associates with a pro-inflammatory phenotype in the lung during HF. The importance of CFTR is supported by observed beneficial therapeutic effects of different CFTR correctors. Remarkably, the choice of CFTR modulator (corrector vs. potentiator) critically determined therapeutic outcome. While increasing CFTR cell surface expression using CFTR correctors attenuated vascular, neuronal, and pulmonary alterations associated with HF, potentiating CFTR channel open times without increasing expression yielded opposing results dependent on cell type and target organ.

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Popular Science Summary

Having a heart attack is still one of the leading causes of death worldwide. However, over the last decades, an increasing number of people has survived such an event, because of improved therapies. These patients often develop a secondary disease, which is known as chronic heart failure. Currently, there are over 64 million people living with heart failure. During heart failure, the heart cannot pump the same amount of blood through the body, so less blood reaches other organs. This results in patients developing problems in organs other than the heart, such as the lung and the brain.

During heart failure, the heart no longer transports the required nutrient and oxygen supply to the body, which in both, the lung and the brain, has negative effects. The lung and the heart are tightly interconnected and problems in one of both organs can cause serious problems in the respective other organ. We found that during heart failure there are increased numbers of immune cells in the body. Whereas immune cells generally protect our bodies from unwanted invaders, they can also be harmful by starting a process called inflammation. During heart failure, such inflammation is often found in the heart and other tissues. In mice with heart failure, we found more immune cells invading the lung. More specifically, we showed that heart failure leads to lower amounts of a specific channel (CFTR) on these immune cells, which seemed to cause unwanted inflammation in the lung. When mice received medication to increase the amount of CFTR on these immune cells, we observed lower inflammation in their lung.

The brain cannot store energy and oxygen for extensive time periods and therefore, a continuous nutrient supply is of utmost importance. If the brain does not receive enough nutrients, its functionality is reduced and in the worst case, brain cells can die. We found that, during heart failure, a reduction of CFTR on blood vessels and nerve cells resulted in a lower blood flow in the brain and the neuronal cells were weakened. Heart failure mice that received a drug, which increased the amount of CFTR on brain cells, showed less such problems.

Interestingly, not all CFTR medications led to the same effects. The best effects were achieved when the amount of CFTR on the different cells in brain and lung was increased. Giving a drug that only keeps the CFTR channel open for a longer time without increasing the amount of CFTR only helped the brain, but not the lung.

In conclusion, increasing the amount of CFTR in brain and lung during heart failure may help to treat problems in the brain and lung of heart failure patients.

Populärvetenskaplig Sammanfattning

En så kallad hjärtattack är fortfarande en av de främsta dödsorsakerna runt om i världen. Under de senaste decennierna har dock överlevnadstalen efter en hjärtattack ökat, huvudsakligen tack vare förbättrade behandlingsstrategier. Dessa patienter utvecklar dock ofta en följdsjukdom, det man kallar kronisk hjärtsvikt. Idag lider över 64 miljoner av hjärtsvikt. Hjärtsvikt innebär att hjärtat inte kan pumpa runt samma mängd blod som ett friskt hjärta, vilket leder till att mindre blod når övriga organ. Detta resulterar i att patienter kan komma att utveckla problem i andra organ mer än i hjärtat, såsom i lungan eller i hjärnan.

Vid hjärtsvikt kan hjärtat inte längre transportera den näring eller syre som kroppen behöver, vilket ger en negativ effekt i både lungorna och hjärnan. Lungan och hjärtat är nära sammankopplade och problem i det ena av de båda organen kan orsaka allvarliga problem i det andra. Vi har sett en ökad mängd av immunceller i kroppen kopplat till hjärtsvikt. Även om immunceller vanligtvis skyddar oss från ovälkomna hot utifrån, kan de också starta en skadlig process, en så kallad inflammation. Vid hjärtsvikt kan inflammationsprocessen uppstå i hjärtat men också i andra vävnader. I denna studie har vi sett att immunceller infiltrerar lungvävnaden när möss lider av hjärtsvikt. Mer specifikt visar vi ocksa att hjärtsvikt leder till lägre uttryck av en specifik kanal, som kallas CFTR, på immuncellerna. Detta verkar ha lett till oönskad inflammation i lungan. När mössen fick läkemedel som ska öka mängden av CFTR-kanalerna på immuncellerna, kunde vi se minskad inflammation i deras lungor.

Våra hjärnor kan inte lagra energi eller syre i längre perioder och är därför beroende av ett konstant flöde av både syre och energi. Om hjärnan inte får tillräckligt med näring kan hjärnfunktionen minska och i värsta fall kan hjärnceller dö. Vi har sett att hjärtsvikt minskar uttrycket av CFTR både på blodkärlen i hjärnan såväl som på hjärncellerna, vilket i sin tur leder till ett minskat blodflöde till hjärnan och en försvagning av hjärncellerna. Dessa problem minskade dock när mössen med hjärtsvikt ges läkemedel som ökar uttrycket av CTFR.

En annan intressant aspekt från denna studie är att inte alla läkemedel som påverkar CFTR ger samma effekt. Den bästa effekten uppnås när mängden av CFTR ökar. Men när man ger läkemedel som gör att CFTR hålls öppen under en längre tid, utan att öka mangden, såg vi bara en förbättringseffekt av det i hjärnan men inte i lungorna.

Sammanfattningsvis, att öka mängden CFTR kan vara en lovande läkemedelsstrategi for att åtgärda följdproblem i hjärnan och lungan hos patienter som lider av hjärtsvikt.

Populairwetenschappelijke Samenvatting

Een hartaanval is nog steeds een van de meest voorkomende doodsoorzaken wereldwijd. Gedurende de laatste decennia zijn er echter steeds meer mensen die een hartaanval overleven door verbeterde medicatie. Deze mensen ontwikkelen vaak een andere ziekte die chronisch hartfalen genoemd wordt. Op dit moment zijn er zo'n 64 miljoen mensen die leven met hartfalen.Tijdens hartfalen kan het hart niet dezelfde hoeveelheid bloed naar de rest van het lichaam pompen als voorheen, waardoor er minder bloed de andere organen bereikt. Dit zorgt ervoor dat patiënten vaker problemen ontwikkelen in andere organen, zoals de longen en de hersenen.

Tijdens hartfalen kan het hart niet voldoende zuurstof en voedingsstoffen vervoeren, wat voor negatieve effecten zorgt in zowel de longen als de hersenen. De longen en het hart zijn sterk met elkaar verbonden en problemen in het ene orgaan kunnen het andere orgaan beïnvloeden. Wij hebben ondervonden dat hartfalen zorgt voor een verhoogd aantal immuuncellen in het lichaam. Normaal gezien beschermen immuuncellen ons lichaam tegen ongewenste indringers van buitenaf, maar deze immuuncellen kunnen ook schade toebrengen door een ontstekingsproces te starten. Tijdens hartfalen vinden we zo een ontsteking vaak in het hart en in andere organen. Bij muizen met hartfalen zien we dat er meer immuuncellen de longen binnendringen. In het bijzonder zien we dat hartfalen leidt tot lagere hoeveelheden van een specifiek kanaal (CFTR) op deze immuuncellen, wat in verband lijkt te staan met een ongewenste vorm van ontsteking in de longen. Wanneer muizen een medicijn toegediend krijgen dat de hoeveelheid van CFTR op deze immuuncellen verhoogt, is de ontsteking niet langer aanwezig in hun longen.

De hersenen kunnen zelf niet veel energie en zuurstof opslaan en daarom is een continue toevoer van voedingsstoffen zeer belangrijk. Een te lage toevoer kan zorgen voor functieverlies en in het ergste geval kunnen hersencellen afsterven. We vonden dat er tijdens hartfalen een verlaagde aanwezigheid van CFTR is op de bloedvaten en de zenuwcellen in de hersenen. Dit resulteerde in een verlaagde bloedtoevoer in de hersenen enerzijds en verzwakte zenuwcellen anderzijds. Muizen met hartfalen die een medicijn kregen dat de hoeveelheid van CFTR verhoogde op de hersencellen, hadden minder van deze problemen.

Interessant om weten is dat niet alle CFTR medicijnen dezelfde effecten hebben. De beste effecten werden bereikt bij het verhogen van de hoeveelheid van CFTR op de verschillende hersen- en longcellen. Het toedienen van medicatie die zorgt dat het CFTR kanaal beter werkt door de openingstijd te verhogen, zonder de hoeveelheid van CFTR te verhogen, hielp enkel in de hersenen, maar niet in de longen.

Als conclusie kunnen we stellen dat het verhogen van CFTR in de hersenen en longen tijdens hartfalen kan een veelbelovende behandeling zijn om problemen in de hersenen en longen van patiënten met hartfalen te verhelpen.

Original Papers Included in the Thesis

I. Lidington D*, Fares JC*, Uhl FE, Dinh DD, Kroetsch JT, Sauvé M, Malik FA, Matthes F, **Vanherle L**, Adel A, Momen A, Zhang H, Aschar-Sobbi R, Foltz WD, Wan H, Sumiyoshi M, Macdonald RL, Husain M, Backx PH, Heximer SP, Meissner A[&], Bolz SS[&]. CFTR Therapeutics Normalize Cerebral Perfusion Deficits in Mouse Models of Heart Failure and Subarachnoid Hemorrhage. *JACC Basic Transl Sci. 2019 Nov 27;4(8):940-958.*

II. **Vanherle L**, Lidington D, Uhl FE, Steiner S, Vassallo S, Skoug C, Duarte JMN, Ramu S, Uller L, Desjardins JF, Connelly KA, Bolz SS, Meissner A. Restoring myocardial infarction-induced long-term memory impairment by targeting the cystic fibrosis transmembrane regulator. *EBioMedicine. 2022 Dec;86:104384.*

III. Uhl FE, **Vanherle L**, Meissner A. Cystic fibrosis transmembrane regulator correction attenuates heart failure-induced lung inflammation. *Front Immunol. 2022 Jul 28;13:928300.*

IV. **Vanherle L,** Matthes F, Uhl FE, Meissner A. Ivacaftor therapy post myocardial infarction augments systemic inflammation and evokes contrasting effects with respect to tissue inflammation in brain and lung. *Biomed Pharmacother. 2023 Jun;162:114628.*

Published Papers and Manuscripts Outside of the Thesis

I. Don-Doncow N, **Vanherle L**, Zhang Y, Meissner A. T-Cell Accumulation in the Hypertensive Brain: A Role for Sphingosine-1-Phosphate-Mediated Chemotaxis. *Int J Mol Sci. 2019 Jan 28;20(3):537.*

II. **Vanherle L***, Matuskova H*, Don-Doncow N, Uhl FE, Meissner A. Improving Cerebrovascular Function to Increase Neuronal Recovery in Neurodegeneration Associated to Cardiovascular Disease. *Front Cell Dev Biol. 2020 Feb 7;8:53.*

III. Jujic A*, Matthes F*, **Vanherle L**, Petzka H, Orho-Melander M, Nilsson PM, Magnusson M, Meissner A. Plasma S1P (Sphingosine-1-Phosphate) Links to Hypertension and Biomarkers of Inflammation and Cardiovascular Disease: Findings From a Translational Investigation. *Hypertension. 2021 Jul;78(1):195-209.*

IV. Don-Doncow N, **Vanherle L**, Matthes F, Petersen SK, Matuskova H, Rattik S, Härtlova A, Meissner A. Simvastatin therapy attenuates memory deficits that associate with brain monocyte infiltration in chronic hypercholesterolemia. *NPJ Aging Mech Dis. 2021 Aug 4;7(1):19.*

V. Uhl FE*, **Vanherle L***, Matthes F, Meissner A. Therapeutic CFTR Correction Normalizes Systemic and Lung-Specific S1P Level Alterations Associated with Heart Failure. *Int J Mol Sci. 2022 Jan 14;23(2):866.*

VI. Meissner A, Garcia-Serrano AM, **Vanherle L**, Rafiee Z, Don-Doncow N, Skoug C, Larsson S, Gottschalk M, Magnusson M, Duarte JMN. Alterations to Cerebral Perfusion, Metabolite Profiles, and Neuronal Morphology in the Hippocampus and Cortex of Male and Female Mice during Chronic Exposure to a High-Salt Diet. *Int J Mol Sci. 2022 Dec 24;24(1):300.*

VII. Skoug C, Erdogan H, **Vanherle L**, Vieira JPP, Matthes F, Eliasson L, Meissner A, Duarte JMN. Density of sphingosine- 1-phosphate receptors is altered in cortical nerve-terminals of insulin-resistant Goto-Kakizaki rats and diet-induced obese mice. *Manuscript in revision*

Abbreviations

CA1	Cornu ammonis 1
CF	Cystic fibrosis
CFTR	Cystic fibrosis transmembrane regulator
CM	Conditioned medium of vehicle-treated microglia
COPD	Chronic obstructive pulmonary disease
DG	Dentate gyrus
EF	Ejection fraction
HF	Heart failure
Iba1	Ionized calcium-binding adapter molecule 1
IL	Interleukin
i.p.	Intraperitoneal
Iva	Ivacaftor
LAD	Left anterior descending
LCM	Conditioned medium of LPS-treated microglia
Lum	Lumacaftor
LPS	Lipopolysaccharide
MI	Myocardial infarction
MOMA	Monocyte/macrophage
NOR	Novel object recognition
o.t.	Oro-tracheal
PCA	Posterior cerebral artery
PSD-95	Postsynaptic density protein 95
RI	Recognition index
TNF-α	Tumor necrosis factor alpha
WT	Wild-type

Introduction

Myocardial Infarction and Heart Failure

Myocardial infarction (MI) is a life-threatening event and currently one of the leading causes of death worldwide[1, 2]. MI is caused by a blockage of one of the coronary arteries, which results in ischemia in a part of the heart, leading to cardiomyocyte cell death[3, 4]. This event is often associated with the development of heart failure (HF) and a characteristic reduction of cardiac ejection fraction (EF). In contrast to acute HF characterized by sudden onset of severe symptoms[5, 6], chronic HF develops slower and worsens gradually over time[6].

While the incidence of HF is still high but stagnating, the overall prevalence is rising nonetheless[7, 8], indicating that the global rate of MI-associated mortality has decreased[1]. Considering survival of HF patients, a recent meta-analysis reported that 5-year survival increased from less than 30% between 1970 and 1979 to nearly 60% between 2000 and 2009[9].

The improved survival rates after MI demonstrate the scientific advancements concerning MI management and long-term survival. Nonetheless, due to increased longevity, many HF patients develop co-morbidities in so-called target organs. In HF patients, the most commonly affected target organs are the brain, the lung, and the kidney[10]. Work presented as part of this thesis focused on HF-associated alterations in cerebral perfusion, neuronal structure, memory function, and pulmonary immune cell environment.

Heart-Brain Axis

Over the last couple of years, a tight interconnection between the heart and the brain has become increasingly apparent. Mounting evidence showing increased risk of cognitive decline in HF patients[11-15] urges for more research concerning underlying molecular mechanisms and potential therapies. Not only does cognitive decline negatively affect quality of life and self-care management in HF patients, but it also limits treatment compliance which further increases hospitalization rates and mortality[12, 15].

As the failing heart is often accompanied by a reduction in cardiac output and impaired autoregulation[16], chronic cerebral hypoperfusion is discussed as one of the mechanisms driving the development of cognitive impairment during HF[16, 17]. It has previously been shown that improving cardiac function (e.g., through heart transplantation) increases cerebral perfusion[18, 19] and subsequently brain function[17, 19, 20]. During the acute phase of HF, activation of neurohormonal compensation mechanisms (e.g., activation of the renin–angiotensin-aldosterone system) is likely beneficial. However, it may be deleterious for the brain in the long run through an increased likelihood of CBF reduction[17]. As a consequence of reduced cerebral perfusion, the brain can experience episodes of hypoxia, which may trigger neuroinflammation[21]. A link between inflammation and cognitive decline has been previously described in several disease models, such as Alzheimer's disease (AD), diabetes, and HF[22-25], but also during aging[26-28]. Here, inflammation has been shown to contribute to cognitive decline. Several pro-inflammatory cytokines have been shown to mediate neurodegeneration or neuroprotection[22], including tumor necrosis factor alpha (TNF-α) and interleukins (ILs). Previous reports suggested that TNF-α is a major contributor to cerebral alterations in a mouse model of HF[29]. While HF increases proximal cerebral artery (PCA) myogenic tone, resulting in reduced cerebral perfusion in wild-type (WT) mice[30], genetic depletion of TNF-α protects from such HF-associated myogenic tone and CBF alterations[29, 31]. In addition, therapeutic scavenging of TNF-α using etanercept showed great potential during HF-mediated cerebral complications by mitigating HF-mediated myogenic tone augmentation[30, 31] and improving cerebral perfusion[30]. Also, etanercept therapy was associated with improvements of HF-mediated neuronal atrophy[29], accompanied by ameliorated memory function[32].

Some of the current therapies to treat HF have been shown to reduce cognitive impairment in other disease models. For example, sodium-glucose cotransporter 2 inhibitors (SGLT2i) significantly ameliorated cognitive deficits[15, 33] in a mixed Alzheimer/diabetes model, and reduced the risk of developing dementia in a population-based cohort study of type 2 diabetes patients[34]. Besides, angiotensin-converting-enzyme inhibitors (ACEi) improved cognitive function during HF, although compared to untreated HF patients, the percentage of patients presenting with improved cognitive scores was marginal (30% vs. 22%)[35].

Despite the availability of those therapies, the incidence of cognitive decline in HF patients remains much higher compared to age matched controls[12]. Therefore, new strategies to target cognitive decline in HF patients are needed. As currently almost all clinical trials on HF medication exclude patients with dementia or cognitive impairment[15], studies that specifically investigate this particular HF patient cohort are urgently needed.

Heart-Lung Axis

The quality of life for patients with HF is often additionally affected by respiratory problems, including shortness of breath, even during merely moderate physical efforts[29, 31, 36], and regular occurrences of pneumonia[37]. Nonetheless, molecular mechanisms underlying pulmonary complications during HF are yet to be elucidated. Recently, extensive effort has led to increased understanding of pulmonary edema etiology during HF[38, 39]. In contrast, knowledge of the pathophysiology of other complications in the lung of HF patients is still lagging. Despite the well-recognized connection between HF and pulmonary manifestations[40, 41], treatment of pulmonary complications in HF patients is difficult due to contraindications of treatment strategies[42].

Current strategies to treat pulmonary complications, occurring during short episodes of decompensated HF, involve loop diuretics (e.g., furosemide) to reduce volume overload and edema[43]. Chronic HF is often associated with increased pulmonary pressures, which can contribute to the development of right ventricular failure[44]. These increased pressures in the lung have been associated with augmented expression of ILs, TNF-α, and transforming growth factor-β (TGF-β), which are key contributors to pulmonary fibrosis development[44, 45]. Available drugs to treat idiopathic pulmonary fibrosis (e.g., Pirfenidone)[46] have shown potential to reduce cardiac fibrosis during HF with preserved EF[47, 48]. However, whether these approaches are also effective to treat HF-mediated pulmonary fibrosis is yet to be investigated. Besides, lung vascular fibrosis and immune cell infiltration into the lung have been observed during HF[45]. Inflammation during HF is generally targeted using anti-inflammatory strategies that have yielded inconsistent results[49-54]. However, additional research and long-term clinical trials to implement beneficial anti-inflammatory treatments in the clinic are needed[49].

The Cystic Fibrosis Transmembrane Regulator (CFTR) as a Molecular Mechanism

Since the discovery of the CFTR channel, most effort has been focusing on its genetic defects that cause cystic fibrosis (CF). Many of the research endeavors have centralized the importance of the CFTR channel primarily on pulmonary epithelial cells[55-57]. More recently, the CF research field has started to investigate epithelial cell CFTR function of other affected organs aside from the lung, such as the gastrointestinal tract and the pancreas where proper CFTR function is vital for ion and fluid homeostasis during digestion[58, 59]. Although CFTR is widely expressed in the human body, for many cell types CFTR function has not yet been investigated

with respect to potential disease associations. CF is characterized by genetic CFTR mutations that cause problems with channel opening, protein folding, or transport of the protein to the plasma membrane[60]. On the other hand, recent evidence spurred interest in CFTR in non-CF contexts as an acquired CFTR dysfunction has been linked to several other pulmonary diseases, such as chronic obstructive pulmonary disease (COPD), chronic bronchitis, and asthma[61-63], as well as HF[31]. Here, it is unclear whether other tissues in the body are affected as well. Therefore, it will be necessary to define whether systemic CFTR modulator application has unwanted off-target effects.

While it is well-known that CF patients present with pulmonary problems for which they are mostly treated, co-morbidities in other organs, including the heart and the brain have been identified. A study including 100 CF patients with differing disease severity concluded that severe CF was accompanied by a reduced fractional shortening, which suggests a reduced contractility, and a non-significant reduction of left ventricular EF[64]. It has been long recognized that CF patients present with nervous system abnormalities[65-67], however, not much research effort has gone into investigation of cerebral perfusion and memory function of CF patients[68]. These manifestations in the heart and brain of CF patients have come to attention only recently as in the last two decades life expectancy of CF patients has increased due to improved treatment strategies[60]. As a result of increased life expectancy, CF patients present with complications of aging which leads to additional therapeutic challenges[69].

Molecular mechanisms suggested to induce acquired CFTR dysfunction mostly include inflammatory signaling. HF-associated CFTR downregulation in different organs, including the brain and the lung (Figure 1A-B) is mediated through TNF-α-dependent mechanisms[31], particularly in the brain. Here, TNF-α is specifically upregulated within the cerebral vessel wall[30] where it mediates vascular tone augmentation. This results in a CBF reduction by altering vessel-specific CFTR expression, which in turn increases the bioavailability of the vasoactive phospholipid sphingosine-1-phosphate (S1P)[30]. Since CFTR represents a critical bottleneck for S1P degradation[31], TNF-α-mediated CFTR downregulation has been proposed as central mechanism for altered vascular responses to elevated extracellular S1P in different vascular beds during HF[31]. In the lung, first evidence supports an acquired CFTR dysfunction by showing a marked reduction of CFTR expression in the epithelium of terminal bronchioli during murine HF (Figure 1C). Sequestration of TNF-α by etanercept (i.e., soluble TNF-α receptor) normalized CFTR expression in brain and lung tissue (Figure 1A-B), reduced augmented cerebrovascular constriction and thus, improved cerebral perfusion[30, 31, 70]. The RENEWAL study[50] combined results from two clinical trials where etanercept was globally assessed for the treatment of HF patients. The studies were terminated as etanercept did not have clear benefits on morbidity and mortality endpoints defined

as part of the trial. Although there was no conclusive evidence that etanercept was harmful, the risk ratio for worsening chronic HF was increased[71-73]. Additionally, higher rates of HF-related hospitalizations and death have been reported in patients receiving infliximab[51, 72, 74], a monoclonal antibody targeting TNF-α. A systematic review, investigating rheumatoid arthritis patients with HF, found no increase in worsening of HF in patients treated with TNF-α targeting therapies (etanercept and infliximab) except in the patients population older than 65 years, who had a higher risk of HF hospitalization and death[72]. A recent case report of a rheumatoid arthritis patients demonstrated that etanercept can lead to new onset HF[75]. Considering those findings, guidelines for treatment of rheumatoid arthritis patients suggest that TNF-α targeting drugs should only be used in patients with HF if no other reasonable treatment options are available[72, 74]. Besides, clinical application of TNF-α targeting therapies is still controversial because of general immunosuppression and TNF-α's importance in proper brain function[76-78]. Therefore, the investigation of other therapeutic targets is warranted.

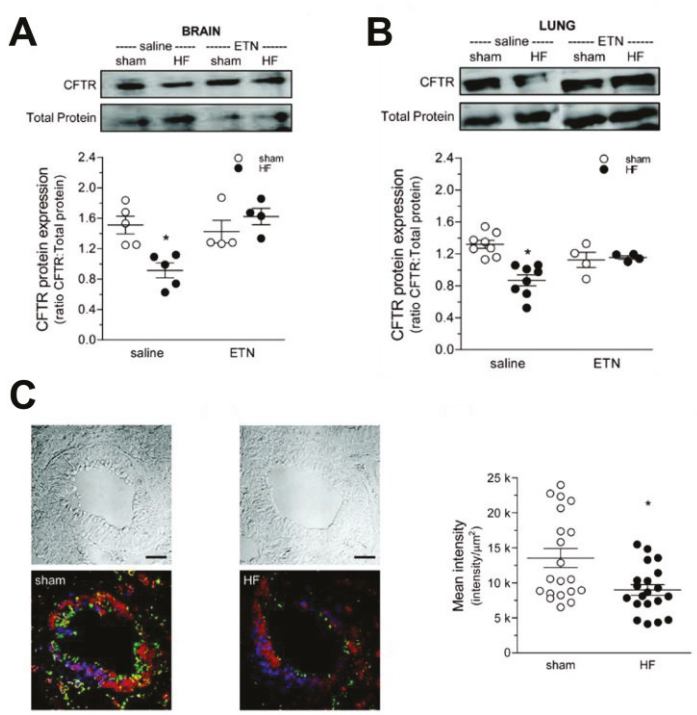

Figure 1: Downregulation of CFTR protein expression in brain and lung during HF. HF associated with the downregulation of CFTR protein expression in **(A)** brain and **(B)** lung tissue; sequestration of tumor necrosis factor-α using systemic etanercept (ETN) treatment abolished this downregulation. **(C)** Representative images of lung slices from sham-operated controls and HF mice, stained for CFTR (green), nuclear factor-κB (cytosolic marker; red), and nuclei (Hoechst 33258; blue). HF significantly reduced CFTR mean intensity in epithelial cells of terminal bronchioli. For **(A-B)** *p≤ 0.05 for multiple, unpaired comparisons with the sham+saline control groups. For **(C),** *p≤ 0.05 for unpaired comparisons between sham and HF groups. *Adapted from Meissner et al.*[31].

Considering TNF-α's central role in regulating CFTR expression[31] during HF, direct targeting of CFTR using approved CFTR modulators appears to be a promising alternative to circumvent unwanted side effects of TNF-α targeting therapy[31]. Currently, five types of CFTR modulator treatments exist (potentiators, correctors, stabilizers, amplifiers, and read-through agents)[60], two of which are approved as single or combination treatments for CF.

CFTR **potentiators** (e.g., ivacaftor) improve channel activity by enhancing the channel's open probability. In combination with correctors, there are various drugs on the market that have been shown to be effective in the context of CF.

CFTR **correctors** (e.g., lumacaftor) can augment CFTR's conformational stability ultimately leading to improved protein folding and increased trafficking of mature CFTR to the plasma membrane. Lumacaftor has been shown to increase WT CFTR expression. Lumacaftor as a monotherapy has not been proven effective in the context of CF, however, in combination with ivacaftor, it is approved as a therapy. Interestingly, the dose of lumacaftor that is used in the combination therapy (i.e., together with ivacaftor) was never tested as monotherapy.

Stabilizers improve anchoring of the CFTR protein at the level of the plasma membrane and simultaneously reduce degradation of the channel. A first CFTR stabilizer for ΔF508-homozygous patients in combination with lumacaftor/ivacaftor is currently in phase II trials[79].

Amplifiers increase the amount of mutant CFTR mRNA, which leads to more substrate for other CFTR modulators[80]. In order to be effective, a combination of amplifiers and potentiators or correctors is necessary, as amplifiers themselves do not have correcting or potentiating capacity. There are currently two amplifiers that show beneficial effects in clinical trials[81, 82]. Whether amplifiers would be beneficial in a non-CF context is yet to be investigated.

The utilization of CFTR modulators outside the CF field may be an important therapeutic strategy for chronic respiratory and non-respiratory diseases with an acquired CFTR dysfunction. Here, the type of CFTR defect is important for the drug type selection. In COPD where both CFTR expression and function are reduced[62], a combination of a CFTR corrector and a stabilizer would be interesting. The corrector that facilitates correct protein folding and the stabilizer which improves anchoring of the protein in the plasma membrane, may be a good combination. There is currently one study that reported promising, yet non-significant, improvements of lung function in COPD patients with chronic bronchitis[61]. HF associated CFTR dysfunction is mainly of proteostatic nature, for which corrector treatment may be most suitable. More research investigating the safety and efficacy of CFTR modulators in non-CF diseases should elucidate their therapeutic potential and repurposing possibilities.

Aims of the Thesis

The link between HF and target organ damage has become much more apparent over the last couple of decades[41, 83-85], however, the molecular mechanisms that are involved remain elusive. The primary aim of this thesis was to investigate acquired CFTR dysfunction as a molecular mechanism involved in the development of target organ damage during HF.

The specific aims of the papers included in the thesis were:

I. Assessing the therapeutic potential of CFTR correctors to restore HF-associated increased myogenic tone and cerebral perfusion deficits in an animal model of HF.

II. Investigating the mechanism of hippocampus-dependent memory dysfunction during HF and the therapeutic potential of restoring CFTR expression.

III. Elucidating the lung phenotype during HF and testing systemic (intraperitoneal) and lung-specific (oro-tracheal) CFTR corrector therapy to treat HF-related alterations.

IV. Verifying whether CFTR potentiation would suffice to reduce HF-induced brain and lung phenotypes.

Newsworthiness of the Thesis

The work in this thesis focused on the investigation of the molecular basis to complications observed in the brain and the lung during HF as there are yet no curative therapies to treat HF-associated cognitive decline or pulmonary inflammation. This sparsity of treatment options is primarily due to the lack of understanding of mechanisms underlying HF-mediated target organ complications. Building on previous work that shows a HF-associated downregulation of CFTR in heart, brain, and lung tissue after MI, cell-specific investigations verified its importance in disease progression and underpinned the benefit of therapeutic targeting of CFTR for target organ damage co-occurring with HF as primary disease.

This thesis specifically shows that CFTR critically regulates cerebrovascular tone and hence, cerebral perfusion with implications for neuronal structure and memory function. Neuron-specific downregulation of CFTR co-occurred with neuroinflammation, altered neuronal arborization, a decline in critical neuronal microstructures, and long-term memory function. Therapeutic CFTR modulation diminished the observed structural and functional changes in the brain.

Besides the brain, HF led to increased pulmonary inflammation, which coincided with an apparent CFTR phenotype specifically in non-alveolar macrophages, presenting with significantly lower CFTR cell surface expression and concurrent pro-inflammatory polarization. Therapeutic correction of CFTR expression induced a reparative phenotype of these cells and reduced overall inflammation in the lung during HF.

Recently reported benefits observed in clinical trials for monotherapy with the CFTR potentiator ivacaftor (i.e., acting through increase of CFTR opening times) prompted its investigation as a vital alternative to CFTR correctors, which are not yet clinically approved as monotherapies. Interestingly, CFTR potentiation using ivacaftor evoked contrasting effects depending on target tissue during HF.

Altogether, these results show that systemic CFTR modulation has the potential to ameliorate HF complications in brain and lung. As CFTR modulator compounds are readily available or have already passed toxicity testing and different phases of clinical trials, repurposing CFTR modulators could be a safe and effective way of treating target organ damage in HF patients.

Key Methods

Ethics (Study I-IV)

The experiments performed in this thesis conform to the EU Directive 2010/63/EU for animal experiments and the ARRIVE 2.0 guidelines. All studies were performed according to the Swedish Animal welfare ACT SFS 1988:534 act, were approved by the institutional ethical committee for animal experiments Malmö/Lund (Dnr 5.8.18-08003/2017, 5.8.18-04938/2021) and conducted in accordance with European animal protection laws.

Heart Failure Mouse Model (Study I-IV)

All studies utilized a mouse model of chronic HF induced by surgical ligation of the left anterior descending (LAD) coronary artery (Figure 2) as previously described[86]. Twelve- to sixteen-week-old C57Bl/6 male mice were sedated using isoflurane, intubated, and ventilated before a lateral thoracotomy was performed. The pericardium was opened, and the LAD was permanently ligated. Following ligation, the thorax was closed, and the mice were extubated upon spontaneous respiration. For sham surgeries, the same procedure was followed without ligating the LAD. Post-surgery, mice were subjected to a buprenorphine-based analgesia protocol.

CFTR modulators were administered systemically (intraperitoneal (i.p.)) or lung-specifically (oro-tracheal (o.t.)) at 10- or 22-weeks post-MI (Figure 2). The following drugs/drug combinations were used: C18 (3mg/kg), lumacaftor (Lum; 3mg/kg), ivacaftor (Iva; 1.875mg/kg), and lumacaftor/ivacaftor (Lum/Iva).

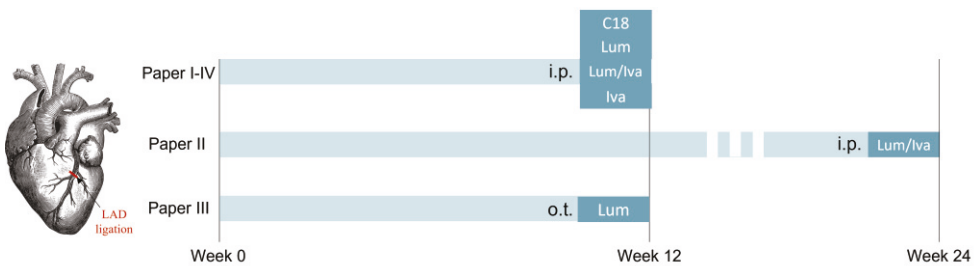

Figure 2: Visual overview of HF mouse model and timeline. *Abbreviations: i.p. – intraperitoneal, Iva – ivacaftor, LAD – left anterior descending Lum – lumacaftor, o.t. – oro-tracheal.*

Cardiac Parameter Assessment (Study I-IV)

Cardiac function was assessed using magnetic resonance imaging (MRI) using a 9.4 T horizontal MR scanner equipped with Bruker BioSpec AVIII electronics, a quadrature volume resonator coil (112/087) for transmission and a 20 mm linear surface loop coil for reception (Bruker, Ettlingen, Germany) as previously described[86]. During scanning, mice were immobilized using isoflurane and were kept at 36–37 °C body temperature while respiration remained between 70–100 bpm. Sequence details can be found in the published papers[87, 88]. Hemodynamic parameters were assessed from the Dicom images using Segment (Medviso, Lund, Sweden)[89]. Left ventricular (LV) ejection fraction was calculated from LV-end-diastolic and LV-end-systolic dimensions.

Novel Object Recognition Test (Study I-II, IV)

Non-spatial short-term or long-term memory was assessed using novel object recognition (NOR) behavioral tests. In order to test hippocampal memory function that involves the activation of cornu ammonis 1 (CA1) neurons[90, 91], a 1-h delay interval was utilized, while cortex-dependent memory was studied using a 5-min delay. Both protocols were executed as adapted from previously described methods[28]. Mice were acclimatized to the test arenas during an open field exploration task (Figure 3, left panel). For long-term memory testing, habituated mice were exposed to two identical objects 24h prior to the test (Figure 3, middle panel). To test short-term memory, this additional "familiarization" day was not part of the protocol as it would have activated memory traces responsible for long-term memory. On the test day, mice were (re-)exposed to (the same) two identical objects and after the respective delay intervals, one of the original objects was replaced with a novel object and mice were allowed to explore the two objects (Figure 3, right panel). The objects and arena were thoroughly cleaned with 70% ethanol between each test, to eliminate potential odor cues. Mice were video tracked with Any Maze software (Stoelting; Dublin, Ireland) that recorded the time spent interacting with the novel (Tn) and original (To) objects. The results were verified by manual Tn and To determination using stopwatches by an observer blinded to the experimental group assignments. The recognition index (RI) was calculated as follows: Tn/[Tn + To]. Animals with total exploration times below 20 s were excluded from analyses.

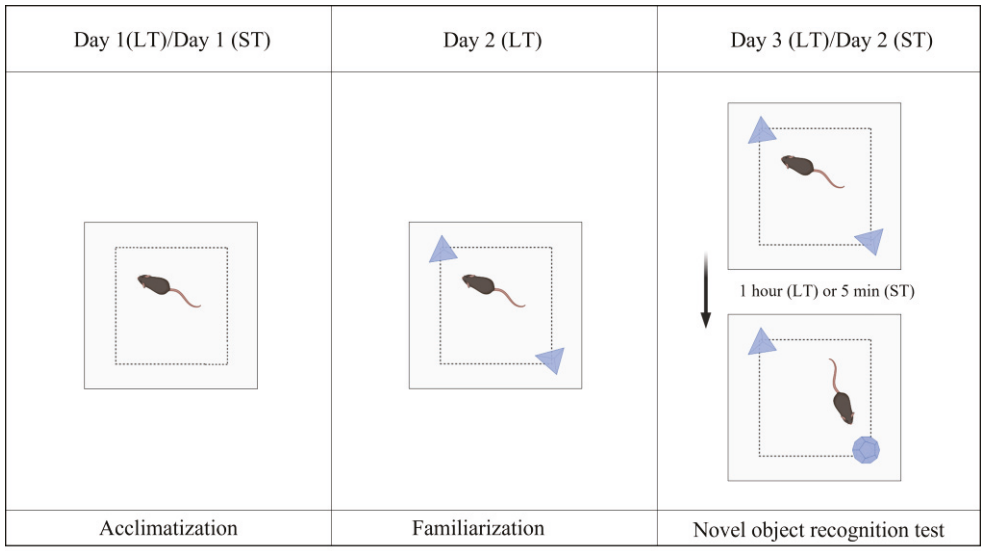

Figure 3: Protocol for long-term (LT) and short-term (ST) memory testing.

Neuronal Arborization and Dendritic Spine Density (Study I-II, IV)

Neuronal dendrite networks and dendritic spine density of pyramidal neurons of the cortex or hippocampus were assessed in coronal Golgi-Cox-stained brain sections (150 µm thickness) as previously described[29, 92]. Cortical or hippocampal neurons were imaged with a Nikon Eclipse Ti2e microscope (Nikon Instruments Europe) and analyzed using Image J (https://imagej.net/Sholl_Analysis, version 3.7.4). The arborization of single neurons from cortex or hippocampal CA1 region were digitally traced using the Simple Neurite Tracer plugin for Image J[93]. The center of the soma was identified, and dendrite arborization (i.e., intersections vs. dendrite lengths) was characterized with a Sholl analysis tool (https://imagej.net/Sholl_Analysis, version 3.7.4), using 5 µm intervals. Additionally, the maximum length of the main dendrite was assessed.

Dendritic spine density (i.e., the number of small protrusions on dendrites) was assessed using 3rd branch order dendritic segments. Spine density was measured as the number of spines per segment normalized to the length of the segment.

Flow Cytometry (Study II-IV)

Lungs were processed using a standard dissociation protocol and red blood cells in lung and blood samples were lysed. N2a cells were collected and washed using PBS.

Samples were blocked (Fc block in FACS buffer) prior to staining with antibodies for 30 min at 4°C (Table 1). Data acquisition was carried out on a BD Accuri C6 Plus cytometer or a BD LSR Fortessa cytometer using FacsDiva software Vision 8.0 (BD Biosciences). Data analysis was performed with FlowJo software (version 10, TreeStar Inc., USA). Cells were plotted on forward versus side scatter and single cells were gated on FSC-A versus FSC-H linearity.

Immunofluorescence Staining, qPCR, and Western Blotting (Study I-IV)

Standard protocols were used for immunofluorescence staining, qPCR, and Western blotting. Antibodies and primers that were used are listed in Tables 1-2.

Statistics (Study I-IV)

Using previous data as guidance[29-31, 94], experimental group sizes were calculated to ensure that all data provided an 80% power level ($1 - \beta > 0.8$) and a two-tailed Type I alpha error of 0.05. The calculation determined that a minimum of 8 animals per group was required for sufficient statistical power for most experimental measures. Due to model-specific mortality, 20% excess was added per group.

Data was analyzed using GraphPad Prism 9 software. Normally distributed data was presented as mean ± SEM and compared using parametric statistical tests. Data that was not normally distributed, as determined by the Shapiro–Wilk test, was presented as median ± interquartile range and compared using non-parametric statistical tests. A Student's t-test or Mann Whitney test was used to compare two independent groups; a one-way analysis of variance (ANOVA) with Tukey post-hoc test or Kruskal Wallis test with Dunn's post-hoc test was used to compare multiple independent groups. Differences were considered significant at $p \leq 0.05$. For all datasets, N represents the number of animals or independent biological samples and n the number of independent measures. All figure data, sample size, and statistical test outcomes are presented in the published papers.

Table 1: Primary antibodies used for flow cytometry (FACS), immunofluorescence staining (IF) and western blotting (WB). AF – Alexa Fluor, CD – cluster of differentiation, CFTR – cystic fibrosis transmembrane regulator, HRP – horseradish peroxidase, Iba-1 – ionized calcium channel binding adaptor protein 1, MAP-2 – microtubule-associated protein 2, MOMA – monocyte/macrophage, SMA – smooth muscle actin, TNF-α – tumour necrosis factor alpha

Antibody	Source	Company	Ordernr.	Application
α-tubulin	Mouse	Cell Signalling Technology	cat# 3873	WB
Alexa Fluor 488 anti-mouse	Goat	Invitrogen	A-11029	FACS, IF
Alexa Fluor 488 anti-rabbit	Goat	Invitrogen	A-11034	IF
Alexa Fluor 594 anti-mouse	Goat	Invitrogen	A-11032	IF
Alexa Fluor 594 anti-rat	Goat	BioLegend	405422	IF
B220 APC-Cy7	Rat	BD	561102	FACS
B220 AF488	Rat	R&D	FAB1217G	FACS
B220 PerCP eFluor710	Rat	Fisher Scientific	46-0452-82	FACS
CD11b PE-Dazzle	Rat	BioLegend	101256	FACS
CD11b PE-Texas-Red	Rat	Fisher Scientific	RM2817	FACS
CD206 PE	Rat	BioLegend	141705	FACS
CD3 eFluor 450	Rat	Fisher Scientific	48-0032-82	FACS
CD45 AF700	Rat	R&D	FAB114N	FACS
CD45 AF488	Rat	R&D	FAB114G	FACS
CD45 PE	Rat	Nordic Biosite	109807	FACS
CD80 BV650	Hamster	BioLegend	104731	FACS
CFTR	Mouse	Fisher Scientific	MA1-935	FACS, IF
CFTR	Rabbit	Cell Signalling Technology	cat# 2269	WB
CFTR	Mouse	CFF foundation – Dr. John Riordan	“Antibody 596”	WB
CFTR AF647 labelled	Mouse	Fisher Scientific	MA1-935	FACS
F4/80 APC	Rat	eBiosciences	17-4801-82	FACS
F4/80 PE	Rat	Fisher Scientific	12-4801-80	FACS
HRP-labelled anti-mouse	Goat	Cell signalling	7076S	WB
HRP-labelled anti-rabbit	Goat	Cell signalling	7074S	WB
Iba-1	Rabbit	Wako	019-19741	IF
Live/Dead Aqua BV510	-	Fisher Scientific	L34966	FACS
Ly6C PE-Cy7	Rat	Fisher Scientific	25-5932-82	FACS
Ly6G APC	Rat	Fisher Scientific	17-9668-82	FACS
Ly6G FITC	Rat	Fisher Scientific	11-9668-82	FACS
MAP-2	Rabbit	Abcam	Ab32454	IF
MOMA	Rat	GeneTex	GTX39773	IF
SiglecF BV421	Rat	BioLegend	155509	FACS
SMA	Mouse	Sigma	A5228	IF
TNF-α	Rabbit	Abcam	ab6671	IF

Table 2: Primers used for qRT-PCR.

Target	Species	Forward primer	Reverse primer
Rpl14	Mouse	GGCTTTAGTGGATGGACCCT	ATTGATATCCGCCTTCTCCC
Cd80	Mouse	GATTTCAATACGACTCGCAACCAC	TGATGACAACGATGACGACGAC
Il18	Mouse	TGAGGCATCCAGGACAAATCAG	AGCACACCACAGGGGAGAAG
Il1b	Mouse	GAAGAGCCCATCCTCTGTGA	TTCATCTCGGAGCCTGTAGTG
Psd95	Mouse	ACCGCTACCAAGATGAAGACAC	CTCCTCATACTCCATCTCCCC
GPI	Human	AGGCTGCTGCCACATAAGGT	CCAAGGCTCCAAGCATGAAT
CD80	Human	CCTGATAACCTGCTCCCATCC	CCTTCTCAATCTCTCATTCCTCC

Summary of Results and Discussion

Due to increased longevity, HF patients often present with complications in vital organs, including the brain and lung. Previously, a HF-mediated downregulation of CFTR has been identified in whole tissue lysates of both organs. This thesis extended these preliminary findings to the cell and tissue level and tested the therapeutic potential of CFTR modulation in HF. In the brain, the cerebral vasculature as well as neuronal cells were studied concerning CFTR expression and their response to CFTR corrector treatment with respect to cerebral blood flow, neuronal structure, and memory function. In the lung, HF led to reduced CFTR expression on infiltrating immune cells that was accompanied by a pro-inflammatory phenotype. CFTR corrector therapy was used to restore CFTR expression, that simultaneously promoted a favorable macrophage polarization. Finally, the therapeutic efficacy of the CFTR potentiator ivacaftor as monotherapy was studied in the context of both target organs.

Therapeutic Targeting of CFTR Normalized Myogenic Tone and Cerebral Perfusion during HF (Paper I)

In accordance with previously published data[31], HF was associated with a significant reduction in cerebral artery CFTR protein expression (Figure 4A) that caused significant augmentation of myogenic tone in PCAs of HF mice (Figure 4B). This cerebrovascular tone augmentation was accompanied by a marked reduction in CBF (Figure 4C). HF mice treated systemically with the CFTR corrector C18 presented with cerebrovascular CFTR expression (Figure 4A), PCA tone (Figure 4B), and CBF (Figure 4C), similar to sham-operated mice. C18 treatment did not influence cardiac function or peripheral resistance and therefore, its therapeutic effect can be attributed to a microvascular mechanism in the brain. Hence, the probability of cardiovascular adverse effects and potential interactions with blood pressure managing medication are unlikely.

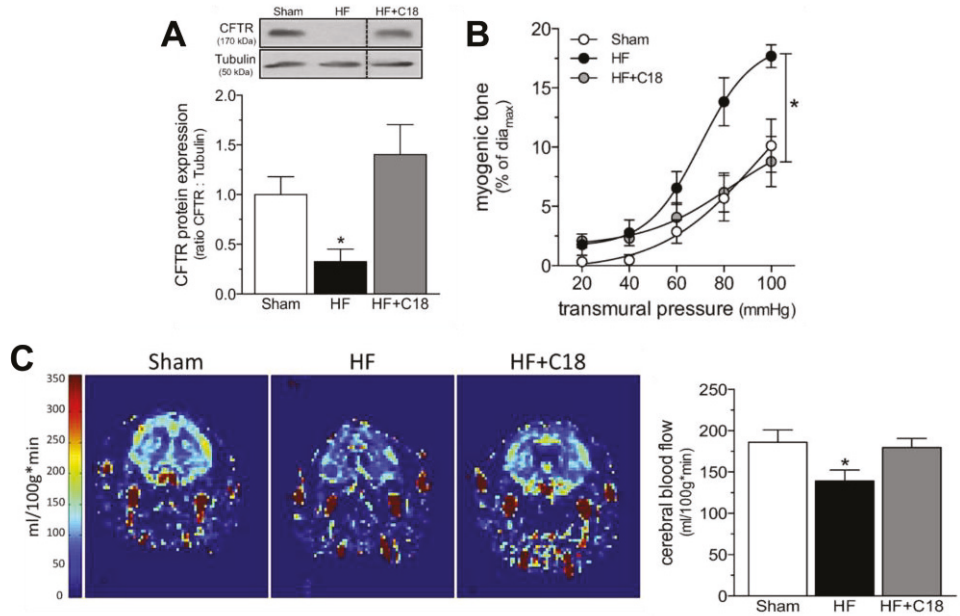

Figure 4: C18 treatment normalized (A) reduced CFTR expression, (B) increased myogenic tone, and (C) cerebral perfusion deficits in HF. (A) Cerebral arteries that were isolated from HF mice (6 weeks post-myocardial infarction) had reduced CFTR protein expression compared to arteries isolated from sham-operated controls. C18 treatment *in vivo* (3 mg/kg intraperitoneally daily for 2 days) eliminated this reduction of CFTR protein expression in cerebral artery. **(B)** C18 therapy *in vivo* reduced myogenic tone in proximal cerebral arteries from HF mice. **(C)** Representative magnetic resonance perfusion maps used to determine forebrain cortical cerebral blood flow. HF led to a reduction in cerebral perfusion, and C18 treatment significantly improved cerebral perfusion in mice with HF. For **(A, C)**, *p≤ 0.05 for unpaired comparisons to sham; for **(B)**, *p≤ 0.05 for unpaired comparisons to HF. *Adapted from Lidington, Fares et al.*[94]

Consistent with previous observations in this model[29], changes in the macro- and microstructures of cortical neurons were observed. An apparent neuronal atrophy was found during HF (Figure 5A-C), which was absent after C18 administration (Figure 5A-C). Similarly, HF was characterized by a reduced dendritic spine density, a phenotype that was no longer observed after treating HF mice with C18. Spines are small, specialized protrusions on the dendrites that play a role in synaptic plasticity and memory processes[95, 96]. A decrease of those structures has been associated with several neurodegenerative diseases, including AD and Parkinson's disease[97-99]. Similar to classical neurodegenerative disorders[97], alterations of dendritic arborization and spine density were accompanied by impaired short-term memory function (Figure 5D). As memory function was the only parameter tested longitudinally, the actual sequence of mechanisms responsible for the observed improvements remains to be determined.

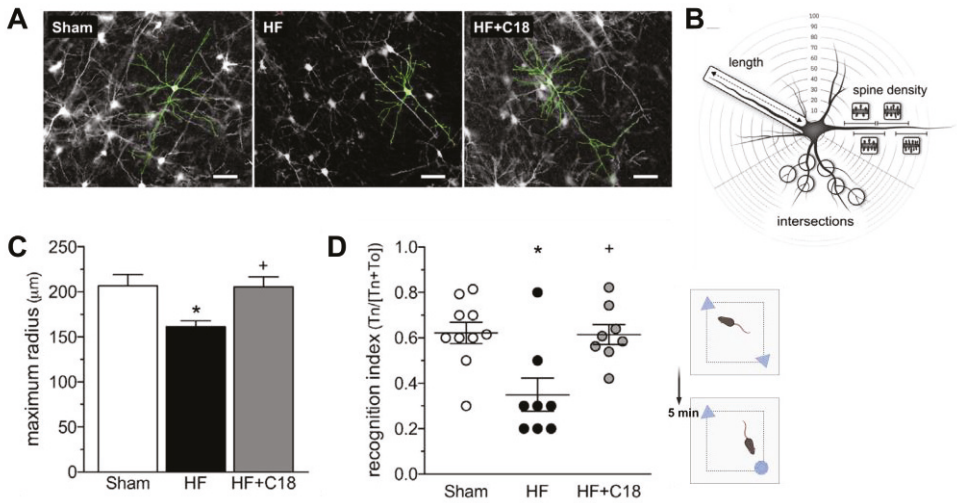

Figure 5: C18 treatment improved neuronal morphology and memory function during HF **(A)** Representative images of Golgi-Cox-stained pyramidal cortical neurons from sham mice, HF mice, and C18-treated HF mice (3 mg/kg intraperitoneally daily for 2 weeks; treatment initiated at 10 weeks post-infarction). The morphology of individual cortical neurons is highlighted with traces superimposed onto the images. **(B)** The images displayed in **(A)** were quantitatively assessed by Sholl analysis at 5µm intervals. **(C)** Sholl analysis revealed a significantly reduced mean dendrite length (i.e., maximum radius) in HF mice compared to sham mice, an effect that was normalized by C18 treatment. **(D)** Relative to sham mice, HF affected rhino-cortical−dependent, short-term memory retention of object familiarity in a nonspatial novel object recognition task, with a 5-min delay interval; the impairment was not present in C18-treated HF mice. *p≤ 0.05 for unpaired comparisons to sham and +p≤ 0.05 for unpaired comparisons between HF and HF+C18 group. Bar over micrographs is 100 µm in Panel A. *Adapted from Lidington, Fares et al.*[94]

Systemic CFTR Modulator Administration Restored Hippocampal Memory Dysfunction during HF (Paper II)

Consistent with data on the cerebral cortex[29, 94], pyramidal hippocampal neurons also presented with neuronal atrophy and reduced dendritic spine density. Functionally, long-term memory was impaired in HF mice. Interestingly, these structural and functional alterations were accompanied by a reduced neuronal CFTR expression in the hippocampal CA1 and dentate gyrus (DG) regions (Figure 6A).

In addition, HF has been linked to neuroinflammation[25, 29, 30], which was corroborated by apparent changes of microglial morphology (Figure 6B) and microglia-specific elevation of pro-inflammatory cytokine expression. *In vitro*, the direct effects of microglia-derived cytokines on neuronal cell alterations, including neuronal CFTR and synaptic marker expression were verified. Conditioned medium of lipopolysaccharide (LPS)- stimulated (100 ng/ml LPS; 48 h) BV2 microglial cells (LCM) resulted in significant downregulation of CFTR in N2a neuronal cells when

compared to N2a cells treated with supernatant of non-stimulated BV2 cells (CM, Figure 6C). As previously reported[100-103], LPS stimulates the release of several cytokines by microglial cells (Supplement Paper II[88]), many of which have been implicated in neurodegeneration[104-108]. For example, IL-8 and IL-1β have been shown to have direct neurotoxic effects[109, 110] and IL-33 has been associated with long-term memory deficits[111]. The LCM-mediated CFTR reduction on N2a cells (Figure 6C) suggests a direct contribution of microglial cytokines to neuron-specific CFTR downregulation. Indeed, CFTR's importance for neuronal integrity was verified by showing reduced postsynaptic density protein 95 (PSD-95) expression after LCM incubation (Figure 6D), which was normalized by CFTR correction (Figure 6C-D). Although the precise role of CFTR in neuronal function is not well known, it has been implicated in hyperexcitability[112, 113]. A previously reported, direct interaction of CFTR with PSD-95 in the context of synaptic integrity[114] suggests an important role of CFTR for general neuronal health[115].

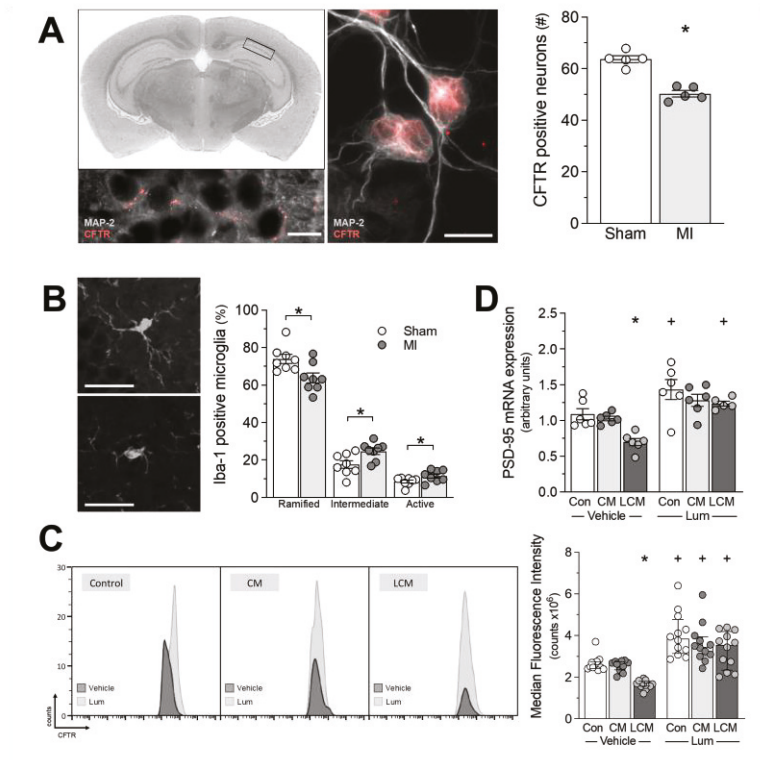

Figure 6: Microglial activation affected neuronal CFTR expression, which was rescued by Lumacaftor. (A) Hippocampal neurons express CFTR; the top-left image shows a coronal brain slice with a hippocampal region marked for immunostaining analysis. The bottom left image shows a representative co-immunostaining for CFTR and microtubule-associated protein 2 (MAP-2; neuronal marker) in cornu ammonis (CA1) hippocampal neurons within a brain slice. The image on the right displays CFTR and MAP-2 co-immunostaining in cultured primary neurons. HF reduced the number CFTR-stained hippocampal neurons. **(B)** Representative images of hippocampal CA1 microglia stained

for ionized calcium-binding adapter molecule-1 (Iba-1) and classified as ramified (top), intermediate (bottom), or active (not shown) based on their morphology in CA1 and dentate gyrus (DG) hippocampus regions. The proportion of ramified microglia was decreased and the proportions of intermediate and active microglia were increased during HF. **(C)** Representative flow cytometry histograms display median fluorescence intensity (MFI) for CFTR cell surface expression in vehicle and lumacaftor-treated N2a neuronal cells. BV2 microglial cells were treated with vehicle or lipopolysaccharide (LPS, 100 ng/ml)) for 48 h; the resulting supernatants were titled conditioned medium (CM) or LPS-conditioned medium (LCM). N2a neuronal cells were then incubated with aforementioned media for 48 h. LCM, but not CM, reduced CFTR MFI and **(D)** PSD-95 mRNA expression, while lumacaftor treatment reversed these LCM-mediated reductions. For **(A-B)** $*p \leq 0.05$ for unpaired comparisons to sham; for **(C-D)** $*p \leq 0.05$ for unpaired comparisons to the respective vehicle- or lumacaftor-treated control; $+p \leq 0.05$ comparing the effect of lumacaftor versus vehicle for Con, CM, and LCM treatment groups. Bar over micrographs is 10 µm in Panel A. *Adapted from Vanherle et al.*[88]

In vivo, the efficiency of two different CFTR modulator treatments to mitigate neuronal CFTR downregulation, structural alterations, and memory deficits was tested. Currently, CFTR modulator therapies are solely approved for the treatment of CF, which is characterized by a genetic CFTR defect. Although most research focuses on CF's pulmonary phenotype, a recent study described that CF patients harbor visual-spatial and executive impairments during cognitive assessments and display subtle brain alterations[116]. Therefore, it is important to investigate the therapeutic potential of the current medications on those disease manifestations. As HF is characterized by a CFTR downregulation at the cell surface, therapy with a CFTR corrector could suffice to treat target organ damage during HF. However, since neither lumacaftor nor other CFTR corrector therapies are currently available as a monotherapy, a combination of CFTR corrector lumacaftor and CFTR potentiator ivacaftor, which is clinically approved and marketed as Orkambi®, was also utilized.

Treatment (lumacaftor or lumacaftor/ivacaftor) was administered daily at 10 weeks post-surgery for two consecutive weeks. Treated HF mice presented with increased neuronal CFTR expression, improved neuronal integrity, and ameliorated memory function compared to their vehicle treated controls. The brain phenotype was found to worsen with time post-MI as evidenced by a lower number of intersections across dendrites in pyramidal CA1 neurons at 24 weeks post-surgery compared to the 12-week timepoint (# intersections at 50µm: $sham_{24wk}$=13±1, HF_{24wk}=8±1; p=0.005 (data from Paper II, Fig. 6C[88]) vs. $sham_{12wk}$= 14±2, HF_{12wk}= 11±2; p=0.317 (data from Paper II, Fig. 1B[88]), Figure 7A). Therefore, CFTR modulator efficacy with respect to a longer time window after surgery was tested. Lumacaftor/ivacaftor combination treatment initiated at 22 weeks post-MI attenuated the HF-induced reduction in dendrite intersections (Figure 7A), dendrite atrophy (Figure 7B), and reduced dendritic spine density (Figure 7C). Functionally, this amelioration resulted in improved memory capacity (Figure 7D).

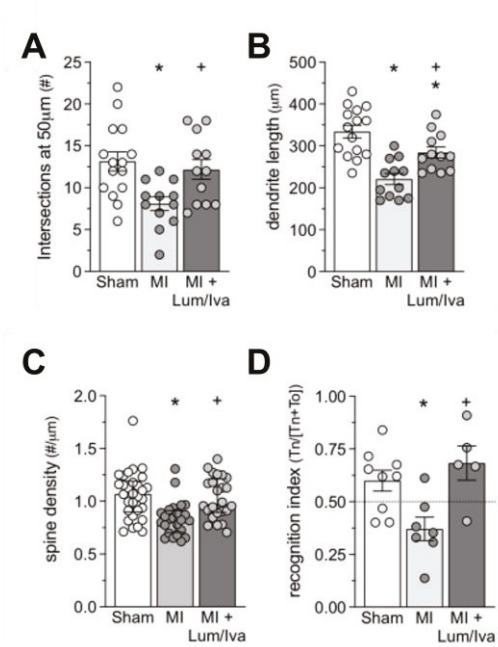

Figure 7: CFTR therapeutics normalized neuronal morphology and memory function following extended treatment delay. (A) HF reduced the number of intersections measured at 50 µm from the soma; lumacaftor/ivacaftor (Lum/Iva) treatment attenuated this reduction. **(B)** Mean dendrite length was reduced in HF mice relative to sham mice; Lum/Iva treatment diminished this reduction. **(C)** Lum/Iva attenuated HF-induced dendrite spine density reduction in pyramidal CA1 neurons of the hippocampus. **(D)** HF impaired hippocampal-dependent retention of object familiarity in a non-spatial novel object recognition task; Lum/Iva treatment reversed this deficit. The dotted line (recognition index= 0.5) represents the level where either object was explored for identical times and thus, mice do not discriminate between novel and alrady seen object. ∗p≤ 0.05 for unpaired comparisons to sham; +p≤ 0.05 for unpaired comparisons between HF and Lum/Iva. *Adapted from Vanherle et al.*[88].

HF was Associated with Lung Tissue Inflammation, which was Attenuated after Restoration of CFTR Expression (Paper III)

At twelve weeks post-surgery, HF mice presented with marked inflammation in the lung as evidenced by increased numbers of monocytes/macrophages (MOMA) verified by the percentage of MOMA-positive cells around the vasculature (Figure 8A). A more specific flowcytometric approach, investigating the inflammatory profile of F4/80^{+} macrophages, revealed significantly higher cell numbers of classically activated CD80^{+} non-alveolar macrophages in the HF lung (Figure 8B). Interestingly, this specific immune cell subset showed a marked reduction of CFTR expression in HF mice compared to naïve mice (Figure 8C). In contrast, CD80 and

CFTR positivity on alveolar macrophages were not affected by HF (Figure 8D-E), suggesting an association between CFTR expression and polarization of non-alveolar macrophages. Studies have shown that CFTR is a critical regulator of inflammatory phenotypes of different myeloid derived cells[117-120]. Additionally, pharmacological inhibition of CFTR increased pro-inflammatory cytokine production in macrophages[121].

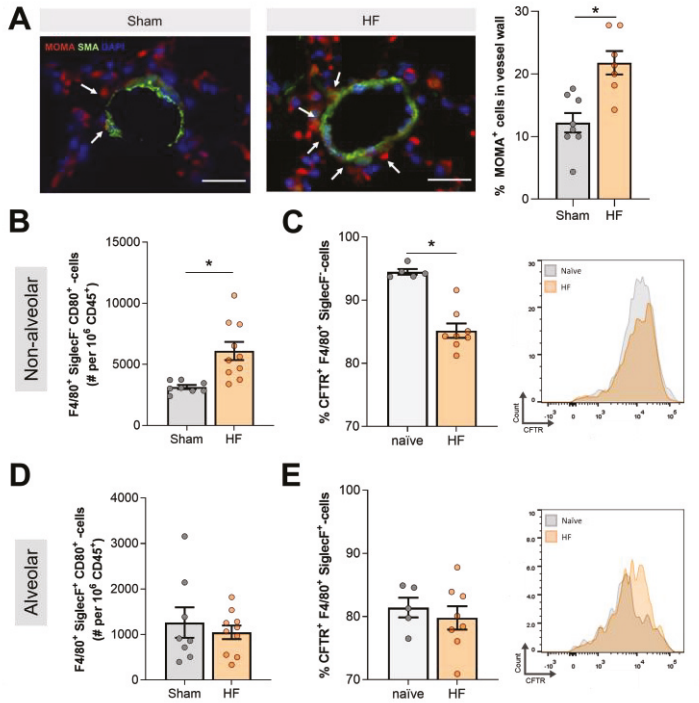

Figure 8: HF associated with pulmonary inflammation and reduced CFTR expression. (A) Representative images of lung sections from sham and HF mice, stained for monocyte/macrophages (MOMA, red), smooth muscle actin (SMA, green), DAPI stained nuclei (blue). Arrows indicate vessel wall-associated MOMA positivity. Quantification of the percentage of MOMA-positive cells in lung vessel wall reveals an increase in the lung of HF mice, compared to their sham controls. HF led to **(B)** an upregulation of F4/80$^+$ SiglecF$^-$ CD80$^+$ classically-activated non-alveolar macrophages in HF mice compared to sham, associated with **(C)** a significant reduction in CFTR expression compared to naïve mice. In contrast, **(D)** the number of F4/80$^+$ SiglecF$^+$ CD80$^+$ classically-activated alveolar macrophages in lung tissue between sham and HF mice remained unchanged, accompanied by **(E)** unaltered CFTR levels between naïve and HF mice. *p≤ 0.05 for unpaired comparisons to sham. Bar over micrographs is 20 µm in Panel A. *Adapted from Uhl et al.*[87].

To test the efficacy of CFTR targeting therapy, HF mice were subjected to systemic (i.p.) or lung-specific (o.t.) treatment using the CFTR corrector lumacaftor. By increasing cell surface CFTR expression, lumacaftor was able to reduce the proportion of infiltrating CD80$^+$ non-alveolar macrophages (Figure 9A). The proportion of alveolar CD80$^+$ macrophages was unaffected by HF or lumacaftor

treatment (Figure 9B). Importantly, lumacaftor increased the percentage of CD206-expressing non-alveolar macrophages (Figure 9C), while statistical significance for alveolar macrophages was not reached ($P_{i.p.}$= 0.22, $P_{o.t.}$= 0.08; Figure 9D). Our results corroborated findings that observed an alternative activation of human monocytes from CF patients after CFTR correction as evidenced by increased IL-10 secretion[122].

In order to reduce potential side effects that could be linked to systemic modulation of CFTR, a lung-specific administration of lumacaftor was assessed. Despite this local application of the drug, no additional beneficial effects were observed when compared to systemic application. In turn, o.t. drug administration led to increased alveolar macrophages numbers (P= 0.007) as well as an increased CD80-positivity of alveolar macrophages ($P_{o.t.}$= 0.06). These observations seem to limit long-term benefits of local CFTR modulator administration. While standard therapies treating lung or heart disease are often complicated by contraindications, CFTR is downregulated in both the heart and the lung[31]. Therefore, restoring CFTR expression is less likely to be a contraindication neither for the heart nor the lung. As recent studies described an acquired CFTR dysfunction in other diseases (e.g., COPD, asthma[61-63]), the possibility of repurposing CF medication could be of great interest to the lung field.

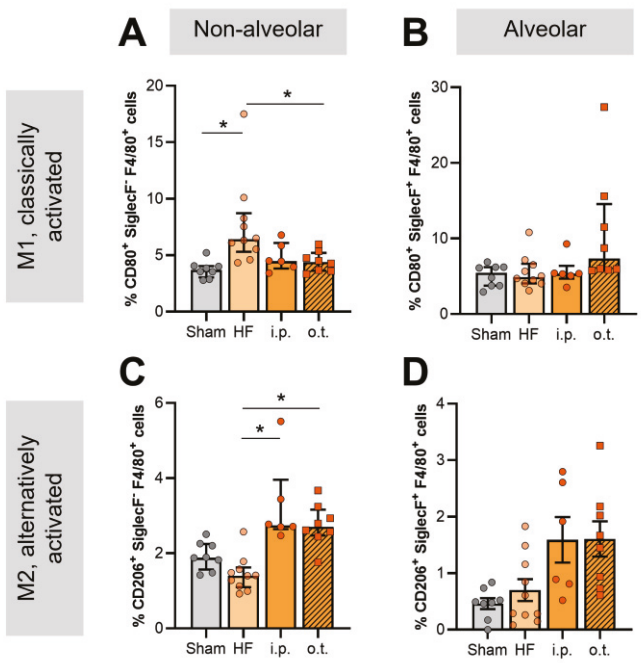

Figure 9: CFTR correction normalized levels of non-alveolar macrophages and increased CD206$^+$ alveolar macrophages. Proportion of pulmonary **(A)** F4/80$^+$ SiglecF$^-$ CD80$^+$ and **(B)** F4/80$^+$-SiglecF$^+$ CD80$^+$ macrophages in sham, heart failure (HF), and Lumacaftor (Lum) treated ((intraperitoneally (i.p.)

or orotracheally (o.t.)). HF increased the proportion of F4/80$^+$ SiglecF$^-$ CD80$^+$ non-alveolar macrophages; local lumacaftor administration attenuated this increase. Percentage of pulmonary **(C)** F4/80$^+$ SiglecF$^-$ CD206$^+$ and **(D)** F4/80$^+$-SiglecF$^+$ CD206$^+$ macrophages in sham, HF, and Lum treated (i.p. and o.t.). Systemic (i.p.) and local (o.t.) lumacaftor administration increased the proportions of F4/80$^+$ SiglecF$^-$ CD206$^+$ cells. *p≤ 0.05 for unpaired comparisons to HF. *Adapted from Uhl et al.[87].*

Systemic Administration of CFTR Potentiator during HF Evoked Contrasting Effects on Brain and Lung (Paper IV)

The CFTR potentiator ivacaftor is the only monotherapy approved for CF patients and is currently in clinical trials for several diseases that present with an acquired CFTR downregulation (e.g., COPD, chronic bronchitis)[61, 62]. Due to the high cost of CFTR modulator drugs[123], increasing demand for CFTR modulators by investigating repurposing potential might lower production cost. This study aimed at testing whether ivacaftor mitigates HF-mediated complications in brain and lung to the same extent as CFTR corrector therapies described above. Ivacaftor therapy alleviated HF-induced neuronal atrophy (Figure 10A), reduced dendritic spine density on CA1 pyramidal neurons (Figure 10B), and ameliorated long-term memory dysfunction (Figure 10C). Although ivacaftor increased dendritic spine density, a direct comparison with lumacaftor revealed a lower drug-to-vehicle ratio after ivacaftor treatment, suggesting that lumacaftor treatment might be more effective at attenuating brain-related alterations during HF.

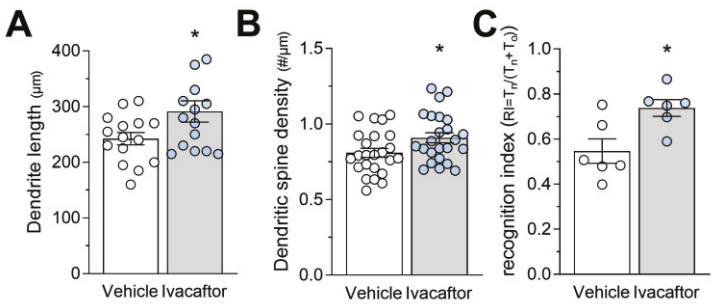

Figure 10: Ivacaftor was protective for HF-induced neurodegeneration and cognitive impairment. HF-mediated **(A)** atrophy of mean dendrite length of pyramidal CA1 neurons, **(B)** reduction of pyramidal CA1 neuron dendrite spine density; and **(C)** hippocampus-dependent memory impairment were ameliorated by ivacaftor administration. *p≤ 0.05 for unpaired comparisons. *Adapted from Vanherle et al.[124].*

In contrast to ivacaftor's positive effect on the brain, an apparent systemic inflammation was observed after ivacaftor treatment, which was absent in lumacaftor-treated HF mice. More specifically, ivacaftor led to an increase of innate

immune cells. This contrasts with lumacaftor effects, which did not alter immune cell numbers compared to vehicle-treated HF mice. Interestingly, the percentage of CFTR-positive circulating adaptive immune cells was markedly lower in comparison to CFTR-positive innate immune cells[87], suggesting a distinct role of CFTR on cells of the innate immune system. Previously published data suggested a connection between reduced CFTR expression and pro-inflammatory immune cell profiles of macrophages in CF and non-CF settings[87, 125, 126]. Human CF monocytes have been shown to be more prone to apoptosis[127], while macrophages from CFTR knockout mice directly contributed to proinflammatory responses[125]. In CF, contradictory results on the use of ivacaftor have complicated clear conclusions. In non-CF settings (e.g., in HF or COPD), the effect of ivacaftor on inflammation is severely understudied. Initial evaluations of ivacaftor in COPD patients with chronic bronchitis focused on lung function and yielded promising, yet non-significant improvements[61]. It would be of great value to investigate ivacaftor's effect on circulating and tissue-specific immune cells during COPD. There is currently only one longitudinal study that assessed systemic inflammation in response to ivacaftor where increases in several inflammatory markers were found[128]. Whether those increased inflammatory markers were a direct effect of ivacaftor or rather a consequence of indirect drug effects is yet to be verified.

Augmenting CFTR cell surface expression by lumacaftor treatment led to an alternative macrophage activation (i.e., increased CD206-positivity) specifically in non-alveolar pulmonary macrophages[87]. The lacking increase of cell surface CFTR expression after ivacaftor treatment may explain increased frequencies of CD80-positive cells observed after ivacaftor treatment.

Contrary to the findings in the systemic circulation and the lung, ivacaftor attenuated HF-related microglial activation as evidenced by increased percentages of ramified microglia which generally indicates microglial cell activation (Paper IV, Fig. 2[124]). As resident macrophages of the brain, microglia belong to the same lineage as circulating monocytes/macrophages and alveolar macrophages. Therefore, ivacaftor's effect on different types of macrophages was tested *in vitro*, using murine macrophages, human monocyte-derived macrophages, and murine microglial cells. LPS treatment induced macrophage activation evidenced by increased CD80 expression (Figure 11 A-C; the dotted line indicates expression levels of control non-LPS treated cells), which was exacerbated by ivacaftor in both human monocyte-derived macrophages (Figure 11A) and murine macrophages (Figure 11B) but not in microglial cells (Figure 11C). While ivacaftor has shown some anti-inflammatory potential in CF[127], the effect of ivacaftor on non-CF cells is understudied. More research to confirm ivacaftor's effects on inflammation in non-CF disease models and clinical trials is warranted.

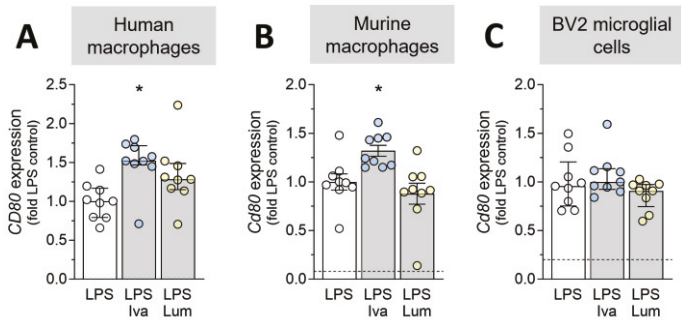

Figure 11: Ivacaftor exacerbated LPS-induced inflammation in human and murine macrophages, but not microglia *in vitro.* Lipopolysaccharide (LPS; 100 ng/ml, for 24 h) augmented CD80 mRNA expression in differentiated human monocyte-derived macrophages (THP-1 cells), murine RAW264.7, and BV2 microglial cells. Dotted lines in the graphs indicate control levels ($Ctrl_{(A)}$=0.0033, $Ctrl_{(B)}$=0.08, $Ctrl_{(C)}$=0.2). Simultaneous application of ivacaftor (Iva, 10 µM), but not lumacaftor (Lum, 10 µM) augmented the LPS response in **(A)** human monocyte-derived macrophages and **(B)** murine RAW264.7 cells. **(C)** Neither simultaneous ivacaftor (Iva, 10 µM) nor lumacaftor treatment (Lum, 10 µM) increased the LPS response in murine BV2 microglial cells. *denotes p≤ 0.05 for unpaired comparisons. *Adapted from Vanherle et al.*[124]

Concluding Remarks and Future Perspectives

Data generated as part of this thesis demonstrates a critical role of CFTR expression during tissue injury in brain and lung associated with HF progression. Downregulation of CFTR in the cerebral vasculature and neuronal cells had implications for myogenic tone, cerebral perfusion, neuronal structure, and memory function. HF was associated with reduced CFTR expression on infiltrating immune cells co-occurring with unfavorable inflammation in the lung. Therapeutic administration of CFTR correctors attenuated tissue damage in brain and lung of HF mice. Specifically, therapeutic CFTR correction restored cerebral CFTR expression, increased cerebral blood flow, improved memory function, and reduced pulmonary inflammation. Interestingly, while increasing CFTR expression had generally positive effects, increasing open probability of CFTR showed opposing results dependent on the target organ.

CFTR is widely expressed in the body and its expression levels differ between organs and different cell types. During HF, the downregulation of CFTR in brain, heart, and lung has previously been shown[31]. Whether CFTR is downregulated in other organs during HF is yet to be determined. In case HF leads to a general CFTR downregulation, systemic CFTR therapy would likely benefit multiple organ systems. If HF does not affect CFTR in other organs with high CFTR expression such as the pancreas, intestine, and kidney, additional research will be needed to assess the consequences of unwanted, increased CFTR expression in those organs with respect to their function and hence, how this may affect HF outcome. However, since CFTR modulators have passed clinical trials, including testing on healthy volunteers, the probability of serious consequences seems small.

Because the lung is often affected by genetic or acquired CFTR dysfunction in a variety of conditions, including CF, COPD, asthma, chronic bronchitis[61-63], as well as HF[31], lung-specific administration is a viable route of CFTR modulator application to mitigate CFTR-related alterations tissue-specifically. Recently, the possibility of pulmonary administration of hydrophobic drugs in the form of spray dried ivacaftor has been investigated[129]. In this thesis, o.t. administration of CFTR modulators increased cell numbers of non-alveolar and alveolar macrophage subsets, including classically activated CD80-positive cells ($p=0.06$[87]). These

findings may limit a long-term o.t. administration of CFTR modulators during HF especially when considering the beneficial macrophage polarization observed after systemic drug administration, which was not associated with potentially adverse proinflammatory macrophage phenotypes.

A few studies have reported positive effects of CFTR modulators with respect to airway inflammation in CF patients[130-132], however, results are inconsistent and study populations tested were generally small[133]. Additionally, the need for supplementary antimicrobial and anti-inflammatory treatments in order to avoid pulmonary exacerbations and dampen airway inflammation during CF has become clear[134-136]. Current studies in CF patients as well as patients with an acquired CFTR dysfunction often focus on mucus clearing capacity and lung function, while the understanding of molecular mechanisms that may contribute to inflammation or its exacerbation is still lagging[61, 132, 137-140].

Due to the relatively small population benefiting from CFTR modulators, the production costs are extremely high. As some of the current therapeutics have shown promising results regarding the attenuation of acquired CFTR dysfunctions, exploring the repurposing potential of the drugs for diseases other than CF could be valuable. Since not only HF, but also COPD, asthma, and chronic bronchitis present with acquired CFTR dysfunction[61-63], clinical trials in those patient populations are urgently needed. When CFTR therapeutics can benefit more people, demand increases, which would ultimately reduce production costs and make the drugs more accessible.

Taken together, CFTR therapeutics show great potential for the treatment of HF-associated target organ damage. Future clinical trials should target the efficacy of CFTR modulation in a patient context.

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