	Serum Iron mean +/- SD			Hgb mean +/- SD			TSAT mean +/- SD		
	Pre-ETI	Post-ETI	P-value	Pre-ETI	Post-ETI	P-value	Pre-ETI	Post-ETI	P-value
Total	46.9+/-23	64.6+/-35	0.040*	12.1+/-1.6	13.3+/-1.3	<0.001*	13.1+/-5.9	22.6+/-21	0.055
No prior modulator	35.7+/-20	81+/-44	0.003*	11.9+/-1.9	13.5+/-1.4	0.005*	11.9+/-4.5	21.2+/-13	0.044*
dF508-homozygous previously on modulator	52.5+/-18	56.6+/-26	0.608	12.3+/-1.4	13.3+/-1.2	0.009*	13.5+/-6.6	25.6+/-28	0.204
Previously on a highly effective modulator	63.5+/-52	39+/-16	0.697	12.2+/-1.1	12.1+/-1.7	0.969	16+/-9.9	12.2+/-8.8	0.824

*p value < 0.05. Hgb = hemoglobin in g/dL; TSAT = transferrin saturation %; ETI = elexacaftor-tezacaftor-ivacaftor; SD = standard deviation.

Table 1: (abstract: 554): Change in iron hemostasis before and after elexacaftor/tezacaftor/ivacaftor.

Conclusion: Iron deficiency is common in patients with CF. There appears to be an increase in mean Hgb after starting elexacaftor/tezacaftor/ivacaftor that is statistically and clinically significant. Future considerations would be to include ferritin in the study and to analyze changes in Hgb of our additional 200-plus patients on elexacaftor/tezacaftor/ivacaftor who did not have iron studies. We also plan to perform a multivariate analysis adjusting the estimated effect of elexacaftor/tezacaftor/ivacaftor for BMI, sex, and age. Limitations of our study include small sample size because of few patients having iron studies. A larger study will probably need multicenter data.

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Clinical trial interest after establishment of modulator therapy: Interim CHEC-SC survey results

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Background: Despite remarkable clinical benefits of the CFTR modulators ivacaftor and elexacaftor/tezacaftor/ivacaftor, additional effective CF therapies are needed. Although approximately 30% of people followed in the CF Patient Registry have enrolled in research studies in the past 3 years, robust elexacaftor/tezacaftor/ivacaftor-associated benefits may affect interest in future trial participation, particularly if designs require halting standard-of-care therapies for extended periods. We assessed the willingness of people receiving elexacaftor/tezacaftor/ivacaftor to enroll in future CF interventional trials and describe how trial design factors and participant characteristics affect interest.
Methods: The Characterizing CFTR Modulated Changes in Sweat Chloride (CHEC-SC) study is an observational study of sweat chloride (SC) response to CFTR modulators, assessing SC and long-term outcome associations. Eligible CHEC-SC participants taking elexacaftor/tezacaftor/ivacaftor for 3 months or longer completed a survey of willingness to enroll in modulator trials of differing durations, including those in which subjects would be required to stop elexacaftor/tezacaftor/ivacaftor and potentially be randomized to placebo. Participants with self-reported use of inhaled antimicrobials (inhABX) were also asked about enrolling in placebo-controlled inhABX trials. Factors possibly influencing willingness (age, sex, lung disease stage, prior trial enrollment, elexacaftor/tezacaftor/ivacaftor SC response) were evaluated and tested using chi-square and logistic regression.
Results: Responses from 608 subjects (51% female) were collected through March. 2021; not all surveys were complete. Most responses (74%) came from people with CF, the rest from caregivers; 47% had previously enrolled in a CF interventional trial. The group had a mean age of 22 ± 12 years, ppFEV$_1$ of 88 ± 25, and an elexacaftor/tezacaftor/ivacaftor-associated SC change of −51 ± 27 mM. Willingness to participate in a new modulator study requiring a placebo was 75% for a 1-month trial (95% CI, 71–78%) and 55% (95% CI, 51–59%) for a 6-month, with slightly higher rates of 79% (95% CI, 75–82%) and 64% (95% CI, 60–68%), respectively for trials versus an active comparator. Past trial experience was strongly associated with enrollment in longer duration trials (P = 0.02). Willingness to participate in placebo-controlled an inhABX trial was similar; 80% (95% CI, 74–85%) would enroll in a 1-month trial and 61% (95% CI, 54–68%) in a 6-month trial.
Conclusion: Interest in short-duration placebo-controlled CF studies remains good among a cohort engaged in an ongoing observational study and with significant prior trial experience, despite availability of elexacaftor/tezacaftor/ivacaftor for this cohort. These data suggest that future CF drug development programs will need to challenge the traditional regulatory framework of extended placebo-controlled studies to establish efficacy, particularly in the setting of new modulator therapies for which there are safety and ethical concerns regarding prolonged withdrawal of elexacaftor/tezacaftor/ivacaftor.
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Development of molecular imaging tools to monitor drug efficacy through assessment of CFTR localization in vivo

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Background: Molecular imaging allows noninvasive visualization of a target molecule in vivo; positron emission tomography (PET) and single photon emission computed tomography (SPECT) are the most sensitive imaging modalities currently available. In the scope of CF, several PET and SPECT studies have been performed, mainly to assess lung inflammation and clearance and aerosol delivery, proving their applicability to CF patients. We aim to establish an innovative molecular imaging approach to CF. This challenging objective will be accomplished through the development, followed by in vitro and in vivo biological evaluation, of noninvasive molecular imaging probes for plasma membrane CFTR. Within our goal of bringing forward new imaging biomarkers for CF, we previously developed a noninvasive small molecule–based radioprobe targeting plasma membrane CFTR through radio labeling of a CFTR inhibitor with ^{99m}Tc. In vitro assessment of the probe's binding to wild-type versus mutant CFTR showed promising results. This is the first proof-of-principle validation of a CF molecular imaging biomarker [1]. More recently, we expanded these studies to further improve the ability to target CFTR with antibody-based probes.
Methods: We report the ongoing development of an antibody-based probe for plasma membrane CFTR through the isolation of CFTR-specific antibody fragments selected by phage display technology and subsequent labeling with a useful radionuclide.
Results: A human naïve phagemid library of single-chain variable fragments (scFvs) was panned for the isolation of plasma membrane CFTR-binding clones. First the library was screened for scFvs against a small peptide, and in a second approach, selection was conducted in the presence of a functional antigen. In both approaches, 3 rounds of phage binding to antigen, washing, elution, and reamplification of phage binders were performed. After expression and purification of positive clones, validation

was performed through a flow cytometry study, but scFvs were not able to detect CFTR efficiently at the cell surface. We then explored an alternative for selecting improved CFTR-specific antibody fragments through development of an immune library. Rabbits were successfully immunized with a CFTR epitope, and we are producing the antibody fragment library. Cell-based pannings will be repeated, and the resulting clones will be expressed and validated. Finally, the scFvs will be radiolabeled with technetium-99 m and their ability to detect CFTR at the cell surface assessed in human bronchial epithelial cells.

Conclusion: These noninvasive molecular imaging probes have the potential to be a useful imaging biomarker in the assessment of early therapy response in drug evaluation.

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Antisense oligonucleotides modulate nonsense-mediated decay and translation termination pathways to restore expression and function of CFTR harboring nonsense mutations

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Background: Nearly 10% of the CF population harbors at least one allele with a CFTR nonsense mutation resulting in generation of a premature termination codon (PTC). PTCs can inactivate gene function due to truncated protein production by premature translation termination followed by rapid destruction of mRNA by the nonsense-mediated decay (NMD) pathway. As such, it is likely that effective therapeutic approaches require NMD pathway inhibition and translational readthrough promotion. We previously showed antisense oligonucleotides (ASOs) targeting NMD and translation termination machinery have therapeutic potential for diseases caused by nonsense mutations [1–3]. Here we aim to assess the effects of ASO-mediated reduction of core and branch-specific NMD factors on the upregulation of nonsense-mutated CFTR mRNA and function. We will also evaluate the therapeutic potential of combining ASOs targeting NMD and translation termination factors to produce functional CFTR protein. Insights into potential branch-specific regulation of CFTR mRNA NMD could provide an opportunity for safer therapeutic approaches due to the regulation of smaller subsets of endogenous mRNA substrates.

Methods: ASOs were developed to deplete NMD factors (SMG1, SMG5-9, UPF1-2, and UPF3A-B) and translation termination factor (TTF) eRF1 efficiently. The CFF-16HBEge cell model system harboring CFTR nonsense mutations was employed [4]. Cells were treated with ASOs targeting NMD factors alone or in combination with TTF eRF1, with or without aminoglycoside readthrough agent G418. CFTR RNA, protein, and functional upregulation was measured. Ribosomal profiling was employed to evaluate the effects of translational readthrough agents on global translation termination.

Results: ASOs targeting core NMD factors SMG1 or branch-specific NMD factor SMG6 stabilized all evaluated CFTR mRNA nonsense variants and significantly enhanced G418-promoted efficacy, resulting in CFTR functional improvement. We also observed that the eRF1-ASO demonstrated synergistic effects with G418, resulting in increased channel activity of all evaluated CFTR nonsense variants. Finally, ASOs targeting branch-specific NMD factor SMG6 in combination with readthrough agents eRF1 ASO and G418 further upregulated CFTR function for each CFTR nonsense mutation.

Conclusion: Inhibition of NMD/PTC pathways by ASOs may be a valuable therapeutic approach for the rescue of CF disease phenotypes caused by CFTR nonsense mutations.

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Carbon monoxide–based therapy primes macrophages to express HO-1 and to resolve lung hyper-inflammation in cystic fibrosis

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Background: Dysregulated macrophage (MΦ) functions contribute to nonresolving airway hyperinflammation, which drives lung tissue damage in CF patients. Heme oxygenase-1 (HO-1), an inducible enzyme that catabolizes heme groups, is inefficiently induced in human and murine CF MΦs in response to infectious triggers. The heme catabolite carbon monoxide (CO) has strong antiinflammatory, antioxidant, and bactericidal properties and initiates a positive feedback loop by induction of HO-1 expression. The objective was to test the mechanism of action and the antiinflammatory therapeutic efficacy of a polyethylene-glycol-modified carboxyhemoglobin-based drug (PP-007) in preclinical CF mouse models. PP-007 is currently in phase I/II clinical trials for diseases associated with hypoxia and inflammation.

Methods: We used bone marrow–derived murine MΦs isolated from wild-type (WT), MyD88-KO, AKT1-KO and CF (B6.KOCFTR^{tm1UNC}) mice. MΦs were preconditioned for 6 hours with 2 mg/mL of PP-007 prior challenging with *Pseudomonas aeruginosa* lipopolysaccharide (LPS). To test PP-007's effects in vivo, CF (n = 30), βENaC-Tg (B6.Cg-Tg-Scgb1a1-Scnn1b-$^{Bouc/J}$, n = 10), and HO-1^{Cx3CR1} (n = 4) mice were pretreated intravenously with a single clinically relevant dose (320 mg/kg) of PP-007 or vehicle. Mice were nebulized daily with 12.5 mg of LPS for 3 days and sacrificed 6, 24, and 48 hours after the last dose. To assess the effect of PP-007 during chronic *P. aeruginosa* lung infection, 10^5 CFU of *P. aeruginosa* M57-15 embedded in agarose beads was instilled intratracheally in PP-007 or vehicle-treated CF mice. Lung inflammation was assessed by neutrophil numbers (flow cytometry) and proinflammatory cytokine concentrations (Luminex) in bronchoalveolar lavage fluid (BALF) samples. Lung bacterial load was assessed by CFU counting.

Results: PP-007 required the combined activation of MyD88 and PI3 K/AKT signaling pathways for optimal induction of HO-1. By rescuing HO-1, PP-007 decreased the expression of proinflammatory cytokines in LPS-treated CF MΦs. In vivo delivery of a single PP-007 dose led to high expression of HO-1 in circulating and lung-recruited monocytes. Treatment with PP-007 did not alter initial neutrophil migration to CF lungs in response to LPS but accelerated their clearance within 24 hours. PP-007-treated CF mice had lower proinflammatory cytokine concentrations (e.g., TNF-α, IL-6, IL-17, IL-12p70, IP-10) in BALF than vehicle-treated mice and were protected from excessive weight loss. The antiinflammatory properties of PP-007 were also recapitulated in the βENaC-Tg mice, which feature CF-like airway mucus obstruction. HO-1^{Cx3CR1} mice, in which HO-1 is specifically knocked out in monocytes and MΦs, have more BALF neutrophils than WT mice in response to LPS. PP-007 treatment fails to reduce lung hyperinflammation in HO-1^{Cx3CR1} mice. These data demonstrate that HO-1 induction in