A Phase 1b Randomized, Double-blind, Placebo-controlled, Dose Escalation Trial of CB-280, an Arginase Inhibitor, in Patients With Cystic Fibrosis

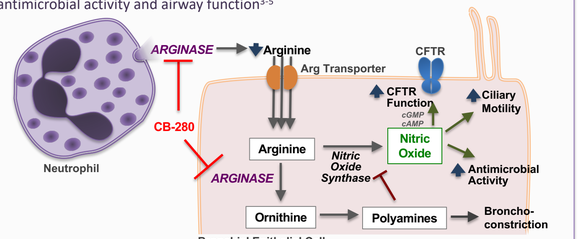
Steven Boas[1], Scott Donaldson[2], Kimberly McBennett[3], Theodore Liou[4], Judie Ann Howrylak[5], Larry G Johnson[6], Charlotte Teneback[7], Allen Dozor[8], Gregory Sawicki[9], Jocel Dumlao[10], Alison Pan[10], Lalith Akella[10], Jing Zhang[10], Susheela Carroll[10], Keith Orford[10], Emil Kuriakose[10], Joel Mermis[11]

[1]Cystic Fibrosis Institute, Glenview, IL; [2]University of North Carolina, Chapel Hill, NC; [3]University Hospital Cleveland Med Ctr, Cleveland, OH; [4]University of Utah Cystic Fibrosis Center, Salt Lake City, UT; [5]Department of Medicine, Penn State Milton S Hersey Medical Center, Hershey, PA; [6]University of Arkansas for Medical Sciences, Little Rock, AR; [7]University of Vermont Med Ctr, Burlington, VT; [8]NY Medical College, Valhalla, NY; [9]Boston Children's Hospital, Boston, MA; [10]Calithera Biosciences, South San Francisco, CA; [11]University of Kansas Medical Center, Kansas City, KS

BACKGROUND

- Deficient airway nitric oxide (NO) production is a known pathophysiologic feature of cystic fibrosis (CF), which leads to impaired host antimicrobial defense, chronic airway infection, and compromised pulmonary function (**Fig. 1**)[1,2]
- L-Arginine is required for NO production by nitric oxide synthase (NOS). Arginase, an abundant enzyme expressed and secreted into airways by neutrophils, depletes L-arginine, contributing to NO deficiency in CF[3,4]

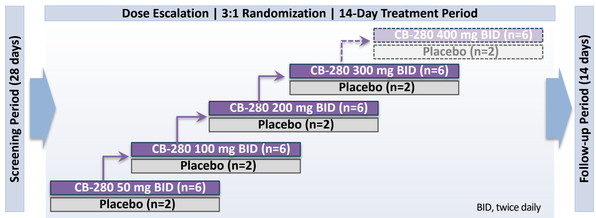
Figure 1. Arginase-dependent regulation of airway homeostasis. High arginase activity in CF reduces availability of arginine as a substrate for NOS, decreasing NO levels. Polyamines (eg, spermine) further decrease NO production by inhibiting NOS, impairing antimicrobial activity and airway function[3-5]



- **CB-280** is an oral, reversible, potent arginase inhibitor that causes sustained increases in systemic arginine levels, thereby promoting airway NO production
- CB-280 significantly improved lung function and reduced *P. aeruginosa* colony-forming units in CF mouse models[6]
- In CF bronchial epithelial cells expressing ΔF508-CFTR, arginase inhibition increased CFTR channel conductance *in vitro* in the presence or absence of CFTR modulators[5]
- While administration of inhaled L-arginine to patients with CF improves FeNO and trends towards improvement in FEV_1, effects are transient owing to arginine consumption by arginase in the airways[7]
- Sustained inhibition of arginase is hypothesized to restore endogenous airway NO production in a continuous fashion, leading to enhanced antimicrobial activity and improved airway function (**Fig. 1**)

METHODS

Figure 2. Study Schema: Sequential Dose Escalation (NCT04279769)



Eligibility

- Adults w/confirmed CF (positive sweat chloride ≥60 mEq/L and/or CFTR genotype w/ 2 CF-causing mutations)
- Percent predicted FEV_1 of 40%-90%
- Oxygen saturation >92%
- Chronic *P. aeruginosa* lung infection (≥1 positive culture in last 2 yrs and >50% of cultures positive thereafter)
- Stable CF medication for ≥28d, incl. CFTR modulators
- No concomitant lung infection w/organisms associated w/more rapid decline in pulmonary status
- No supplemental oxygen/inhaled NO within 28 days

Endpoints

Primary: Safety & tolerability
Secondary: Pharmacokinetics (PK)/pharmacodynamics
Exploratory: Clinical & biological indices of disease modulation based on changes in:

- Airway NO
- Sweat chloride
- Biomarkers of L-arginine/arginase/NO metabolism
- Sputum microbiology
- CF Questionnaire-Revised (CFQ-R) respiratory domain

CFTR, CF transmembrane conductance regulator; FeNO, fractional exhaled nitric oxide; FEV_1, forced expiratory volume in 1 sec

RESULTS

Patients

Table 1. Patient Demographics and Baseline Characteristics

Parameter	Pooled Placebo (n=6)	Cohort 1 50 mg (n=6)	Cohort 2 100 mg (n=6)	Cohort 3 200 mg (n=6)	All Patients (N=24)
Age, yr, median (range)	34.0 (27-51)	31.0 (23-37)	31.5 (22-50)	32.5 (28-44)	31.5 (22-51)
Gender, n (%)					
Male	1 (17)	2 (33)	2 (33)	3 (50)	8 (33)
Female	5 (83)	4 (67)	4 (67)	3 (50)	16 (67)
Race					
White	6 (100)	5 (83)	6 (100)	6 (100)	23 (96)
Asian	0	1 (17)	0	0	1 (4)
Genotype					
ΔF508 homozygous	5 (83)	3 (50)	3 (50)	4 (67)	15 (63)
ΔF508 heterozygous	1 (17)	1 (17)	2 (33)	1 (17)	5 (21)
Other	0	2 (33)	1 (17)	1 (17)	4 (17)
$ppFEV_1$, mean (SD)	51.6 (17.0)	57.2 (12.1)	66.2 (17.5)	54.2 (10.0)	57.3 (14.6)
$ppFEV_1$ category					
<40%*	3 (50)	0	1 (17)	0	4 (17)
40% to <70%	2 (33)	5 (83)	1 (17)	6 (100)	14 (58)
70% to 90%	1 (17)	1 (17)	4 (67)	0	6 (25)
CFTR modulator, n (%)	6 (100)	5 (83)	5 (83)	6 (100)	22 (91)

CFTR, cystic fibrosis transmembrane conductance regulator; $ppFEV_1$, percent predicted forced expiratory volume in 1 second; SD, standard deviation.
*Including patients whose ppFEV1 was >40% at screening but dropped to <40% before dosing on Day 1

Pharmacokinetics

- The mean exposures (AUC and C_{max}) to CB-280 increased roughly dose-proportionally after the first dose and at steady state from 50 mg to 200 mg (**Fig. 3**)
- Drug accumulation was observed after multiple BID dosing over 2 weeks, and the accumulation ratio ranged from 1.5 to 1.8 across cohorts
- Steady state C_{trough} exceeds IC_{90} for plasma arginase inhibition at doses of 100 mg and above, achieving continuous target coverage in plasma

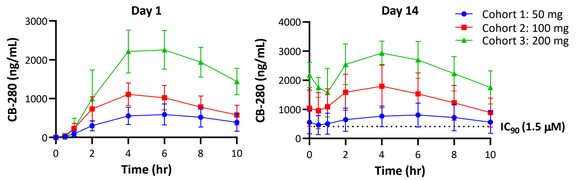


Figure 3. Plasma Pharmacokinetics. Mean plasma concentration of CB-280. Error bars = SD.

Pharmacodynamics

- Strong plasma arginase inhibition at steady state trough for all cohorts (80-99%) (**Fig. 4**)
- Significant and dose-proportional increase in plasma arginine on Days 8 and 14 (treatment period); trending down to baseline levels on Day 21 (follow-up period) (**Fig. 5**)

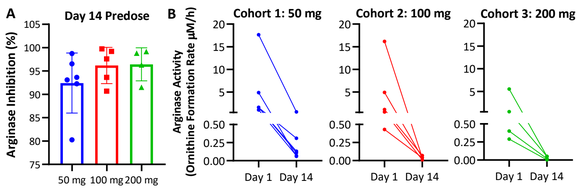


Figure 4. Plasma Pharmacodynamics. (A) Mean plasma arginase inhibition. Error bars = SD. (B) Individual changes in plasma arginase activity on Days 1 and 14. Plasma samples from patients (Days 1 and 14 predose) were incubated with L-arginine-$^{13}C_6$ as substrate and monitored for formation of L-ornithine-$^{13}C_5$. Data points for 2 patients (200 mg cohort) excluded due to unmeasurable ornithine-$^{13}C_5$ levels at all time points (< 0.1 µM).

Pharmacodynamics (cont.)

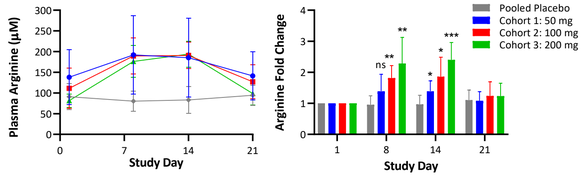


Figure 5. Plasma Pharmacodynamics – Arginine. Mean absolute plasma arginine levels (left) and fold changes (compared to Day 1 predose, right) in samples collected at predose (trough) on indicated study days. Error bars =SD. Paired t-test, $*P < 0.05$, $**P < 0.01$, $***P < 0.001$.

Safety

- All 24 patients completed the study with no patient prematurely discontinuing the treatment phase
- All 24 patients received at least 20 of the 28 planned doses required to be evaluable for dose-limiting toxicity (DLT) assessment
- TEAEs in 8 (44%) of patients on CB-280 vs. 2 (33%) on placebo (**Table 2**)
- No Grade ≥3 events, DLTs, serious TEAEs, or major adverse changes on lab assessments, ECG, or vital signs occurred in patients on CB-280
- No evidence of clinically significant urea cycle inhibition*

Table 2. Treatment-Emergent Adverse Events (TEAEs)

Adverse Event, n (%)	Pooled Placebo (n=6)	CB-280 Cohort 1 50 mg (n=6)	CB-280 Cohort 2 100 mg (n=6)	CB-280 Cohort 3 200 mg (n=6)	CB-280 Overall (N=18)
Any TEAEs	2 (33)	2 (33)	1 (17)	5 (83)	8 (44)
Any related TEAEs	0	1 (17)	1 (17)	3 (50)	5 (28)
Abdominal pain lower	0	0	0	1 (17)	1 (6)
Acne	0	0	1 (17)	0	1 (6)
ALT increased	1 (17)	0	0	1 (17)	1 (6)
AST increased	0	0	0	1 (17)	1 (6)
Blood ALP increased	1 (17)	0	0	1 (17)	1 (6)
Chest discomfort	0	0	0	1 (17)	1 (6)
Dizziness	0	1 (17)	0	0	1 (6)
Dry mouth	0	1 (17)	0	0	1 (6)
Fatigue	0	0	0	1 (17)	1 (6)
Hematuria	0	0	0	1 (17)	1 (6)
Hypertension	0	1 (17)	0	0	1 (6)
Mood altered	0	0	0	1 (17)	1 (6)
Musculoskeletal chest pain	0	0	0	1 (17)	1 (6)
Nasopharyngitis	0	0	0	1 (17)	1 (6)
Nausea	1 (17)	0	0	1 (17)	1 (6)
Pain	0	0	0	1 (17)	1 (6)
Proteinuria	0	0	0	1 (17)	1 (6)
Rash	0	0	0	1 (17)	1 (6)
Sinusitis	0	1 (17)	0	0	1 (6)
Sputum increased	1 (17)	1 (17)	0	0	1 (6)
Abdominal pain	1 (17)	0	0	0	0
Abdominal pain upper	1 (17)	0	0	0	0
Headache	1 (17)	0	0	0	0
Vomiting	1 (17)	0	0	0	0

ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; TEAE, treatment-emergent adverse event

*defined as plasma ammonia ≥ 2x upper limit of normal [ULN] AND ≥ 2x baseline with concomitant urinary orotic acid ≥10x ULN (fasting) or ≥ 40x ULN (fed state) OR fasting urinary orotic acid > 10x ULN on 2 measurements OR any urinary orotic acid > 40x ULN on 2 measurements

Spirometry

- No decline in FEV_1 (a safety endpoint) on treatment with CB-280
- Small positive trend in $ppFEV_1$ observed with pooled CB-280 vs. placebo (**Fig. 6**)

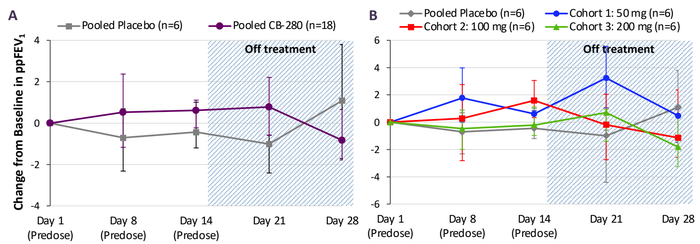
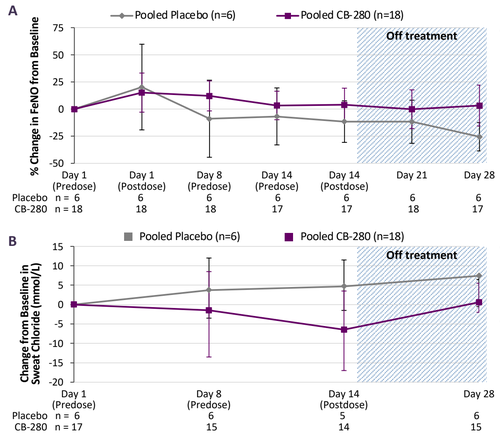


Figure 6. Mean changes in % predicted FEV_1. Pooled placebo vs. (A) pooled CB-280 cohorts or (B) CB-280 Cohorts 1, 2, 3. One sample missing from Cohort 2 on Day 14. Error bars = interquartile range (IQR).

Biomarkers



- Slight trend towards increased FeNO in pooled CB-280 cohorts (**Fig. 7A**)
- Slight trend towards decreased sweat chloride in pooled CB-280 cohorts vs. placebo (**Fig. 7B**)
- Mean decrease of 6.5 mmol/L by day 14 for pooled CB-280 cohorts

Figure 7. Mean changes from baseline in (A) FeNO and (B) sweat chloride in pooled placebo vs. pooled CB-280 cohorts. Postdose samples taken at 6 ± 1 hours. Error bars = 95% confidence interval (Panel A) and IQR (Panel B)

- Insufficient paired sputum samples for evaluation of changes in microbiology

CONCLUSIONS

- CB-280 was well tolerated in this study, with no patient on CB-280 experiencing a DLT in Cohorts 1-3
- CB-280 exhibited linear PK and dose-proportional increases in plasma arginine
- Pharmacodynamic biomarkers trended toward improvement with CB-280 vs. placebo:
  - Slight trend towards increased FeNO
  - Slight trend towards decreased sweat chloride
- Early positive trend in FEV_1 with CB-280 vs. placebo
- The study is ongoing, with Cohort 4 (300 mg BID) currently enrolling

REFERENCES: 1. Korten et al. *J Cyst Fibros*. 2018;17:105-108. 2. Schairer et al. *Virulence* 2012;3:271-279; 3. Ahmadi et al. *Am J Respir Cell Mol Biol*. 2019;61:755-764. 4. Grasemann et al. *Am J Respir Crit Care Med*. 2005;172:1523-1528. 5. Wu et al. *Mol Pharmacol*. 2019;96:515-525. 6. Brown et al. Emory University. Unpublished data. 7. Grasemann et al. *J Cystic Fibr*. 2013;12:468-474.

ACKNOWLEDGMENTS: We thank the patients and families, investigators, and coordinators for participation in the study, and Lou Ann Brown, Emory University, for the preclinical mouse model experiments. Ingrid Koo, PhD, provided editorial support for the poster.